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Comparison of Means of Streptococcus Mutans Count at Different Time Interval in Polycarbonate Bracket with Herbal and Fluoridated Toothpaste.

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Abstract
Aim*: The purpose of this study was to ascertain if toothpastes have an effect on Streptoccus mutans count in orthodontic patients.* ***Materials and Methods****: The study comprised of 30 patients (15 males and 15 females) who were undergoing orthodontic treatment in department were selected. A total of 120 teeth were included in the study. Randomized, prospective, cross sectional single blinded microbiological assay study with each patient acting his /her own control in this study.* ***Results****: Paired T test compared the means of Streptococcus mutans count around polycarbonate bracket at different time intervals.* ***Conclusion****: Polycarbonate bracket had more reduction of Streptococcus mutans with conventional toothpaste as compare to the herbal toothpaste.*

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I. Introduction

Orthodontic patients are faced with the hazard of increased retention of food particles and plaque accumulation due to the presence of multiple attachments like brackets and other auxiliaries in the oral cavity forming encatchment areas for plaque. This results in oral ecological changes with low pH environment and increased proportions and absolute number of salivary mutans. The orthodontist as the clinician is continuously challenged to curb and eliminate White Spot Lesions (WSL) in their patients during orthodontic treatment

Streptococcus mutans is a potent initiator of caries because there are a variety of virulence factors unique to the bacterium and play an important role in caries initiation. Firstly[1], Streptococcusmutans is an anaerobic bacterium known to produce lactic acid as part of its metabolism. Secondly, there is the ability of Streptococcusmutans to bind to tooth surfaces in the presence of sucrose by the formation of water-insoluble glucans, a polysaccharide that aids in binding the bacterium to the tooth[1].The most important virulence factor is the acidophilcity of Streptococcus mutans. Unlike the majority of oral microorganisms, Streptococcus mutansthrives under acidic conditions and becomes the dominant bacterium in cultures with permanently reduced pH[2].

Though, brushing teeth twice a day is considered reasonably effective in plaque and bacterial count reduction, the common prevalence of gingival inflammation in orthodontic patients often suggests inadequate oral hygiene procedures in most patients. As bacteria in dental plaque is one of the main factors causing periodontal inflammation; careful plaque control is very important .The extrinsic variables affecting the bacterial count (besides the confounding intrinsic host factors) may be considered to be the type of tooth brush used by the patient and the method of brushing, the effect of the dentifrice used and the quality and quantity of orthodontic attachments in the oral cavity.

Conventionally orthodontic patients use some form of cleansing equipment like a toothbrush aided with dentifrices of their choice. The market is flooded with conventional, therapeutic (fluoridated and non-fluoridated) and 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[2].

Hence, this study project wasdesigned as a microbiological assay of Streptococcus mutans with an objective to study the performance and measure the efficacy of two common toothpastes- Neem, Meswak, Babool and Pomegranate based herbal toothpaste and a Fluoride based conventional toothpaste with conventional design Ceramic orthodontic brackets.

II. Aim And Objectives

To ascertain if dentifrices have an effect on Streptococcus mutans count in orthodontic patients with polycarbonate brackets.

III. 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.

I. Dentifrices

S.No	Details	Code
1	**Conventional Dentifrice** (Colgate Palmolive) containing fluoride.	**X** (**White**)
2	**Himalaya Herbals Dentifrice** (Himalaya Global Holding Ltd.) Containing Neem, Meswak Babool and Pomegranate	**Y** (**Blue**)

II. Bracket Type

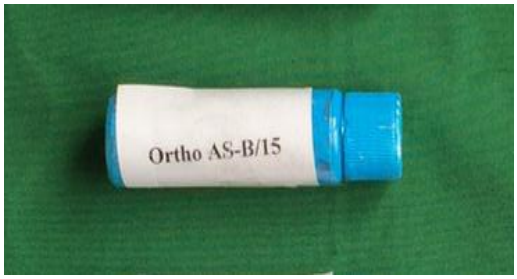
S.No	Bracket type
1	**Polycarbonate** Rhomboidal MBT

Polycabonate bracket

Steps and Time Interval of Study

- Each group consists of 30 teeth with 30 brackets to be tested.
- Each patient served as his/her own control as 1 types of bracket were tested in the same mouth at the same time period, with the 2 types of dentifrices.
- Each patient had tooth No's 45 included in the study with stainless steel bracket bonded.
- Dentifrices tested were Conventional fluoride based (control group) and Herbal based.
- The dentifrices were dispensed into 5ml bottles coded as **X** for Conventional toothpaste –Colgate and **Y** for Herbal toothpaste –Neem, Babool, Meswak and Pomegranate. Color Coding of Dentifrices
- Conventional tooth paste was considered as control group.

S.No	N	Type	Bracket Bonded on Tooth Number
I	30	**Polycarbonate bracket**	45



CODES	COLOR	DENTIFRICES
X	White	**Conventional Fluoride BasedToothpaste** (Colgate Palmolive)
Y	Silvervff Blue	**Herbal Based Toothpaste** (Herbal Global Holding Ltd Toothpaste)

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.

TOOTHPASTE	TIME INTERVAL
Conventional (X)	3rd to 8th Day
Herbal (Y)	10th to 15th Day

Table Shows: Time Interval of Tooth Paste Usage

Plaque Collection Method

- Patients were requested to refrain from eating or drinking 1 hour prior to sample collection.
- Plaque sample was collected by Four Pass Technique 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.

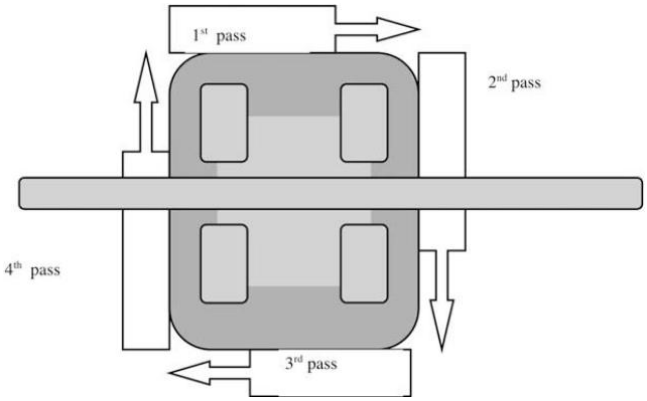


Fig. No.3: Showing Four Pass Technique

Plaque Collection Method

- 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.4) 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.

Fig. No.5: Plaque Collection and Transportation

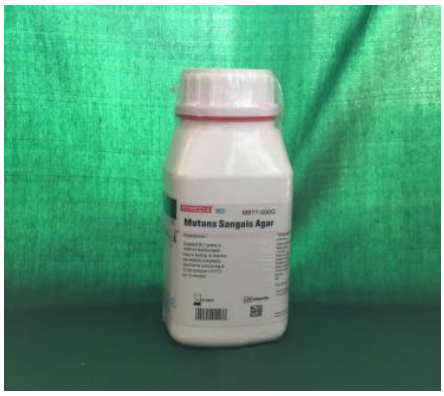
Sample Count	Time Interval	Day Count
Sample **No.1** (baseline without use of study dentifrices)	**(T_1)** (Start of study)	Day : **1**
Sample **No.2**	**T_2**	Day : **2**
Sample **No.3**	**T_3**	Day : **8**
Sample **No.4**	**T_4**	Day : **10**
Sample **No.5**	**T_5**	Day : **15**

No.6: Time Interval of Plaque Collection

Plaque collection and transportation

- Plaque sample placed in 5ml sterilized vials with1ml distill water.
- Sterilized vials were transported in icebox to the lab.
- The bacteriological study was conducted by Dilution Plating Method.
- The growth media used was Mutans-Sanguis Agar.

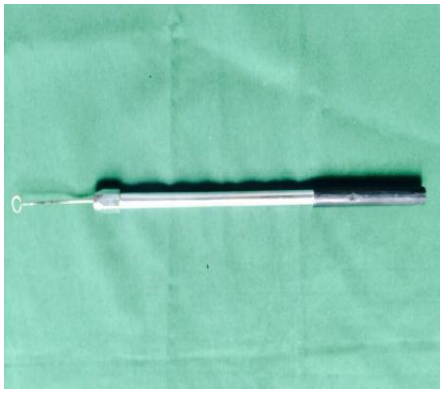
Hot Plate



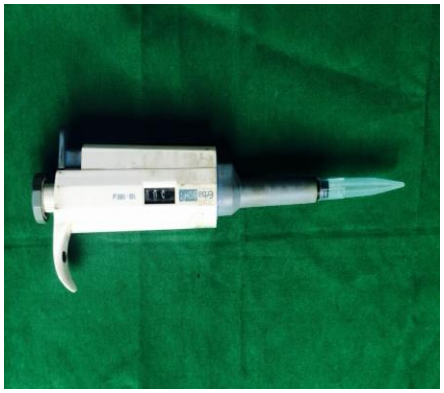
MutansSanguis Agar

Laminar Air Flow

Distilled Water



Wire Loop



Micropipette

Fig. No. 6: Laboratory Equipment's&Consumable

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 No.7: Laboratory Equipment's

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

Table No. 8: Laboratory Consumable

Fig No. 7: Sterilization of Diluted Agar Medium in Autoclave

Fig No. 10: Petridishes Placed Inside Incubator

Fig No. 8: Solidification of Agar Medium in Laminar Air Flow

Fig No. 11: Incubator

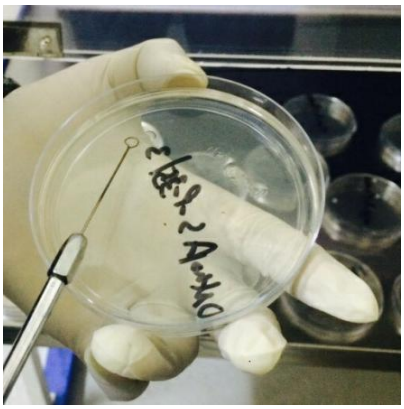


Fig No. 9: Spreading of Plaque Sample over Petridish

Lab Procedures

a) Protocol under Autoclave

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

b) Protocol under Laminar Air Flow

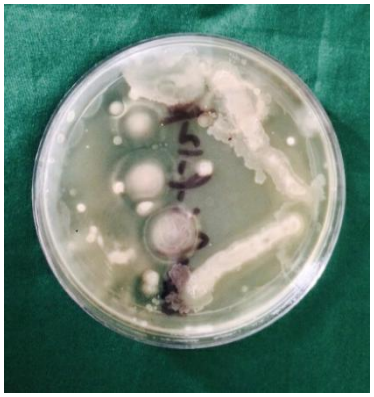
- Cotton plug (Absorbent) and Home foil was removed from the top of conical flask and melted agar was poured in petridish for solidification in laminar air flow for 10-15 minutes at 37^0C.
- Then with the help of sterilized wire loop, plaque sample was spreadover the petridish.

c) Protocol Under Incubator

- 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

- Then growth was mixed with 1 ml of distill water and then incubated again for 24 hours in incubator.
- With the help of micropipette 10 micro liter was spread over slide and then covered with cover slip
- Then colony count was done under the microscope with the help of 40 X (High power lens)
- The data was collected for all the groups. Colony counting was done by researcher to obtain the number of Streptococcus mutans in the sample tested and then random count of samples was done repeatedly in order to avoid observer error.



Day 1

Day 8

Day 2

Day 10

Day 15

Colonies of Streptococcus mutans

Statistical test-

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

- T-test was used to compare efficacy of same brackets against Streptococcus mutans in conventional and herbal group.
- The two-way ANOVA identifies if there is a significant interaction effect between both conventional and herbal group.

IV. Results

Table 9:

Days	Mean difference	t	d.f.	P value
Day 1 - Day 2	.10000	.619	29	.541*
Day 1 - Day 8	.96667	7.370	29	.000***
Day 2 - Day 8	.86667	4.709	29	.000***
Day 1 - Day 10	.13333	.941	29	.354*
Day 1 - Day 15	.96667	6.547	29	.000***
Day 10 - Day 15	.83333	5.473	29	.000***
Day 2 - Day 10	.03333	.372	29	.712*
Day 8 - Day 15	.00000	.000	29	1.000*

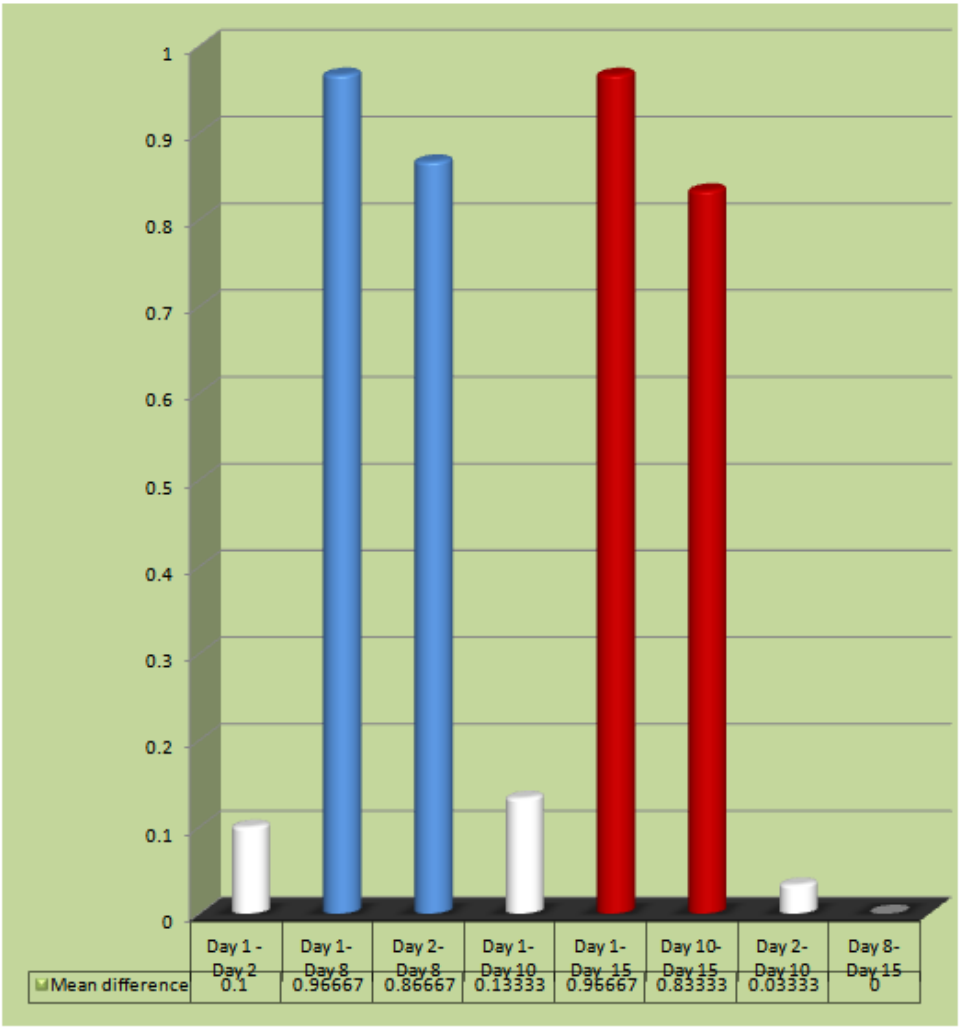
***Highly Significant p <0.001, **Significant p < 0.05,*Not Significant p >0.05

Table9: Comparison of Means of Streptococcus mutans Count between Two Days in Polycarbonate Bracket by Paired T –Test

Table 9 represents-

- Paired T test compared the means of Streptococcus mutans count around PC bracket at different time intervals.
- Difference between Day 2 and Day 8 with fluoride based dentifrice was highly significant statistically
- Difference between Day10 & Day 15 with herbal dentifrice was highly significant statistically
- Difference between Day 1 & Day 8 and Day 1 & Day 15 were also highly statistically significant.

Graph No. 4: Comparison of Means of Streptococcus mutansCount between Two Days inPolycarbonate Bracket



Graph No. 4 shows

- The mean difference between Day 2 & Day 8 is 0.8666 and between Day 10 & Day 15 is 0.83333.
- This shows PC has more reduction of Streptococcus mutans with conventional toothpasteas compare to theherbal toothpaste.

V. 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. mutansis the most cariogenic. ***Loesche et al***[7]showed significant association between plaque levels of S. mutansand caries. Although WSL occur in caries development irrespective of orthodontic treatment, it is during orthodontic treatment that they are extremely common and a prime concern for the clinician. The brackets, bands, archwires and elastomeric modules of fixed orthodontic appliances provide additional surface area for bacteria to develop and thus accelerate the accumulation of plaque and the formation of lesions in areas that would normally have a low risk of caries. This is especially so when combined with a poor oral hygiene. However, ***Rosenbloom*** [9] differed and suggested that orthodontic treatment does not result in any long-term elevations of S. mutans levels.

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 dentifices had any role in reducing bacterial oral microflora. Hence ceramic bracket material was tested. These brackets were of the conventional design.. The dentifrices tested were (i) Herbal commercially available product containing Babool, Neem, Meswak and Pomegranate and(ii) Fluoride containing Colgate was used as the control as it is often considered the most common household toothpaste, specially in rural/semi urban India.

Hence, it was decided to research if any differences existed in the performance of the common fluoridated dentifrice and an herbal product with a potent combination of neem, babool, meswak and pomegranate.

The teeth which were chosen for the present study were 12, as the study done ***Khalid***[11] 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 .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 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*** [11] used Mitis Salivarius agar for bacterial culturing Streptococcusmutans around SLB systems. ***Syed & Loesche***[13]tested different culture media sucrose blood agar, N2C agar, Schaedler agar, and Mitis Salivarius agar and found the Mitis Salivaris Agar a suitable media for differential culture. ***Emilson & Bratthall***[14] tested total cultivable flora and the selective media of Mitis Salivaris Agar and found it acceptable as a culture media for Streptococcusmutans. Some researchers like ***Wade et al*** [15] incorporated Bacitracin for superior results.

The results of the current research study showed significant reduction of *Streptococcusmutans* counts around Polycarbonate bracket with both conventional toothpaste and herbal toothpaste .The results of current study were in contrast ***Papaioannon et al***[16]conducted three adhesion experiments using stainless steel, ceramic, and plastic orthodontic brackets and found that there were consistently no differences in the adherence of *Streptococcusmutans* to stainless steel, ceramic, or plastic brackets. ***Gastel*** [17] concluded that orthodontic brackets serve as different loci for biofilm formation though significant differences were noted between the different types of brackets. This is similar to the findings of the current study as Strep mutans counts were different on different bracket design and materials with different levels of statistical significance.The results of current study were in contrary to the study done by ***Joon***[21] who investigated the adhesion levels of cariogenic streptococci strains to monocrystalline sapphire, polycrystalline alumina, stainless steel, plastic, and titanium brackets and found that the adhesion amounts were highest in the plastic brackets and lowest in the monocrystalline sapphire brackets.

Herbal products for oral health care seem to have a potential in this direction ***Delphine*** [12]show an increase of *Streptococcusmutans* values during the orthodontic treatment and follow up and the whole dental workforce should be aware that preventive measures are of paramount importance .***Wilson*** [14] and ***Alves et al*** [15]found the antibacterial effects of chlorhexidine mouthwash on *Streptococcusmutan*s in orthodontic patients having the best antimicrobial effect. However, chlorhexidine has the disadvantage of staining the teeth and making them unacceptable to many orthodontic patients. Herbal tooth pastes (Meswak) was compared[13]and found to be highly effective. In patients with poor oral health, periodontal conditions and patients averse to stains, herbal dentifrices may be considered a good alternative. ***Subramaniam et al***[7]neem stressed that different concentration of neem leaves extract influenced the inhibition of *Streptococcusmutans* ***Elangovan*** **et al** , using the disc diffusion method proved that the antimicrobial activity of neem, meswak, and mango extracts increased as their concentrations increased.

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

VI. Conclusion

- This shows Polycarbonate has more reduction of Streptococcus mutans with conventional toothpastes compare to the herbal toothpaste

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