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Original Article

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

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Abstract

Objectives: The purpose of the current study was to determine how different bracket materials (Polycarbonate and Ceramic) affected the number of Streptococcus mutans in orthodontic patients using herbal toothpaste.

Material and methods: Thirty individuals receiving fixed orthodontic treatment were included in this prospective analysis. One tooth from each patient's first and fourth quadrant of the mouth, totaling 60 teeth, were included in the study. Plaque samples were taken at varied intervals of time. The dilution plating method was used to conduct the bacteriological study. SPSS software tallied the data and performed statistical analyses on it. Each group was compared using a one-way ANOVA. Individual herbal dentifrices were compared across different brackets using multiple comparison tests (Tukey HSD).

Results: Streptococcus mutans count was found to be same Ceramic brackets at mean difference 2.6667 & Polycarbonate brackets at mean difference 2.6667 among 30 orthodontic patients using herbal tooth paste. No statistically significant difference was found between them,

Conclusion: With herbal tooth paste, both ceramic and polycarbonate brackets behaved well and were clinically effective in lowering Streptococcus mutans colony counts nearby.

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Keywords: Streptococcus Mutans¸ Herbal toothpaste¸ Ceramic brackets¸ Polycarbonate brackets.

INTRODUCTION

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.[1]

Pomegranate is currently finding important applications in the field of dental health. Clinical studies[2] 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).[2]

Babool (Acacia nilotica) is a medium size thorny tree found in the drier parts of India[6]. Babul contains tannin and gallic acid and the leaves, bark and the gum of the tree all have medicinal qualities of antibacterial, antihistaminic, antiinflammatory, astringent and hemostatic properties and is helpful in cases of gingivitis and periodontitis.[3]

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. Orthodontic brackets have been found to induce specific changes in the oral environment[4] 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 ceramic, and polycarbonate 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 polycarbonate and Ceramic orthodontic brackets.

MATERIAL AND METHOD

Nature of Study

Microbiological assay study that is prospective, randomised, cross-sectional, and single-blinded, with each patient serving as their own independent control.

Area of Study

Research and Microbiological Assay were carried out in Divya Jyoti Hospital by the Department of Orthodontics and Dentofacial Orthopedic

Ethical Approval

Institutional Committee (IEC No. DJD/IEC/2014/A-001) gave its approval for this investigation. Each participant provided written consent prior to participating.

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

N = ($Z\alpha + Z\beta$) 2 x S2 x2 /d2

N= (1.96+1.282)2x (0.5)2 x 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 undergoing orthodontic treatment from the Department, 15 of whom were men and 15 of whom were women, with a mean age of 15 to 25 years, were chosen. One tooth from each patient's first and fourth quadrants of the mouth was included in the study, for a total of 60 teeth.

Inclusion criteria
• Patient with comparable socioeconomic status and similar dietary preferences.
• One month without any oral or parenteral antibiotics for the patients.
• There are no systemic or periodontal diseases.
• Patients who have undergone alignment and levelling but are not crowded.

Ceramic **Polycarbonate**
Fig. No.1: Brackets Included In Study

S.No	N	Type	Bracket Bonded on Tooth Number
Group I	30	**Ceramic** Rhomboidal MBT	35
Group II	30	**Polycarbonate** Rhomboidal MBT	45

Table shows: Sample Grouping

Bonding Technique
The labial and buccal surfaces of the teeth were acid etched for 30 seconds with 37% phosphoric acid gel (3MTM ESPETM), followed by 30 seconds of rinsing and 20 seconds of drying with oil-free compressed air. With an applicator tip, Ortho Solo (Ormco) primer was applied to the teeth, and Transbond XT (3M Unitek, Monrovia adhesive) was put to the bracket base. The bracket was then positioned on the tooth while being sufficiently squeezed by the positioning tweezer to force the flash out of the bracket. Explorer was used to carefully remove the flash, making sure that it was completely gone. The bracket used in the investigation was bonded by a single operator to ensure consistency in the pressure used to discharge the flash. Light was used to cure the teeth for 20 sec.

Oral Hygiene Instructions

- The respondents received oral hygiene instructions and were urged not to use any additional oral hygiene products, such as mouthwash.
- The study schedule called for the individuals to use toothpaste to clean their teeth twice daily as part of a routine oral hygiene regimen.
- The patients were instructed to thoroughly rinse after each meal.

TOOTHPASTE	TIME INTERVAL
Herbal	3rd to 8th Day

Table shows: Time Interval of Tooth Paste Usage

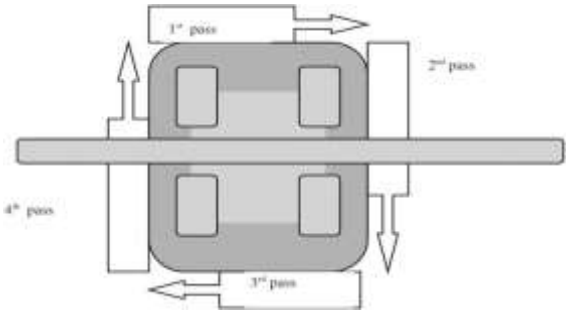

Fig. No.2: Showing Four Pass Technique

Plaque Collection Method

• Patients were instructed to skip eating and drinking for one hour before sample collection.
• At midday 11a.m., a plaque sample was obtained using the Four Pass Technique (Fig. No. 2).
• The explorer tip is moved around the bracket's perimeter at the bracket-tooth interface in this procedure.
• To avoid overtaxing the instrument tip, four passes are made along the tooth at the bracket interface at the gingival, mesial, distal, and occlusal aspects.
• This approach is thought to be successful in acquiring the entire plaque. Plaque samples were put in distilled water-filled, sterile vials

Ice Box


Ice Box

Fig. No.3: Plaque collection and transportation

- Plaque samples were put into 5 ml sterilised vials with 1 ml of distil water.
- Vials that had been sterilised were delivered to the lab in an icebox.
- The dilution plating method was used to conduct the bacteriological study.
- Mutans-Sanguis Agar was the growth medium employed

Table shows: Time Interval of Plaque Collection

S.No	Item
1	Autoclave
2	Hotplate
3	Petridish
4	Micropipette
5	Laminar flow Cabinet
6	Conical flask
7	Cotton Plug
8	Sterilized Wire loop
9	Incubator
10	Disposable gloves
11	U shape flask
12	Disposable Mouth mask

Table shows: Laboratory Equipment's

S.No	Item
1	Mitis Sanguis Agar (Himedia)
2	Distilled Water

Table shows: Laboratory Consumable

Sample Count	Time Interval	Day Count
Sample **No. 1** (baseline without use of study dentifrices)	**(T1)** (Start of study)	Day : **1**
Sample **No. 2**	**T2**	Day : **3**
Sample **No. 3**	**T3**	Day : **8**

Hot Plate

Mutans Sanguis Agar

Laminar Air Flow

Distilled Water

Wire Loop

Micropipette

Fig. No. 4: Laboratory Equipment's & Consumable

Fig No. 5: Sterilization of Diluted Agar Medium in Autoclave

Fig No. 6: Petridishes Placed Inside Incubator

Fig No. 7: Solidification of Agar Medium in Laminar Air Flow

Fig No. 8: Incubator

Fig No. 9: Spreading of Plaque Sample over Petridish

Lab Procedures

a) Protocol under Autoclave

• One litre of distilled water was combined with 100 grammes of Mitis Sanguis Agar (Himedia), and the mixture was autoclave sterilised at 121 degrees for 20 minutes.
• Home foil was placed on top of a cotton plug (Absorbent) and liquid agar in a conical flask.

b) Laminar Air Flow Protocol

• The cotton plug (Absorbent) and home foil were taken off the conical flask's top, and melted agar was then poured into the petridish for solidification in laminar air flow for ten to fifteen minutes at 37 °C.
• The plaque sample was applied to the petridish using a sterilised wire loop.

C) Protocol Under Incubator

- Petridishes containing samples of agar made in the preceding step were sealed with Parafilm "M" and incubated in an incubator for 48 hours at 37 °C.

D) Data Collection

- Data collection was done by mixing the growth with 1 ml of distil water and re-incubating it for 24 hours.
- A 10 microliter sample was applied with a micropipette to a slide before being covered with a cover slip.
- Colony counts were performed using a 40 X (high power) lens and a microscope. Data was gathered and summarised for each group. The researcher counted colonies to determine how many Streptococcus mutans were present in the sample. In order to verify for intra observer error, samples were randomly recounted at various time intervals. No significant errors were discovered.

Statistical test-

- SPSS software tallied the data and performed statistical analyses on it.
- For each time period in the study, one-way analysis of variance (one-way ANOVA) was utilised to compare the various groups (Ceramic and Polycarbonate) (T1,T2 & T3).
- The brackets dentifrice combination's effectiveness was compared using a T-test against the colony count of Streptococcus mutans.
- Two groups of brackets with herbal dentrifices (Ceramic, Polycarbonate) were compared using a two-way analysis of variance (ANOVA) test.

- Individual comparisons between the several brackets using herbal dentifrices were made using multiple comparison tests (Tukey HSD).
- Using a one-way ANOVA, the significance of the mean count of Streptococcus mutans around two brackets tested.

Bracket	Day 1	Day 3	Day 8
C	3.5555	3.3667	2.6667
PC	3.6333	3.5555	2.6667

***Highly Significant p <0.001, **Significant p < 0.05,*Not Significant p >0.05

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

- **Day 1(Baseline):** The Streptococcus mutans count was high in PC bracket at 3.6333 and low in C bracket at 3.4333 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 8 Herbal:** Both the values were similar at 2.6667 to that of baseline at Day 8 no statistical significance.

Day	Group	Group	Mean Difference	P value
3	**C**	**PC**	0.0000	1.000*

***Highly Significant p <0.001, **Significant p < 0.05,*Not Significant p >0.05

Day 3: Herbal based toothpaste showed no statistically significant difference in inter group comparison of the brackets.

Day	Group	Group	Mean Difference	P value
8	**C**	**P C**	0.00000	1.000*

***Highly Significant p <0.001, **Significant p < 0.05,*Not Significant p >0.05

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

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

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

Value	Inference
>0.9	Excellent
>0.8	Good

> 0.7	Acceptable
> 0.6	Questionable
>0 .5	Poor
< 0.5	Unacceptable

Table shows: Rule of George and Mallery

Reliability Statistics		
Cronbach's Alpha	Cronbach's Alpha Based on Standardized Items	No. of Items
0.771	0.780	1

Table shows: Cronbach's alpha reliability test.

- The Cronbach's Alpha reliability test was used to evaluate the statistical test retest validity and determine whether the streptococcus mutans colony count had decreased. The result, which was discovered to be 0.771, indicates that the data's reliability is satisfactory.

DISCUSSION

There are several different biomaterial bracket kinds on the market. According to published research, bracket material 8 may have a significant impact on how much plaque sticks to fixed appliances, how much bacteria adhere to it, and how likely it is that WSL would develop. Electrostatic and hydrophobic interactions account for a large portion of bacteria's initial attraction for solid surfaces. Bacteria like S. mutans are attracted more readily to surfaces with high surface free energy.

In order to decrease enamel demineralization and WSL, the clinician is searching for a beneficial mix of dentifrices, bracket material, and/or bracket design. The purpose of this study was to determine whether the bracket material had any influence on bacterial oral microflora. So, ceramic and polycarbonate bracket materials were examined. These brackets have a traditional shape. The commercially available, herbal dentifrices examined included Babool, Neem, Meswak, and Pomegranate. The participating subjects were instructed to follow a certain oral hygiene routine and asked to use the offered dentifrices within the allotted times. This single blinded prospective microbiological assay was carried out on blinded patients to determine whether any combination of bracket material/design and dentifrice might lessen the microbial count of Streptococcus mutans in the mouth during the test period. Since it is commonly known that elastomeric rings gather more microorganisms, both brackets to arch wire ligations were completed using ligature wire. The teeth used in the current investigation were 45 and 35 because, according to Khalaf's study, 9 the canines on the maxilla and the lateral incisors on the mandible and maxilla as well as the first molars had the highest incidence of WSLs due to plaque deposition. Plaque was collected using the Four Pass technique as per usual procedure, and the laboratory's microbial flora was evaluated using the Diluting and Palating method.10 Streptococcus mutans has been cultured in the lab on a variety of growth media. 11 Accuracy in colony counting and microbe isolation is hampered by actual microbe isolation Accuracy in colony counting and microbe isolation is hampered by actual microbe isolation. Because of their similar sizes, Strep. sanguinis and Strep. mutans may compete for the same oral environment niche or coexist together. While Strep. mutans is a pathogen linked to dental caries and WSL, Strep. sanguinis is a commensal of the oral cavity and may be helpful by preventing the growth of Strep. mutans. Therefore, every research using lab cultures must select an adequate culture media in order to produce reliable research results. In the current research we used Mitis Salivaris Agar 12 which is a differential culture media and differentially allows the growth of Strep. mutans and inhibits Strep. sanguinis his or her own control.

The results of the current research assessing the performance of orthodontic brackets of Streptococcus mutans around ceramic brackets using herbal toothpaste revealed an extremely

substantial reduction with ceramic and polycarconate brackets. These findings were consistent with a study by Jurela et al.9 that found no evidence of a significant difference in the number of colony-forming units of S. mutans and S. sobrinus in stimulated saliva samples between patients wearing stainless steel brackets and patients wearing polycarbonate brackets

When using herbal toothpaste, the current research study's findings indicated a highly significant reduction around Polycarbonate brackets. The study by Papaioannon et al 10 where three adhesion experiments using ceramic, and plastic orthodontic brackets was done and it was found that there were consistently no differences in the adherence of Streptococcus mutans to stainless steel, ceramic, or plastic brackets. . The results of the current research study showed significant reduction around Ceramic bracket highly significant reduction when herbal toothpaste is used**.** These results were in consonance to the study done by *Anhoury et al*[11]

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 study looked at whether using herbal dentifrices with bracket biomaterials made of ceramic and polycarbonate may reduce the prevalence of Strep mutans. Strict selection criteria were used to enrol 30 people in the study. Each person's teeth Nos. 45 and 35 were linked with a particular bracket, and 60 teeth total—30 teeth per bracket in each study group—were included in the analysis. The tests on the herbal dentifrices & 2 variables of brackets were performed at the same time interval in all participants, which increased accuracy in this research design. The results can be summed up as follows:
- The Streptococcus mutans count around the various brackets at baseline for herbal toothpaste showed no statistically significant difference.
- The bacterial colony counts of Strep mutans around the cearmic and Polycarbonate bracket showed a statistically significant difference on Day 3 (i.e., 24 hours after the usage of herbal toothpaste).
- By Day 8, the Strep mutan colony counts surrounding both brackets were significantly decreased from baseline values on Day 1 and both brackets had equally well results with the herbal-based tooth paste. Tests of intergroup comparability showed no discernible difference between the brackets.
- Herbal tooth paste has decreased the amount of S. mutans colony forming units near both brackets.

As a result, it can be said that both ceramic and polycarbonate brackets worked effectively with herbal toothpaste to lower Strep mutans colony numbers around them.

During orthodontic therapy, it may be recommended to use herbal remedies containing neem, babool, pomegranate, and meswak along with polycarbonate and ceramic brackets.

The study revealed that even while a statistically significant decrease in bacteria counts was discovered 24 hours after the introduction of the dentifrice, patient counselling regarding consistent discipline in dental hygiene is essential.

To gain knowledge for creating products with a sustained release and an antibacterial activity that reduces counts for an extended length of time, more research with various doses of the active components has to be conducted on bigger and more diverse sample groups. This new invention would be especially helpful for individuals with poor periodontal health and those who struggle to maintain good oral hygiene while undergoing orthodontic treatment.

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Authors's contribution
SA¸orthodontic treatment of patients and follow-up¸plaque sample collection¸transportation of plaque samples at different interval to microbiological lab ¸ interpretation of data and writing of the protocol and manuscript. KRR participated in the concept design¸supervised the workflow and reviewed the research .ST participated in reviewing the paper.

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Availability of data and materials
The patients and the material were provided by the institute after the approval by the head of the department

Declarations
Ethics approval and consent to participate. All patients gave their informed consent to take part and the study got ethical clearance from the institutional ethical committee Id- (IEC No DJD/IEC/2014/A-001).

Consent for publication -
All patients gave their informed consent to take part.
Competing interests-
The authors declare that they have no competing interests.

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