Original Article

To Ascertain If Bracket Material Difference (Polycarbonate 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.S08.595

Abstract

Objectives: The purpose of the current study was to determine how different bracket materials (Polycarbonate and Stainless Steel) 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 slightly low with stainless steel brackets at mean difference 2.5333 & high with polycarbonate brackets at mean difference 2.6667 among 30 orthodontic patients using herbal tooth paste. A statistically significant difference between them, however, was not present.

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

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

INTRODUCTION

Patients of traditional orthodontics typically use a toothbrush and their preferred dentifrices as well as other cleaning supplies. There are an abundance of Ayurvedic goods on the market. The patient is typically free to choose his own toothpaste throughout orthodontic treatment, with the majority of orthodontists only prescribing orthodontic-specific toothpaste and mouthwash. A number of carefully conducted research trials have shown that using herbal dentifrices to clean teeth decreases gingivitis and supragingival plaque1. Meswak, a medicinal herbal plant, has been used for ages as a means of maintaining good mouth hygiene. A chemical examination of S. persica revealed the presence of many substances with antibacterial properties. Salvidorine and trimethylamine, which are found in Meswak (Salvidora persica), have been demonstrated to have antibacterial properties on cariogenic2 bacteria such Streptococcus mutans. These active ingredients have been demonstrated to promote periodontal health, lessen the buildup of biofilm-like dental plaque, and have fungistatic activity against Candida albicans2. The World Health Organization (WHO) recommended using the meswak due to its benefits for maintaining oral hygiene and scientific studies that have supported its antibacterial and plaque-preventing abilities. Meswak is a natural, economical, and eco-friendly way to maintain good oral hygiene. The use of meswak stick and other natural herbs is expanding quickly in both developing and developed countries due to its free availability and unique chemical structure. According to research, meswak is just as useful as—or possibly even more effective than—the standard dental hygiene tools used today. The use of meswak as an effective technique for oral hygiene has been acknowledged and advised by the World Health Organization (WHO). 3

A tropical evergreen tree native to India called neem (Azadirachtaindica) is also present in other southeast Asian nations. Neem has been utilised in Ayurvedic medicine for more than 4,000 years and is referred to as "the village pharmacy" in India due to its many therapeutic uses. In Sanskrit, neem is also known as "arista," which means "perfect, complete, and imperishable." A substance found in the seeds' bark and leaves has been shown to have antibacterial, antiviral, antipyretic, anti-inflammatory, anti-ulcer, and antifungal properties3. Neem's long-standing reputation as a cavity preventer was explained by the discovery that it might lessen Streptococcal bacteria's capacity to proliferate on the surface of teeth.

Presently, the field of dental health is seeing significant applications for pomegranates. According to clinical studies4, this well-known antioxidant tackles tooth decay's molecular origins with surprising vigour. Numerous cultures throughout the world have made substantial use of pomegranate fruit in folk medicine. One of the first medical writings, the Egyptian Ebers Papyrus, mentions the therapeutic power of pomegranates (1500 BC). 4 A medium-sized thorny tree called babool (Acacia nilotica) can be found in the drier regions of India5. The leaves, bark, and gum of the babool tree all have therapeutic capabilities of antibacterial, antihistaminic, anti-inflammatory, astringent, and hemostatic properties and are useful in cases of gingivitis and periodontitis. Babool also contains tannin and gallic acid. 6

The presence of orthodontic appliances is another crucial element for microbial colonisation. In addition to the adherent patterns of bacteria on various types of orthodontic brackets—determined by design and material—interactions between salivary components in the pellicle and properties of the different microorganisms also have a significant impact on the adhesion of oral microorganisms to bracket surfaces. Metallic braces have been shown to cause particular alterations in the oral environment6, including pH levels that are lower, plaque buildup that is higher, and S. mutans colonisation that is higher. Recent research on potential variations in the initial affinity and long-term adherence of bacteria on metal and polycarbonate brackets, however, came up inconclusive.

Therefore, this research project was created as a traditional Streptococcus mutans microbiological assay with the goal of evaluating the performance and efficacy of Neem, Meswak, Babool, and Pomegranate based herbal toothpaste with Orthodontic brackets made of polycarbonate and stainless steel..

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 = (Zα +Zβ) 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.

Stainless steel **Polycarbonate**

Fig. No.1: Brackets Included In Study

.No	N	Type	Bracket Bonded on Tooth Number
Group I	30	**Stainless Steel** Rhomboidal MBT	12
Group II	30	**Polycarbonate** Rhomboidal MBT	45

Table No.1: 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 No.2: Time Interval of Tooth Paste Usage

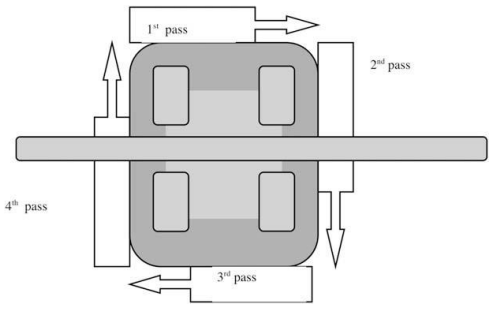

Fig. No.2: Showing Four Pass Technique

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. 4).

• 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

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 No.3: Time Interval of Plaque Collection

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

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.4: Laboratory Equipment's

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

Table No. 5: Laboratory Consumable

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: Medium Solidification of Agar in Laminar Air Flow

Fig No. 8: Incubator

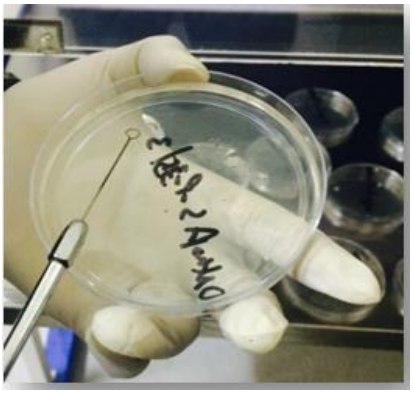

Fig No. 9: Spreading of Plaque Sample over Petridish

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 370C.
• The plaque sample was applied to the petridish using a sterilised wire loop.

b) 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.

c) 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 intraobserver 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 (SS 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 (SS, 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.

Day	Bracket	N	Mean	Std. Deviation	P value
1	SS	30	3.5000	±.50855	0.391*
	PC	30	3.6333	±.49013	
2	SS	30	3.3333	±.47946	0.025**
	PC	30	3.5333	±.50742	
3	SS	30	3.3667	±.49013	0.431*
	PC	30	3.5000	±.50855	
8	SS	30	2.5333	±.50742	0.532*
	PC	30	2.6667	±.66089	

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

Table No. 6: Comparisons of Means of Streptococcus mutans Count in Two Brackets at Different Time Interval (One Way ANOVA)

Bracket	Day 1	Day 3	Day 8
SS	3.5555	3.3667	2.5333
PC	3.6333	3.5555	2.6667

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

Table No.7: 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 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 8 Herbal :** low with SS at 2.5333 & high with PC at 2.6667.

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

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

Table No.8: Multiple Comparisons of Means of Streptococcus mutans Count in Two Brackets on Day 3 by Tukey HSD Test

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	**SS**	**P C**	0.00000	1.000*

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

Table No. 9 : 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,

Days			Mean difference	T	d.f.	P value
Day 1	-	**Day 3**	0.13333	1.000	29	0.326*
Day 1	-	**Day 8**	0.96667	6.547	29	0.000***
Day 3	-	**Day 8**	0.83333	5.767	29	0.000***

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

Table No. 10: Comparison of Means of Streptococcus mutans Count at Different Time Intervals around Stainless Steel Bracket by Paired T – Test**.**

- Paired T test compared the means of Streptococcus mutans count around SS 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 significant.

Days			Mean difference	T	d.f.	P value
Day 1	-	**Day 3**	0.06667	0.494	29	0.625*
Day 1	-	**Day 8**	0.76667	6.707	29	0.000***
Day 3	-	**Day 8**	0.70000	5.114	29	0.000***

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

Table No. 11: Comparison of Means of Streptococcus mutans Count at Different Time Interval in Polycarbonate Bracket by Paired T – Test

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- 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 No. 12: 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 No. 12: 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

Enamel white spot lesions (WSL) close to orthodontic brackets are frequently caused by plaque building up around the brackets. S. mutans is the most cariogenic among the numerous bacteria that make up plaque. Significant correlation

between S. mutans plaque levels and caries was shown by Loesche et al. 7 in their study. Although WSL develop throughout caries development regardless of orthodontic treatment, they are more prevalent and of primary concern to clinicians during orthodontic treatment. Microorganisms are a significant factor in the development of WSL and dental caries. Although complete eradication of microorganisms from the oral cavity is challenging, their number can be decreased through a variety of preventative methods, making it less cariogenic. 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 stainless steel 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 12 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. It aids in separating Enterococcus from salivary Mitis. Streptococcus mutans was cultured on Mitis Salivarius agar by Fadia et al.13 in the vicinity of SLB systems. Different culture media, including sucrose blood agar, N2C agar, Schaedler agar, and Mitis Salivarius agar, were evaluated by Syed & Loesche 14 who discovered that the latter was the best for differential culture. The selective media of Mitis Salivaris Agar was put to the test by Emilson & Bratthall 15 and was shown to be suitable as a culture medium for S mutans. For better outcomes, some researchers—like Wade et al 16—incorporated Bacitracin. The current investigation was distinctive since herbal dentifrice was applied to both brackets being examined simultaneously while each participant served as his or her own control.

Colony counting in vivo may be very time-consuming and labor-intensive if numerous factors are being examined, and it may not always be ethical to test items or treatments in vivo if they have not been approved for use in humans. Nitin et al. (17) employed the disc diffusion method, which determines whether bacteria are impacted by antibiotics, to investigate the antibacterial efficacy of herbal extracts against dental infections. In line with Pujari's 12 study, which concluded that the diluting and plating approach is the most reliable way for growing Streptococcus mutans, the diluting and plating method was used in the current investigation. The results of the current research assessing the performance of orthodontic brackets of Stainless Streptococcus mutans around stainless steel brackets using herbal toothpaste revealed an extremely substantial reduction with steel and polycarconate brackets. These findings were consistent with a study by Jurela et al.18 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 19 where three adhesion experiments using stainless steel, 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. 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 stainless steel 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. 12 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 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 stainless steel and polycarbonate brackets worked effectively with herbal toothpaste to lower Strep mutans colony numbers around them. Although there was a minor improvement in the reduction of S mutans on stainless steel, there was no discernible difference between the brackets in terms of performance.
During orthodontic therapy, it may be recommended to use herbal remedies containing neem, babool, pomegranate, and meswak along with steel 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.

ACKNOWLEDGEMENTS

The authors thank the microbiological department of the institution for helping in conducting bacteriological study .The authors also thank Mr Manoj Sharma for his immence help in the statistical analysis.

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.

Funding

No funding was provided for the study

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.

REFERENCES

1. Ryan KJ, Ray CG. Normal Microbial Flora. In Sherris Medical Microbiology: An Introduction to Infectious Diseases. McGraw-Hill, USA, 2004; 4:141–148.
2. Berardi V et al. Antimicrobial Efficacy of Different Toothpastes and Mouthrinses: An In Vitro Study. Dent Res J.2011; 8(2): 85–94.
3. Sukkarwalla A, Ali SM, Lundberg P. Efficacy of Meswak on Oral Pathogens Dent Res J. 2013; 10(3): 314–320.
4. Botelho MA et al. Differential cytotoxicity: a component of neem oil whose action is exerted at the cell membrane level. Molecules. 2008; 14: 122-133.
5. Taha GI et al. The effect of Pomegranate Peels Extract against Streptococcus Mutans and Adherence to tooth surface in Comparison to Chlorhexidine gluconate. Advances in Life Science and Technology. 2015; 35:23-31.
6. Chatterjee A et al. To evaluate the antigingivitis and antipalque effect of an Azadirachta indica mouthrinse on plaque induced gingivitis: A double-blind, randomized, controlled trial. J Indian Soc Periodontol. 2011; 15(4):398–401.
7. Loesche WJ, Rowan J, Loos PJ. Association of Streptococcus mutans with human dental decay. Infection and Immunity. 1975; 11(6):1252-1260.
8. Ervedi, Heymann GC, Grauer D A. Contemporary Review of White Spot Lesions in Orthodontics J Esthet Restor Dent 2013;25:85–95.
9. Khalaf K. Factors Affecting the Formation, Severity and Location of White Spot Lesions during Orthodontic Treatment with Fixed Appliances. J Oral Maxillofac Res. 2014; 5(1):4.
10. Pellegrini P, Sauerwein R, Tyler FT. Plaque retention by self-ligating vs elastomeric orthodontic brackets: Quantitative comparison of oral bacteria and detection with adenosine triphosphate-driven bioluminescence. Am J Orthod Dentofacial Orthop 2009; 135:426.e1-e9.

11. Hoover CI, Newbrun E. Survival of bacteria from human dental plaque under various transport condition, J Clin Micro. 1977; 6(3)212-218.
12. Pujari S. Bacteria Present In a Sample by Serial Dilution Agar Plating Method or Total Plate Count (TPC). Int J Microbiology.2015; 6(2):101-103.
13. Fadia D, Vandekar M, Vaid N, Vassess D. Plaque accumulation and Streptococcus mutanslevels around self-ligating bracket clips and elastomeric modules: A randomized controlled trial. APOS Trends Orthod. 2015; 5:97-102.
14. Syed SA. Efficiency of various growth media in recovering oral bacterial flora from human dental plaque .Appl Microbiol .1973; 26(4):459-465.
15. Emilson CG, Bratthall D. Growth of Streptococcus mutans on various selective media. J. Clin. Microbiol.1976;4(1):95-98
16. Wade W. G, Aldred M. J, Walker D. M. An improved medium for isolation of Streptococcus mutans J.Med. Microbiol. 1986; 22:319-323.
17. Nitin CM, Adhikrao VY. Polyherbal toothpaste: Oral hygiene product. Indian J Dent Res 2010; 21:380-4.
18. Jurela A, DarioRD, Pejda S et al. The effect of two different bracket types on the salivary levels of S mutans and S sobrinus in the early phase of orthodontic treatment Angle Orthod.2013; 83(1)140-145.
19. Papaioannou W. Adhesion of Streptococcus mutans to Different Types of Brackets. Angle Orthod 2007; 77 (6) 1090-1095.