COMPARATIVE EVALUATION OF PROBIOTIC AND CHLORHEXIDINE MOUTHWASHES EFFECT ON THE ADHESION OF STREPTOCOCCUS MUTANS ON STAINLESS STEEL BRACKETS- A CLINICAL TRIAL

DISSERTATION SUBMITTED TO

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

For Partial fulfilment of the requirements for the degree of

MASTER OF DENTAL SURGERY

BRANCH - V

ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS

THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI – 600 032

2017 – 2020

CERTIFICATE

This is to certify that **Dr. M RAMYA** Post graduate student **(2017-2020)** in the Department of Orthodontics and Dentofacial Orthopaedics, Tamil Nadu Government Dental College and Hospital, Chennai – 600003 has done this dissertation titled **"Comparative evaluation of probiotic and chlorhexidine mouthwashes effect on the adhesion of streptococcus mutans on stainless steel brackets- A Clinical trial"** under my direct guidance and supervision for partial fulfilment of the M.D.S. degree examination in April 2020 as per the regulations laid down by The Tamil Nadu Dr. M.G.R. Medical University, Chennai – 600032 for **M.D.S., Orthodontics and Dentofacial Orthopaedics (Branch – V)** degree examination.

Guided by

Dr. B. Balashanmugam, M.D.S.
Professor of Orthodontics and Dentofacial Orthopaedics,
Tamil Nadu Government Dental College &
Hospital, Chennai - 600003

Dr. Sridhar Premkumar, M.D.S.,
Professor and Head,
Dept. of orthodontics and Dentofacial Orthopedics
Tamil Nadu Government Dental College &
Hospital, Chennai - 600003

Dr. G.Vimala, M.D.S.,
Principal,
Tamil Nadu Government Dental College &
Hospital, Chennai - 600003

DECLARATION

I, **Dr M RAMYA**, do hereby declare that the dissertation titled **"Comparative evaluation of probiotic and chlorhexidine mouthwashes effect on the adhesion of streptococcus mutans on stainless steel brackets- A Clinical trial"** was done in the Department of Orthodontics, Tamil Nadu Government Dental College & Hospital, Chennai-600003. I have utilized the facilities provided in the Government Dental College for the study in partial fulfilment of the requirements for the degree of Master of Dental Surgery in the specialty of Orthodontics and Dentofacial Orthopaedics (Branch V) during the course period **2017-2020** under the conceptualization and guidance of my dissertation guide, **Professor Dr. B. BALASHANMUGAM, MDS.,**

I declare that no part of the dissertation will be utilized for gaining financial assistance for research or other promotions without obtaining prior permission from The Tamil Nadu Government Dental College & Hospital.

I also declare that no part of this work will be published either in the print or electronic media except with those who have been actively involved in this dissertation work and I firmly affirm that the right to preserve or publish this work rests solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai 600 003, but with the vested right that I shall be cited as the author(s).

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ACKNOWLEDGEMENT

I seek the blessings of the **ALMIGHTY GOD** without whose benevolence this study would not have been possible.

With my heartfelt respect, immeasurable gratitude and honour, I thank my benevolent guide, **Dr. B. BALASHANMUGAM, M.D.S.,** Professor, Department of Orthodontics and Dentofacial orthopedics, Tamil Nadu Government Dental College and Hospital, Chennai – 3, for his astute guidance, support and encouragement throughout my post graduate course and to bring this dissertation to a successful completion.

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I also thank my post graduate colleagues for their help and constant support.

TRIPARTITE AGREEMENT

This agreement herein after the "Agreement" is entered into on this..... day of December 2019 between the Tamil Nadu Government Dental College and Hospital represented by its **Principal** having address at Tamil Nadu Government Dental College and Hospital, Chennai-03, (hereafter referred to as, "the college")

And

Dr. B. BALASHANMUGAM aged 48 years working as professor at the college, having residence address at 8-B,Crescent road, Shenoy nagar,Chennai-600030, Tamil Nadu (Herein after referred to as the 'Principal investigator')

And

Dr. M. Ramya aged 40 years currently studying as postgraduate student in Department of Orthodontics in Tamil Nadu Government Dental College and Hospital (Herein after referred to as the 'PG/Research student and co- investigator').

Whereas the 'PG/Research student as part of his curriculum undertakes to research **"** Comparative evaluation of probiotic and chlorhexidine mouthwashes effect on the adhesion of streptococcus mutans on stainless steel brackets- A Clinical trial **"** for which purpose the PG/Principal investigator shall act as principal investigator and the college shall provide the requisite infrastructure based on availability and also provide facility to the PG/Research student as to the extent possible as a Co-investigator.

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10. In witness where of the parties herein above mentioned have on this the day month and year herein above mentioned set their hands to this agreement in the presence of the following two witnesses.

Principal **PG Student**

Witnesses **Student Guide**

1.
2.

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LIST OF ABBREVIATIONS

Abbreviation	Full form
CPP-ACP	Casein Phosphopeptide-amorphous calcium phosphate
Ag + TiO2	Silver + titanium dioxide
BisGMA	Bis-Glycidyl methacrylate
CFU	Colony forming unit
CLSM	confocal laser scanning microscopy
LB	Lactobacilli
MS	Mutans streptococci
PCR	Polymerase chain reaction
QAMS	quaternary ammonium methacryloxy silicate
RT-PCR	Real Time-Polymerse chain reaction
S. Sobrinus	Streptococcus sobrinus
S.D.	Standard deviation
S.Mutans	Streptococcus mutans

TEGDMA	Triethylene glycol dimethacrylate
WSL	White spot lesions

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IRB Reference No: 7/IRB/2017

Project Title:
Comparing and evaluating adhesion of Streptococcus mutans on stainless steel brackets by using probiotic and chlorhexidine mouthwashes – A clinical trial.

Principal Investigator: Dr.M.Ramya

Review: New/Revised/Expedited

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Decision of the IRB: Approved

Recommended time period: One Year

30.11.17

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Instances where selected sources appear:

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CERTIFICATE -II

This is to certify that this dissertation work titled **"COMPARATIVE EVALUATION OF PROBIOTIC AND CHLORHEXIDINE MOUTHWASHES EFFECT ON THE ADHESION OF STREPTOCOCCUS MUTANS ON STAINLESS STEEL BRACKETS- A CLINICAL TRIAL"** study by the candidate **DR. M.RAMYA** ,with Registration number _____ for the award of **MASTER OF DENTAL SURGERY** in the branch of **ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS (BRANCH V**). I personally verified the urkund.com website for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and results shows 09(Nine only) percentage of plagiarism from in the dissertation

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INTRODUCTION

The discipline of Orthodontics is concerned with aligning teeth to appropriate positions by using various metallic and ceramic appliances, which stay in the patient's mouth for prolonged periods of time. While the clinician tries to complete the procedure in the shortest time possible, it still takes more than six months to accomplish the goals in tooth movement. While this has been accepted by the patient, clinician also has various other responsibilities with regard to preservation of existing structures. The triad of form, function and esthetics can be fully realized, only if all the aspects of orthodontic appliance are completely considered.

Usually, the concern about the appliance would relate to placement, adjustment and careful removal of the appliance. As it is of complex shape, proper cleaning becomes a challenge to the patient as well as the clinician. In the yesteryears many strategies have been devised to aid in the mechanical cleaning of the appliance. They all met with limited success due to various known reasons. Since, predominantly patients are adolescents and children, demanding such intricate work may not be feasible. Though parents can assist it, it has practical difficulties. The diet is already rendered soft to protect the orthodontic appliance, making it sticky due to absence of fiber. In addition, irregular shape of the appliance makes it difficult for natural cleansing mechanisms to carry out their job. Also, such irregular shapes have higher surface area, encouraging plaque accumulation, shifting the balance of health to negative side.

The consequence of such adhesion and plaque formation is the dissolution of enamel and compromise in periodontal health.[1] Therefore, preservation of existing structures, viz. tooth structure and periodontium is a difficult task. Various methods have been attempted to aid in the reduction of plaque accumulation with limited success. Mechanical debridement is often seen as the most successful procedure. Modified toothbrushes and powered toothbrushes have been tried over the decades with various improvements made in the material, shape and arrangement of fibers etc.[2, 3, 4] Even ultrasonic brushes have been tried for the same.[5]

While it is true that mechanical cleansing is superior, it may not be always feasible to perform by the patient. In order to address this issue, mechanical means are augmented by chemical plaque control. Various disinfectants have been used in this connection. Chlorhexidine, triclosan and other agents have been extensively tried to check the growth of biofilm.[6] Herbal mouthwashes have also been tried to this effect.[7] In addition, chlorhexidine has been tried with dental varnish to prevent plaque formation.[8] Other chemicals that inhibit flora are extensively tried, like quaternary ammonium compounds.[9] Use of large amounts of such mouthwashes for prolonged time may not be a health option and hence the focus was shifted to re-mineralize the enamel, so that despite the presence of flora caries may not occur.[10] Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) has been tried to accomplish remineralization.[11] Also, in some cases the appliance had been coated with nano-silver to prevent plaque accumulation.[12]

Nevertheless, the freedom from caries originating due to orthodontic treatment is still on the rise.[13] In order to handle this problem effectively, it is required to have a technique that would work on carious flora and inhibit them without any adverse effect on the patient. Recently, the paradigm has shifted in methods that handle plaque.[14] The concept of increasing the number of favorable flora in the oral cavity that shifts the balance towards healthy enamel and gums is being experimented widely. However, investigators have used various bacterial species to accomplish the same. This technique is observed to work not only on bacterial species but also on fungal species.[15] Lactobacillus salivarius has been used to control caries. Various authors have tried various species commonly available in their locality.[16]

If this modality of plaque control is effective in orthodontic patients, it could result in large reduction of incidence caries and gingivitis, thereby reducing the treatment time, requirement of patient compliance and expenses associated with countering such infections. All these are essential elements in a developing economy like India. This reduction in cost and complications can result in more number of people resorting to orthodontic therapy and also widens the clinical options in adults with higher susceptibility to caries and periodontal diseases.

While success has been demonstrated in this technique in non-orthodontic patients, its application in orthodontics has not been thoroughly explored. Placement of appliance offers a new binding surface for oral flora and they can have a different ecosystem building

up on the wire and brackets.[17] In addition, there is an increase in acidogenic bacteria in dental plaque subsequent to placement of appliance.[1] Hence it cannot be generalized that use of probiotics would result in plaque control in orthodontic patients.

Various reports have suggested use of coating agents for appliances to reduce plaque accumulation on the appliance.[18] Lindel et al., (2011) have shown difference in plaque accumulation on metal and ceramics.[19] Dittmer et al., (2015) have clearly shown various materials used in orthodontic appliances can show difference in plaque accumulation.[20]

Moreover, as the flora in oral cavity is widely variable, large numbers of studies have to be performed to standardize the procedure and to elaborate any external influence on the treatment regimen. This study is hence attempted to fill that lacunae in the literature pertaining to efficacy of probiotics in reducing bacterial plaque on fixed orthodontic appliances.

AIMS AND OBJECTIVES

Aims

To evaluate and compare the minimal adhesion of *Streptococcus mutans* over the stainless steel brackets between probiotic and chlorhexidine mouth washes.

Objectives

Evaluate the adhesion of *Streptococcus mutans* on the stainless steel bracket for 30 days in patients using probiotic mouthwashes

Evaluate the adhesion of *Streptococcus mutans* on the stainless steel bracket for 30 days in patients using chlorhexidine mouthwashes

Compare the values obtained from RT-PCR

REVIEW OF LITERATURE

Fournier et al., (1998)[21] studied the affinity of Streptococcus mutans to orthodontic brackets made from metal, plastic, and ceramic using labelling with [3H] thymidine. They found that the initial affinity of S. mutans to metal brackets was statistically significantly lower than that to plastic and porcelain brackets with and without saliva coating.

Rupf et al., (2001)[22] have developed and performed species specific PCR reactions on streptococcus mutans and streptococcus sobrinus and used membrane fatty acid spectra (MFAS) and peroxidase reaction (PR) after aerobic and anaerobic incubation. They identified 423 strains of Streptococcus mutans and 2 strains of Streptococcus sobrinus.

Ahn et al., (2005)[23] have analysed the adhesion of cariogenic streptococci to orthodontic metal brackets in terms of the type of bacterial strains, the incubation time, and saliva coating. They observed a characteristic binding pattern according to the type of bacterial strains used. Therefore, they concluded that each strain of cariogenic streptococci has a characteristic adhesion pattern and the type of bacterial strain, the incubation time, and saliva influenced the adhesion. The implication of this study is that any effort made to decrease bacterial adhesion cannot be universal and species specific changes should be borne in mind.

Ahn et al., (2007)[24] have investigated the adhesion levels of 4 cariogenic streptococci strains to various orthodontic brackets with respect to bracket type, bacterial strain, incubation time, and saliva coating. Five bracket types (monocrystalline sapphire, polycrystalline alumina, stainless steel, plastic, and titanium) were used in the study. From the results of the study, the cariogenic streptococci strain was observed to have a characteristic adhesion pattern. Highest adhesion was seen in plastic brackets and lowest in the monocrystalline sapphire brackets.

Brusca et al., (2007)[25] have attempted to define the capacity of different bracket materials to modify the growth and adherence of microorganisms. They used 3 types of brackets, viz. metallic, ceramic, and composite. Streptococcus mutans and Candida albicans were used to study the phenomenon. They saw that adherence of Streptococcus mutans was not modified by the different brackets. On the other hand Candida albicans adhered more to composite and less to other materials - viz. composite > ceramic > metallic.

Ahn et al., (2007)[26] have analyzed the prevalence of cariogenic streptococci adhering to incisor brackets in 80 samples collected at debonding. They used PCR to obtain the results. They found that the prevalence of cariogenic streptococci was not significantly associated with the oral hygiene indexes at debonding. This observation has profound

implication to current study. The count of bacteria need not necessarily be reflected in the oral hygiene of the patient.

Faltermeier et al., (2008)[27] have studied the susceptibility of various plastic bracket materials to the adherence of Streptococcus mutans. They studied the adhesion on polyoxymethylene, polycarbonate, high-density polyethylene, and an experimental polymer (90% polyethylene). They used fluorescence dye for counting and Alamar Blue/resazurin assay for determining the quantity of bacterial adhesion. They could not find any significant differences in the quantities of S mutans adhering to these polymers.

Lim et al., (2008)[28] have investigate the adhesion of 2 cariogenic streptococci strains to 7 orthodontic raw materials (3 light-cured orthodontic adhesives, 3 bracket raw materials, and hydroxyapatite) with respect to bacterial species, incubation time, and saliva coating. It was an in vitro study. They found that adhesion of cariogenic streptococci was significantly higher for bonding adhesives than for bracket materials, and adhesion to resin-modified glass ionomer was the highest.

Magno et al., (2008)[29] have investigated in vivo the contamination by Streptococcus mutans of Super Slick elastomeric rings (TP Orthodontics, LaPorte, Ind), manufactured with Metafasix technology (TP Orthodontics), using microbial culture and scanning electron microscopy (SEM). Their results showed that the Super Slick

elastomeric rings had statistically significant greater S mutans contamination than the conventional elastomeric rings. They could not observe any formation of S mutans colonies in the elastomeric rings removed directly from their original packages. Based on their investigations, they concluded that there was no clinical evidence that Super Slick elastomeric rings are effective in reducing bacterial biofilm formation on their surfaces, they do not recommend their use in orthodontic therapy for that purpose.

van Gastel et al., (2009)[30] have observed the differences in total bacterial counts and capacity for biofilm formation amongst seven commercially available bracket systems (Damon, Clarity, Mystique, Speed, Victory MBT, Micro-loc and Generus). were compared. They stored brackets in the culture medium (Brain heart infusion agar) and incubated for 72 hours and the amounts of aerobe and anaerobe bacteria were determined by counting the colony-forming units (CFU). Group with low adhesion were Victory MBT, Micro-loc and Generus. the group with high adhesion of were Damon, Clarity, Mystique. Speed exhibited intermediate adhesion. Hence significant differences were noted between the different types of brackets.

Lee et al., (2009)[31] have used Surface roughness and surface free energy characteristics to investigate nine different orthodontic materials (four orthodontic adhesives, three bracket raw materials, hydroxyapatite blocks, and bovine incisors) using confocal laser scanning microscopy and sessile drop method. Their results demonstrated

that surface free energy characteristics play an important role in the initial MS adhesion to orthodontic materials.

Chapman et al., (2010)[17] have evaluated the incidence and severity of WSLs by examining pretreatment and posttreatment digital photographs in a total of 332 consecutive finished patients from a university graduate orthodontic clinic. They found that the agreement between direct clinical examination and digital photo data was excellent. They found that risk factors for the development of incipient caries during orthodontic treatment were young age (preadolescent) at the start of treatment, number of poor hygiene citations during treatment, unfavorable clinical outcome score, white ethnic group, and inadequate oral hygiene at the initial pretreatment examination.

Demling et al., (2010)[18] have investigated the biofilm adhesion on polytetrafluoroethylene (PTFE) coated orthodontic brackets in 13 adolescent patients and evaluated for 8 weeks. Quantitative biofilm formation was analysed with the Rutherford backscattering detection (RBSD) method and scanning electron microscopy technique. Their results indicated that PTFE coating of brackets reduces biofilm adhesion to a minimum.

Lindel et al., (2011)[19] have evaluated stainless steel and ceramic brackets form biofilm adhesion in 20 adolescent subjects. They quantitatively analyzed for biofilm

coverage with the Rutherford backscattering detection method. They found that total biofilm formation was 12.5% on the surface of metal and 5.6% on ceramic brackets. Their results indicated that ceramic brackets exhibit less long-term biofilm accumulation than metal brackets.

Yang et al., (2011)[32] have tested whether orthodontic bonding has any effect on the initial adhesion of mutans streptococci in the presence of saliva in an in vitro study. They saw that the adhesion was influenced by the bonding steps and the presence of saliva. Implication of this study is that its a trilogy that infleneces bacterial adhesion, viz. Bacterial factors, Salivary consistency and the surface characteristics of the material.

do Rosário Junior et al., (2011)[33] have evaluated the influence of saliva obtained from caries-free and caries-active individuals on the adhesion rates of Streptococcus mutans to metallic brackets. They assessed adhesion rates by crystal violet retention technique. They showed that saliva from caries-active patients tends to increase the mutans adhesion to surfaces. The implication is that, when orthodontic treatment is performed, prior caries history will decide the intensity of anti caries measures to be prescribed. Moreover, when plaque or bacterial adhesion is studies, previous caries is an important confounding factor.

Velazquez-Enriquez et al., (2012)[34] have quantitatively determined the independent bacterial colonization of S. mutans and S. sobrinus in orthodontic composite resins - viz. Enlight, Grengloo, Kurasper F, BeautyOrtho Bond, Transbond CC, Turbo Bond II, Blugloo. They used radioactive marker to codify the bacteria (^3H) for quantitative analysis. Accordingto the results of the study, "Enlight", obtained the lowest adherence of S. mutans and S. sobrinus, which may reduce the enamel demineralization and the risk of white spot lesion formation.

Passariello and Gigola (2013)[35] have compared the early bacterial adhesion and biofilm formation of common and uncommon periodontal pathogens on a 15 different commercial brackets in vitro. They used quantitative real time PCR after extraction of bacterial DNA. They found that materials significantly influenced bacterial adhesiveness in a species-specific way. They saw that titanium and gold brackets constantly yielded the lowest while other materials were not as effective as these in controlling bacterial adhesion. Hence they recommend use of brackets made of gold, titanium, and ceramics.

Baka et al., (2013)[36] have evaluated the effects of self-ligating brackets and conventional brackets ligated with stainless steel ligatures on dental plaque retention and microbial flora in 20 boys with a mean age of 14.2 ± 1.5. They obtained supragingival plaque samples at baseline and 3 months after bonding for the detection of bacteria; and used quantitative analysis for Streptococcus mutans, Streptococcus sobrinus, Lactobacillus casei, and Lactobacillus acidophilus using real-time polymerase chain reaction. They

concluded that Self-ligating brackets and conventional brackets ligated with stainless steel ligatures do not differ with regard to dental plaque retention.

Condò et al., (2013)[37] have determined in vivo, the retention of plaque on three different elastic ligatures, in comparison with stainless steel ligature, to determine a possible association between type of ligatures and accumulation of microorganisms. They used ring-shape, clear, latex ligatures (Leone® Spa), ring-shape, grey, polyurethane ligatures (Micerium® Spa) and grey, polyurethane, Slide low-friction ligatures (Leone® Spa), compared with stainless steel ligatures (Leone® Spa) used as control. The study was conducted on 40 orthodontic patients. They quantified the presence of bacterial slime by spectrophotometric method (crystal violet-Bouin's fixative) and Scanning Electron Microscopy (SEM). They concluded that Elastomeric ligatures showed a significant lower susceptibility to plaque adhesion, in comparison to the stainless steel of the metallic ligatures.

Ghasempour et al., (2014)[38] have evaluated the reduction in the level of mutans streptococci due to regular consumption of probiotic Kefir drink. 22 healthy volunteers aged 22-32 years with good oral hygiene were enrolled in the trial. Saliva was sampled before and after interventions. The acidity and the count of MS were assessed. Based upon the results of the study, they suggest that the Kefir drink can inhibit salivary MS similar to sodium fluoride rinse.

do Nascimento et al., (2014)[39] have published a systematic review on whether the design of brackets (conventional or self-ligating) influences adhesion and formation of Streptococcus mutans colonies. They found 6 eligible articles and have reported based on them. They concluded that there is no evidence for a possible influence of the design of the brackets (conventional or self-ligating) over colony formation and adhesion of Streptococcus mutans. This implies that it is the material aspect but not the design aspect that favours or impedes colony formation. But, this may not be true of plaque formation.

Jacobo et al., (2014)[40] have determined the in vitro antibacterial effectiveness of the orthodontic bonding Transbond XT (3M Unitek) and four self-etching adhesives with possible use in orthodontic bonding (Clearfil Protect Bond, Clearfil Self-etching Bond, Transbond Plus Self-Etching Primer; iBond) against Streptococcus mutans and Lactobacillus gasseri in order to compare that capacity among the adhesives and with respect to Transbond XT. They have also determined the bacterial adhesion capacity of these microorganisms to the tested adhesives. They used scanning electron microscopy to study bacterial adhesion. Clearfil Protect Bond and iBond produced a clear growth inhibition halo against S.mutans and L. gasseri. According to them iBond was the only tested product to which the bacteria adhere profusely, particularly S. mutans.

Gizani et al., (2016)[14] have studied the effect of daily intake of lozenges containing probiotic bacteria on white spot lesion (WSL) formation as well as on salivary lactobacilli and mutans streptococci counts, in patients undergoing orthodontic treatment with fixed

appliances, in a randomized double-blind placebo-controlled study design with two parallel arms. They sample size was 85. The study duration was 17 months, the intervention was that the subjects in the test group were instructed to take one probiotic lozenge containing two strains of Lactobacillus reuteri once daily. Dental plaque, WSL, and salivary MS and LB levels were recorded at baseline and immediately after debonding. Within the limitations of the study, they concluded that daily intake of probiotic lozenges did not inflence the WSL during orthodontic treatment with fixed appliances.

Dittmer et al., (2015)[20] have compared early biofilm formation amongst 3 biomaterials used in contemporary fixed orthodontic treatment, viz. stainless steel, gold and ceramic. They inserted splints intraorally for 48 h, retrieved and biofilms were stained with a two color fluorescence assay for bacterial viability and analyzed by using confocal laser scanning microscopy (CLSM). Their results indicated that initial biofilm formation seemed to be less on stainless steel surfaces compared with other traditional materials in a short-term observation.

Jung et al., (2015)[41] have analyzed in vivo mutans streptococci adhesion to self-ligating ceramic brackets - Clarity-SL and Clippy-C and the relationships between bacterial adhesion and oral hygiene indices. Adhesions of Streptococcus mutans, S. sobrinus, and total bacteria were quantitatively determined using real-time polymerase chain reaction after genomic DNA was extracted. They computed the correlation coefficients to determine the relationships of bacterial adhesion to oral hygiene indices. They found that oral hygiene

indices were not significantly correlated with adhesions of bacteria to self-ligating ceramic brackets

Bai and Vaz (2015)[42] have analysed the bacterial adhesion on the Super Slick and Safe-T-Ties and compared it with their unmodified counterparts, in a sample of 30 subjects, aged between 12 to 25 years. They found significant difference in the S. mutans and Lactobacilli counts in both surface modified and unmodified elastomeric modules. Hence, they have concluded that modifications of these elastomers using the Metafasix or OrthoShield Technology, was better than their unmodified counterparts.

Jongsma et al., (2015)[43] have compared in vivo biofilm formation on single-strand and multi-strand retention wires with different oral health-care regimens. They found that use of antibacterial toothpastes marginally reduced the amount of biofilm on both wire types, but significantly reduced the viability of the biofilm organisms. Also, they observed that additional use of the mouth- rinse did not result in significant changes in biofilm amount or viability. They saw major shifts in biofilm composition induced by combining a stannous fluoride- or triclosan-containing toothpaste with the mouth -rinse.

Saruttichart et al., (2016)[5] have compared the effectiveness of a motionless ultrasonic toothbrush to a manual toothbrush in reducing dental plaque, gingival inflammation, and mutans streptococci in patients with fixed orthodontic appliances. 25

subjects participated in the study, that was conducted for 30 days. According to their results, On the bracket side, the motionless ultrasonic toothbrush showed a significantly higher mean plaque index bracket score after 30-day usage than baseline, while the manual toothbrush group showed no difference between the before and after brushing periods. Therefore, they concluded that manual toothbrushing performed better than brushing with the motionless ultrasonic toothbrush in plaque removal on the bracket side in orthodontic patients. Surprisingly, no difference could be observed in terms of gingival status and the numbers of mutans streptococci.

Liu et al., (2016)[9] have conducted a double-blind randomised clinical trial to determine the in vivo antimicrobial efficacy of quaternary ammonium methacryloxy silicate-containing orthodontic acrylic by using custom-made removable retainers, worn intraorally by 32 human subjects. In 48 hours, the disks showed that the QAMS-containing acrylic exhibits favourable antimicrobial activity against plaque biofilms in vivo.

Singh et al., (2016)[10] had reported a study that on efficacy of fluoride toothpaste alone and in combination with fluoride varnish and CPP-ACP plus crème in the remineralization of post-orthodontic WSLs in 45 subjects, evaluating immediately after debonding and subsequently after 1, 3, and 6 months of their use. They found that the use of fluoride varnish and CPP-ACP plus crème in addition to twice daily use of fluoride toothpaste had no additional benefit in the remineralization of post-orthodontic WSLs.

Esenlik et al., (2016)[11] have conducted a prospective randomised controlled clinical trial to test the efficacy of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) paste applied in-office to prevent white spot lesions (WSL) in patients undergoing fixed orthodontic treatment in a total of 57 patients. They recorded pretreatment plaque, gingival and bleeding indices oral hygiene habits. They found that there was a lower incidence of WSL in the experimental group compared to the control group.

Farhadian et al., (2016)[12] have evaluated the effect of silver nanoparticles incorporated into acrylic baseplates of orthodontic retainers on Streptococcus mutans colony-forming units in a study participated by 66 Sixty-six orthodontic patients at the debonding stage. They considered only those patients who revealed no clinical evidence of dental caries, periodontal pockets, or systemic disease. They intended to compare the number of S mutans colony-forming units between the 2 groups 7 weeks after retainer delivery. They analysed 29 patients in the control group and 32 in the intervention group were analyzed. They found that adding silver nanoparticles to the acrylic plate of retainers had a strong antimicrobial effect against S mutans under clinical conditions.

Jung et al., (2016)[44] have analyzed the adhesion of periodontopathogens to self-ligating brackets (Clarity-SL, Clippy-C and Damon Q) and have attempted to identify the relationships between bacterial adhesion and oral hygiene indexes. They collected central incisor brackets from from 60 patients at debonding after the plaque and gingival indexes

were measured. Adhesions of Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium nucleatum, and Tannerella forsythia were quantitatively determined using real-time polymerase chain reactions. Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Prevotella intermedia adhered more to the Damon-Q brackets in the mandibular area.

Mei et al., (2017)[1] have investigated the amount and the distribution of biofilm in patients wearing fixed appliances and its relation with age, gender, frequency of tooth brushing, and patient motivation. They had conducted their study on 52 patients, comprising of 30 females and 22 males. They used a questionnaire to collect information from subjects. According to them, gingival, mesial, and distal areas accumulated more biofilm than occlusal areas. Also, lower amount of biofilm was found in females, adults and "self-motivated" patients, compared with males, children, and "family-motivated" patients. Therefore, they concluded that patients wearing fixed orthodontic appliances have clinically significant amount of biofilm accumulation.

Jurišić et al., (2018)[6] have evaluated the efficacy of two formulations of chlorhexidine 0.2% (CHX) mouthrinses in terms of oral hygiene and gingival health status in adolescents with fixed orthodontic appliances wearing two different types of brackets during 18 weeks, in 80 subjects, randomly divided into metal-stainless steel group and ceramic groups. They assessed according to gingival index and oral hygiene index-simplified recorded prior to the placement of the appliance, at 6 weeks, 18 weeks post

placement. They found statistically significant decrease in GI and OHI-S indices after 6 weeks and then increase after 18 weeks for all groups. They also found that the ceramic brackets as well as usage of CHX-ADS resulted in improved gingival status.

Tupinamba et al., (2017)[45] have modified metallic surface of orthodontic brackets with Plasma-polymerized film deposition. They used Hexamethyldisiloxane polymer to do the deposition using Plasma-Enhanced Chemical Vapor Deposition radio frequency technique. They used Scanning Electron Microscopy for observing bacterial adhesion, Confocal Interferometry and surface wettability, by goniometry. They found that plasma-polymerized film deposition was only effective on reducing surface roughness and bacterial adhesion in conventional brackets compared to self -ligating brackets.

Khoroushi and Kachuie (2017)[46] have clearly reviewed the currently used methods to manage enamel demineralization during and after orthodontic treatment. They have considered good oral hygiene habits, and prophylaxis with topical fluorides, including high-fluoride toothpastes, fluoride mouthwashes, gels, varnishes, fluoride-containing bonding materials, and elastic ligatures, application of casein phosphopeptides-amorphous calcium phosphate, antiseptics, probiotics, polyols, sealants, laser, tooth bleaching agents, resin infiltration, and microabrasion. They have included probiotics in the effective methods for control of WSLs.

Fatani et al., (2017)[47] have evaluated the significant role of different brackets in reducing enamel demineralization indirectly. They found that there was a significant reduction in adhesion, biofilm formation and growth of tested bacterial species on brackets coated with Ag + TiO2. Their Scanning electron microscopy showed less bacteria attached with the surface coated with Ag + TiO2. Thea also tested the biocompatibility of such bracket materials against gingival fibroblast cell cultures and found positive results.

Altmann et al., (2017)[48] have assessed the remineralizing potential and antibacterial effect of a newly developed orthodontic adhesive. They added 1,3,5-tryacryloylhexahydro-1,3,5-triazine and phosphate invert glass containing niobium pentoxide were added to "75 wt% BisGMA, 25 wt% TEGDMA, 5 wt% fummed silica, and photo-initiator system". The modified group presented with a reduction in bacterial growth when compared with the control. They saw mineral deposit on those materials in 28 days. They concluded that the orthodontic adhesive which they developed had antibacterial activity and mineral deposition, which could hence be a reliable choice for brackets adhesion.

Cunha et al., (2018)[2] have compared the effect of single-tufted toothbrush combined or not with a conventional toothbrush to control dental biofilm in healthy orthodontic patients, in 20 subjects undergoing orthodontic therapy. They recorded stained plaque index, visible plaque index and gingival bleeding index. From the results of the

study, they concluded that the combination of single-tufted and conventional toothbrushes was effective for controlling dental biofilm formation in orthodontic patients.

Naik et al., (2018)[4] have evaluated the effectiveness of different bristle designs of toothbrushes and the periodontal status among patients undergoing fixed orthodontic treatment by using a randomized controlled trial, participated by 45 adolescents undergoing fixed orthodontic treatment. They found that toothbrush with crisscross bristles exhibited maximum plaque reduction.

Niazi et al., (2018)[7] have effectively compared the antiplaque effects of two herbal mouthwashes containing Salvadora persica and Azadirachta indica, respectively, with two synthetic mouthwashes containing either chlorhexidine or cetylpyridinium in a triple-blind, randomised controlled trial, in 100 patients undergoing orthodontic treatment. Plaque accumulation was scored three times according to the Modified Bonded Bracket Plaque Index: at the start, after the toothbrush-toothpaste trial, and at the end of mouthwash trial. They found that, compared to other mouthwashes, Salvadora persica miswak-based mouthwash showed a maximum reduction in the plaque scores among orthodontic patients.

Lipták et al., (2018)[8] had evaluated the effects of Cervitec Plus® on the level of mutans streptococcus (SM) and lactobacillus (LB) colonies and the development of white spot lesions (WSLs) in patients with fixed orthodontic appliances in 32 volunteers of mean

age 16.5 ± 2.75 years. Bacterial colonies were determined in saliva and plaque using CRT Bacteria(Ivoclar-Vivadent, Schaan, Liechtenstein) and the number of WSLs was registered. With this longitudinal study, they concluded that the monthly use of Cervitec Plus® could result in a significant improvement in oral health of orthodontic patients.

Sajedinejad et al., (2018)[16] have conducted a randomized double-blind placebo control trial to test a mouthwash containing L. salivarius NK02 at a dose level of 10^8 ((CFU) ml-1), monitoring over a period of 4 weeks. It was apparent that the probiotic mouthwash was able to inhibit the bacterial growth on both saliva and sub-gingival crevice and exhibited antibacterial activity against A. actinomycetemcomitans. Their study period was 4 weeks and collected samples from saliva and subgingival plaques. They suggest that probiotic mouthwash is healthy for daily use as an alternative for maintaining dental and periodontal health.

Sharma et al., (2018)[49] have assessed the bacterial adhesion on elastomeric ligatures with special reference to coloured elastomeric rings during orthodontic treatment in a sample size of 240, using standard techniques for bacterial counts. The study showed colour and material dependent bacterial colonization on orthodontic modules. While bacterial adhesions have been extensively studied on brackets, this study has studied on elastics.

Erbe et al., (2019)[3] have conducted a 2-arm parallel trial to determine the plaque removal efficacy (main outcome) and the motivation assessment (secondary outcome) comparing a manual versus an interactive power toothbrush in orthodontic patients, in 60 adolescents with fixed orthodontic appliance in both the arches. Subjects used either an interactive power toothbrush (Oral-B Professional Care 6000, D36/EB20) with Bluetooth technology or a regular manual toothbrush (Oral-B Indicator 35 soft). They assessed plaque removal using Turesky Modification of the Quigley-Hein Plaque Index at baseline, 2 and 6 weeks. Based on the results, they have concluded that the interactive power toothbrush generated increased brushing times and significantly greater plaque removal compared with manual brush.

Enerbäck et al., (2019)[13] have evaluated the effects of orthodontic treatment and different fluoride regimens on caries risk and caries risk factors, including cariogenic bacteria in a Three-armed, parallel group, randomized, controlled trial, with group I (Control group), 1450 ppm fluoride (F) toothpaste; group II, 1450 ppm F toothpaste plus 0.2 per cent sodium fluoride (NaF) mouth rinse; and group III, 5000 ppm F toothpaste, analysed for a period of 3 months. 255 subjects were analysed. They suggest everyday use of high-fluoride toothpaste (5000 ppm F) or mouth rinse (0.2% NaF) in combination with ordinary toothpaste for optimal protection from caries.

DE Sanctis et al., (2019)[15] have failed to demonstrate any effect of Lactobacillus brevis in preventing radiation induced mucositis in subjects receiving radio-chemotherapy

for head and neck cancer. They remark that although modulating homeostasis of the salivary microbiota in the oral cavity seems attractive, it clearly needs further study.

Goyal et al., (2019)[50] have evaluated the effect of amine fluoride and probiotic mouthwashes on levels of P. gingivalis during orthodontic treatment, using real time-polymerase chain reaction (RT-PCR) in a randomised controlled trial model, performed in 45 subjects. They found that levels of P. gingivalis were significantly decreased with probiotic mouth wash and hence recommend probiotics as an adjunctive measure for caries control during orthodontic therapy.

Shah et al., (2019)[51] have compared the efficacy of probiotic and chlorhexidine oral rinses in orthodontic patients in a randomized control trial with 10 subjects in probiotic group, 10 in chlorhexidine group and 10 in control group. They saw that probiotics are equally efficient as chlorhexidine as adjunctive chemical plaque control agent.

MATERIALS AND METHODS

SOURCE OF DATA-

The study population was selected from the outpatient section of the Department of orthodontics and dentofacial orthopaedics, Tamilnadu Government Dental College & Hospital, Chennai, Tamilnadu, India. This clinical trial study was performed using 30 subjects

STUDY GROUPS:

- Group A: study group USING CHLORHEXIDINE MOUTH WASH – 15 subjects
- Group B:study group USING PROBIOTIC MOUTH WASH – 15 subjects

- **Products used for the mouthwash**

  HEXIDINE mouthwash -0.2% chlorhexidine gluconate

  BIFILAC :•Bacillus Mesentericus (1 Million Spores)

  •Clostridium Butyricum (2 Million Spores)

  •Lactobacillus Sporogens (50 Million Spores)

  •Streptococcus Faecalis (30 Million Spores)

Method of collection of data :

- One group of patients (Group A) was given the chlorhexidine mouth wash, and the other group (Group B) was given the probiotic mouth wash. The patients were instructed to rinse their mouth with these mouth washes in the morning at night (b.i.d), after brushing their teeth with a prescribed tooth paste.

- The patients were asked to brush twice daily for 2 min; this was demonstrated by the operator. They were instructed to avoid chewing gums, lozenges, and antibiotics and to restrict intake of any food or beverage 30 min to 1 h, before and after having the mouth wash during the study. The mouthwash were to be administered to the patients from day 1 after the first plaque sample had been assessed and continued until day 30. Plaque samples were to be again taken and evaluated at the end of day 30. At each appointment, the elastomeric modules were carefully removed, and archwires were disengaged.

- Plaque samples were collected from the labial surfaces of the maxillary lateral incisors surrounding the stainless steel brackets with a sterile scaler using a four pass technique..

- All participants was given a monitoring sheet, where participant and parent to put tick mark each time after the use of mouthwash both at morning and at night.

- All participant was also given a monitoring sheet ,where participant and parent to put tick mark on consuming only vegetarian diet daily during the study.

- The plaque samples were suspended in 1 ml of sterile phosphate buffer saline (0.12 M NaCl, 0.01 M $Na_2 HPO_4$, 5 mM $KH_2 PO_4$ pH 7.5) and sealed for transport for polymerase chain reaction (RT-PCR). The PCR values were obtained as CFU/ml. The values were tabulated, and the statistical analysis was performed

Criteria For Selection

Inclusion Criteria

- Orthodontic treatment with the pre adjusted edgewise appliance .
- Complete permanent dentition
- Good general health (no significant medical history or drug use during the last month)
- No anti-inflammatory or antibiotic medications taken in the month before the study
- No chewing gum or mouthwash used in the last week and during the study
- Habit of brushing twice daily with prescribed toothpaste
- Patient with good periodontal condition

Exclusion Criteria

- Patient with poor periodontal condition

- Patient with known medical condition, for example, subacute bacterial endocarditis, diabetes, valvular disease, anemia

.

Statistical Analysis

Statistics was calculated using Microsoft Excel Version 2010. Mean and Standard deviation was determined. Pretreatment and Post treatment microbial count was analysed using Wilcoxon signed Rank test. Variation between control and test group before treatment was computed using Mann Whitney test. Similarly, variation between post treatment values of both groups were computed using Mann Whitney test.

Methodology for PCR Analysis

1. Cell pellet wash buffer: 1X PBS (Phosphate Buffer Saline, pH7.5) (Cat#P3813, Sigma-Aldrich, USA)
2. DNA extraction buffer:
    a. Bacterial lysis buffer: 100mM of Tris (pH8), 25mM EDTA and 2% SDS (Cat# NA2110, Sigma-Aldrich, USA).
    b. 10mg/ml of lysozyme (Cat#L6876, Sigma-Aldrich, USA)
    c. 20mg / ml of Proteinase K (Cat#P18030S, Macher Nagel, Germany).
3. Organic Reagents:
    a. Absolute Ethanol

4. Gel Electrophoresis Buffer: Tris – Acetic Acid EDTA pH 7.5 buffer (Cat# B49, ThermoFischer Scientific, USA)
5. Agarose Gel:
    a. Agarose *(Medox Fine Chemicals, Chennai, India)*
    b. Ethidium Bromide (DNA staining dye) *(Medox Fine Chemicals, Chennai, India)*
6. Gel Electrophoresis Equipment:
    a. Agarose gel casting unit with casting tray *(Medox Fine Chemicals, Chennai, India)*
    b. Electrophoresis chamber *(Medox Fine Chemicals, Chennai, India)*
7. DNA loading dye *(Medox Fine Chemicals, Chennai, India)*
8. UV transilluminator (to see DNA bands)
    a. Gel Documentation system with UV Camera (Gelstan)
9. PCR reagents:
    a. Emerald Master mix (cat# RR310A, Takara, Japan)
    b. Luna qPCR-Master Mix (Cat#M3003S, New England Biolabs, USA)
    c. SYBR Green qPCR-Master Mix (Cat#RR820B, Takara Bio, Japan)
10. Plasticwares:
    a. 1.5ml and 2ml microfuge tubes (Tarsons, West Bengal, India)
    b. 0.5ml thin walled PCR tubes (Abgene, United Kingdom)
    c. Microtips (Tarsons, West Bengal, India)
11. Micropipettes (to dispense reagents in micro volumes) (Eppendorf, Germany)
12. Micro centrifuge (Eppendorf, Germany)

13. Vortex machine (Vortex Genie 2, Germany)
14. Dry heat incubator (Labline, India)
15. Conventional Thermal Cycler (Takara, Japan)
16. QUBIT fluorometer (Invitrogen, USA).
17. Real Time Thermal Cycler (Qiagen Rotorgene Q, Germany)

Methods:

DNA extraction

Plaque samples were collected in 1.5ml sterile DNase/RNase free tube and stored at 4°C until transported to laboratory for DNA extraction. At the time of DNA extraction, the samples were centrifuged at 10,000 rpm for 3 minutes at room temperature. The pellet thus obtained was washed once with sterile 1X PBS (Phosphate Buffer Saline, pH7.5) (Cat#P3813, Sigma-Aldrich, USA) before being subjected to DNA extraction with lysis buffer containing 100mM of Tris (pH8), 25mM EDTA and 2% SDS were digested with 10mg/ml of lysozyme (Cat#L6876, Sigma-Aldrich, USA) at 37°C for 30 minutes. Following cell lysis 20mg / ml of Proteinase K was added and the lysates were incubated at 57°C for 2 hours to digest all protein components present in the lysate. Subsequently, the lysates were transferred to DNA extraction columns as per recommendation of the manufacturer after addition of binding buffer (Cat# NA2110, Sigma-Aldrich, USA). The total amount of DNA present in each of the sample was quantified with Qubit fluorometer (Life Technologies, USA).

Amplification and quantitation of S.mutans

In order to identify the quantitative presence of the above bacteria in the saliva samples, an equal concentration (2 nanogram) of total genomic DNA was subjected to polymerase chain reaction (PCR) amplification of the 16S rRNA gene hypervariable regions V1 to V9 with the following set of primers: Forward: AGTTTGATCCTGGCTCAG, and Reverse: TACCTTGTTACGACTT under the following conditions. After an initial denaturation at 94°C for 4 min, the samples were subjected to 40 cycles of 94°C for 45 s, 48°C for 45 s, 72°C for 2 m, with a final extension at 72°C for 5 min. The PCR amplified products were subsequently quantified with Qubit fluorometer to find the concentration in each sample. All PCR amplicons were then diluted to obtain 2 nanogram concentrations in all samples, which were then used as template in the next round of qPCR with species specific primers. Real time polymerase chain reaction (qPCR) amplification was performed with a pair of S.mutans species specific primers as shown in table 1

Bacteria	**Primer sequence**[22]
S.mutans	GGTCAGGAAAGTCTGGAGTAAAAGGCTA
	GCGTTAGCTCCGGCACTAAGCC

Table 1: Primer sequences used to analyze the quantitative presence in saliva of patients

10µM of each of the above primers were added to Luna qPCR-Master Mix in 10µl reaction, and samples were analyzed in Rotor Gene Q real time PCR equipment (Qiagen, Germany). The following universal amplification condition was used: after an initial denaturation at 95°C for 10 min, samples were amplified for 35 cycles at 95°C for 20s, 56°C for 20s, and 72°C for 25s.

Establishment of standard:

In order to quantitatively determine the copy numbers of each bacteria (relative to each other and among the samples), a standard curve was established with serial dilutions of PCR product amplified from V5-V6 region of 16s rRNA gene representing 789 to 1068 base pairs of E.coli genome. The following pair of primers was used: Forward: TAGATACCCSSGTAGTCC (789–806), Reverse: CTGACGRCRGCCATGC (1053–1068). The amplification produces a 279 base pair PCR product. The following amplification condition was used: after an initial denaturation at 95°C for 10 min, samples were amplified for 35 cycles at 95°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds with a final extension at 72°C for 4 minutes. The V5-V6 PCR amplicon was gel purified (cat#NA1111, Sigma-Aldrich, USA) and eluted in 40µl of elution buffer. The concentration of gel eluate was determined by quantifying 1µl of the eluate by Qubit fluorometer (Invitrogen, Austria) using QuantiFluor ONE dsDNA system (cat#E4871, Promega, USA). Copy number of PCR amplicons present in nanograms of V5-V6 gel eluate was determined by using the following formula:

(nanograms per microliter) x 6.022 x 1023 / (length of amplicon in base pairs) x 1 x 109 x 650

After determining the copy numbers, serial dilutions of the V5-V6 eluate was made to obtain concentrations from 1 x 106 to 1 x 101. These serial diluted samples were then analyzed by realtime PCR in the presence of QuantiNova SYBR Green PCR Kit (Cat#208052, Qiagen, Germany) in Qiagen 5-plex rotor gene real time PCR system to establish a linear standard graph. The following amplification condition was used: after an initial denaturation at 95°C for 5 minutes, the standards were subjected to 40 cycles of amplification at 95°C for 15 seconds and 60°C for 30 seconds. Linear standard curve thus obtained was stored in the system to be used as reference during sample amplification process.

Melt Curve Analysis to confirm specificity of amplification: To confirm for the specificity of amplification of S.mutans, the samples were subjected to melt curve analysis, which involved a ramp step that ranged between 60°C to 95°C with an initial hold for 90 seconds followed by a rise of 1°C at each step with a 5 second hold to enable the melting process. Analysis of melt curve showed distinct peak intensities for S.mutans between 85°C to 90°C.

Interpretation of qPCR to determine the quantitative presence of bacteria in samples: The quantitative presence of each bacteria among the samples was determined by comparing the normalized fluorescence value with that of a linear standard graph obtained

from running known concentration of control DNA (refer above). The entire comparison procedure was performed with the in-built Qiagen Rotor Gene Q real time PCR system software. Upon comparison, the software expresses the quantity of bacteria as copy numbers. For example, if sample “A” has a higher concentration of S.mutans relative to sample “B”, sample “A” will produce higher fluorescence than sample “B”. The software detects this higher fluorescence in sample “A” and expresses the same as higher copy number of S. mutans in sample “A”.

COLOUR PLATES

Fig. 1. The chlorhexidine mouth wash used in the study

Fig. 2. The probiotic used for mouthwash in the study

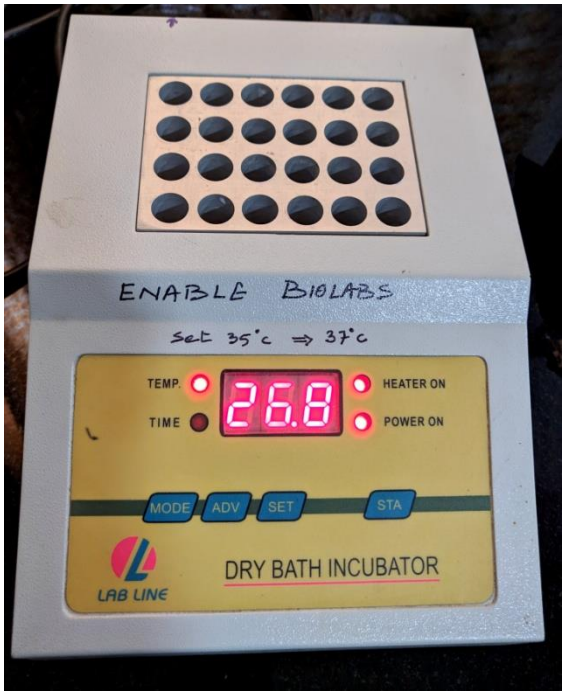


Fig.3. The incubator used in the study

Fig.4. The set of micropipettes used in the study

Fig.5. Centrifuge used in the study

Fig.6. Containter with the primer

Fig.7. The vortex instrument

Fig 8- Qubit fluorometer used in the study

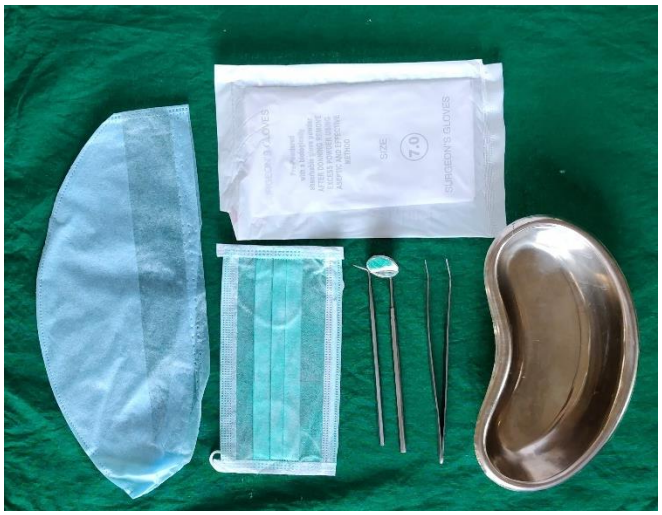


Fig 9: Armamentarium -Sterile scalar

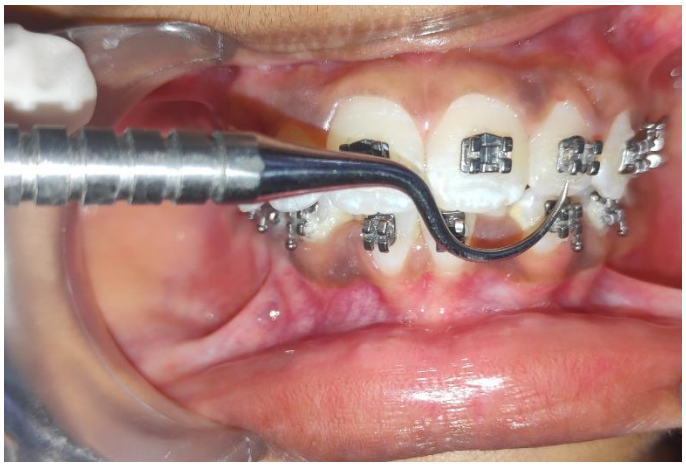


Fig 10 -Collection of plaque samples from the labial surfaces of the maxillary lateral incisors surrounding the stainless steel brackets with a sterile scalar using a four pass technique

RESULTS

Table 2: Test for normality using Kolmogorov Smirnov test

	Group A		**Group B**	
	Pre treatment count	**Post treatment count**	**Pre treatment count**	**Post treatment count**
Kolmogorov smirnov D	0.00112	0.00615	0.00078	0.00069
P Value	0.47832	0.42202	0.47462	0.47825
Implication	Not normally distributed	Not normally distributed	Not normally distributed	Not normally distributed

All four groups of values were significantly different from normal distribution and hence non parametric statistics were computed to analyse the results.

Table 3. Comparison of the groups

Group	Pre treatment		Post Treatment		P Value (Pre Vs Post)	P Value (Pre)	P Value (Post)
	Mean Count	S.D.	Mean	S.D.	(Wilcoxon signed Rank)	(Mann Whitney)	(Mann Whitney)
A	196146.9	196146.90	767.1	1611.31	0.01507#	0.65169	0.4406
B	84013.1	277100.74	16816.6	62556.69	0.10458	-	-

- Significant

Both Group A and Group B had no significant difference preoperatively, implying that there was no significant bias in the patient selection. . The difference between pre and post use of the mouthwash in group A was statistically significant. In group B, there was statistically no difference between pre and post use of probiotic mouth wash. In addition, comparison of post test values did not differ either. That implied that the results are inconclusive about the use of probiotics in place of mouthwashes.

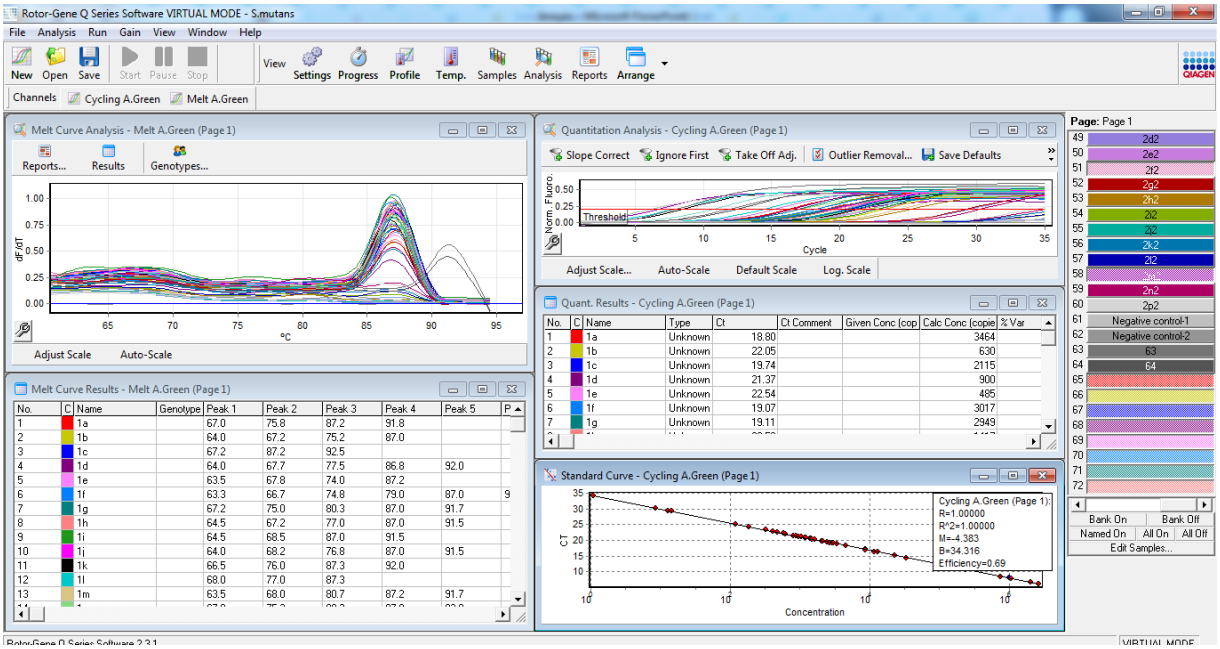


Fig.11 Screen shot of data analysis

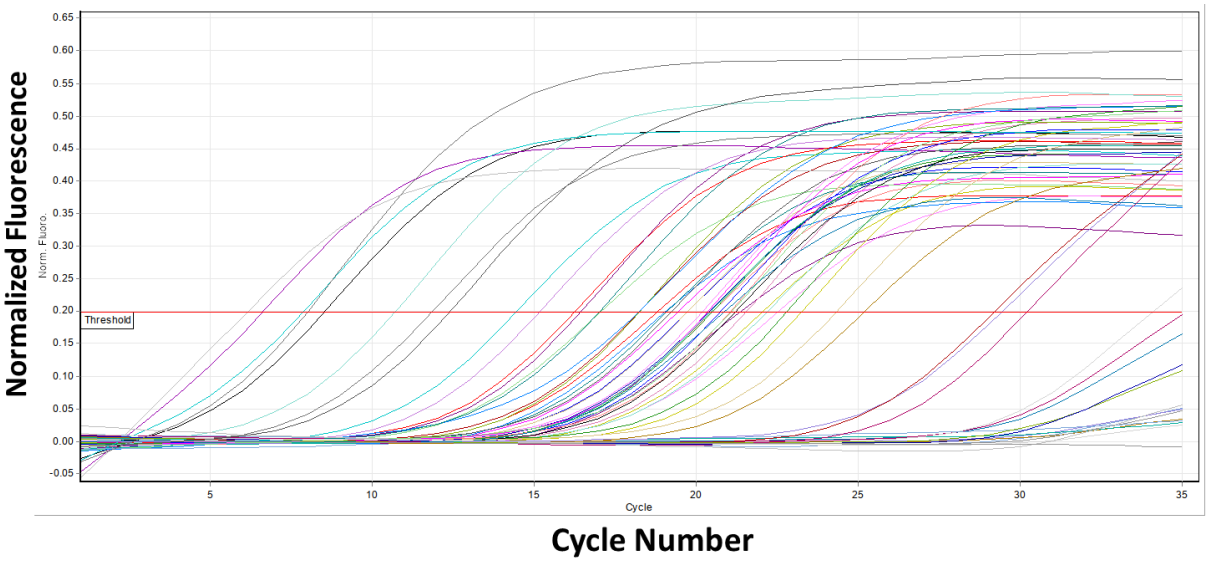


Fig.12 Amplification Curves of S.mutans

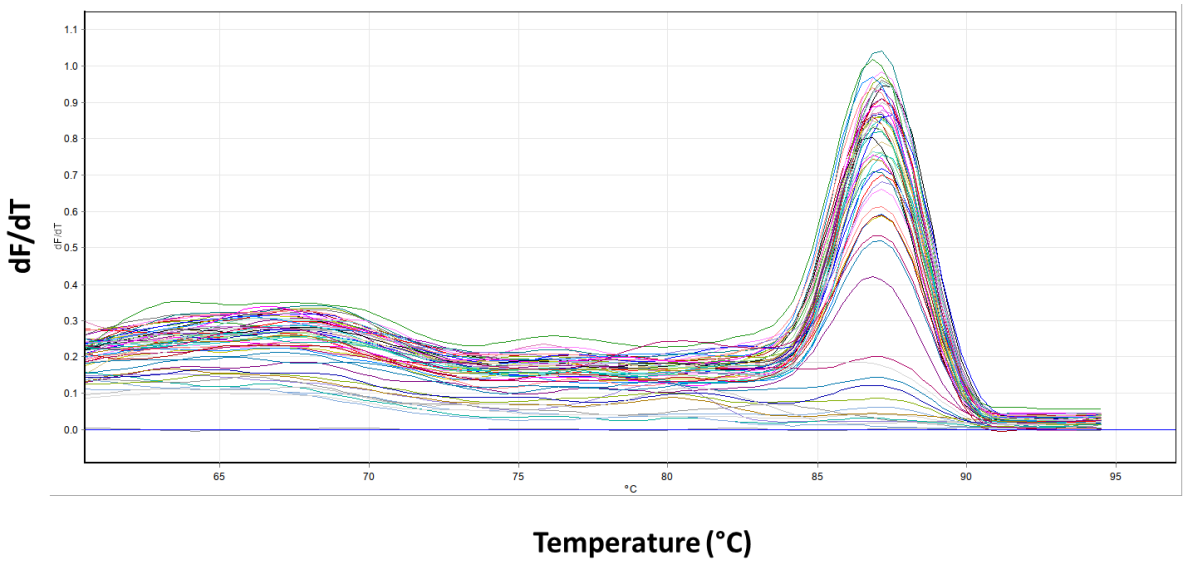


Fig.13 MELT CURVE ANALYSIS of S. mutans. dF/dT indicates rate of change of fluorescence with respect to temperature

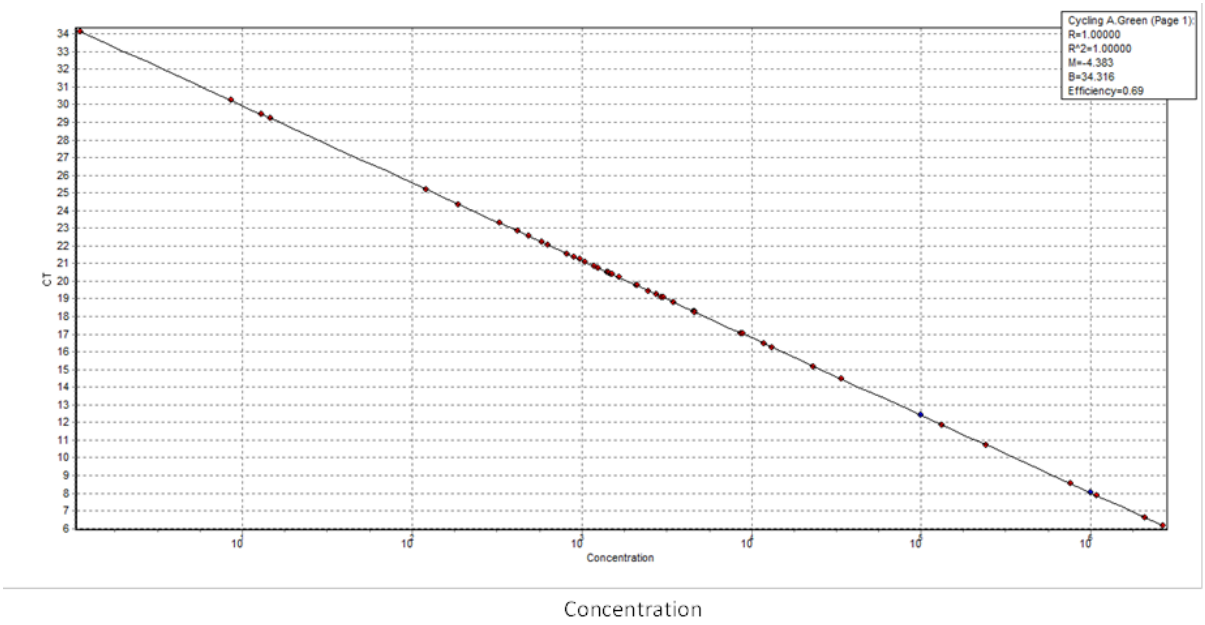
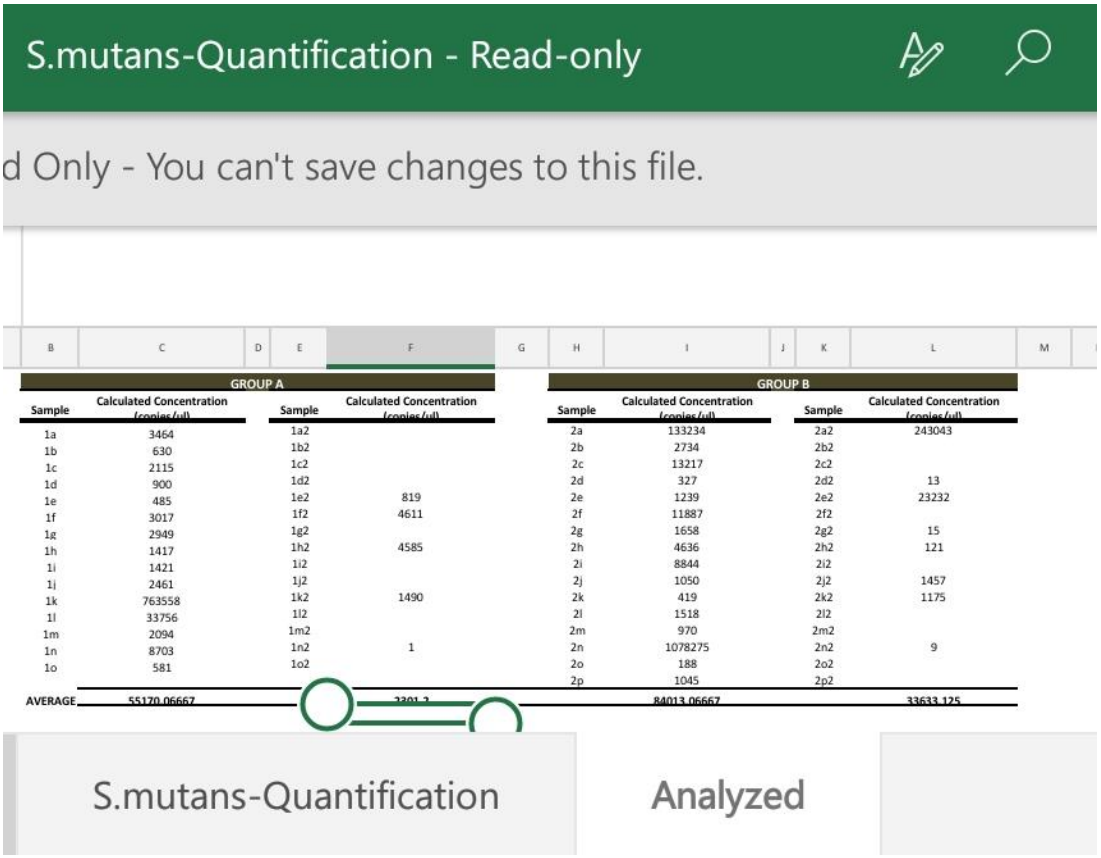


Fig.14. Linear Graph of Standard used to Quantify S. mutans



GROUP A				GROUP B			
Sample	Calculated Concentration (copies/ul)	Sample	Calculated Concentration (copies/ul)	Sample	Calculated Concentration (copies/ul)	Sample	Calculated Concentration (copies/ul)
1a	3464	1a2		2a	133234	2a2	243043
1b	630	1b2		2b	2734	2b2	
1c	2115	1c2		2c	13217	2c2	
1d	900	1d2		2d	327	2d2	13
1e	485	1e2	819	2e	1239	2e2	23232
1f	3017	1f2	4611	2f	11887	2f2	
1g	2949	1g2		2g	1658	2g2	15
1h	1417	1h2	4585	2h	4636	2h2	121
1i	1421	1i2		2i	8844	2i2	
1j	2461	1j2		2j	1050	2j2	1457
1k	763558	1k2	1490	2k	419	2k2	1175
1l	33756	1l2		2l	1518	2l2	
1m	2094	1m2		2m	970	2m2	
1n	8703	1n2	1	2n	1078275	2n2	9
1o	581	1o2		2o	188	2o2	
				2p	1045	2p2	
AVERAGE	55170.06667		2301.2		84013.06667		33633.125

Fig 15: Screenshot of the s.mutans quantification analysed

DISCUSSION

Orthodontic brackets have always been a place of concern in terms of infective floral adhesion. According to Ahn et al., (2005)[23], they observed a characteristic binding pattern on these brackets according to species. Ahn et al., (2007)[24] have also observed that the cariogenic streptococci strain to have a characteristic adhesion pattern and the highest adhesion was seen in plastic brackets and lowest in the monocrystalline sapphire brackets. Therefore, initial attempts were made to modify the material.

Brusca et al., (2007)[25] have used 3 types of brackets, viz. metallic, ceramic, and composite and found that adherence of Streptococcus mutans was independant of material type and candida adhered more to composites. But, earlier, Fournier et al., (1998)[21] have shown that the initial affinity of S. mutans to metal brackets was statistically significantly lower than that to plastic and porcelain brackets with and without saliva coating. Numerous in vitro studies have been reported on bacterial adhesion like Lim et al., (2008)[28] who have investigated the adhesion of 2 cariogenic streptococci strains to 7 orthodontic raw materials and van Gastel et al., (2009)[30], who observed total bacterial counts and capacity for biofilm formation amongst seven commercially available bracket systems. The all found significant differences among their materials of study.

However, from these literatures it is seen that the pattern may be different for various strains and species also. Hence, sampling one material for a species is the best way to evaluate a technique for reducing bacterial adhesion. The selection of species and strain for in vitro studies have hence always been a problem. Therefore, molecular techniques were resorted to by several authors.

Ahn et al., (2007)[26] have conducted PCR studies at de-bonding stage; have analyzed the prevalence of cariogenic streptococci adhering to incisor brackets in 80 samples collected at de-bonding. They showed that the prevalence of cariogenic streptococci was not associated with the oral hygiene indexes at de-bonding. Hence, the count of bacteria need not necessarily imply higher cariogenic potential and also the count of bacteria need not necessarily be reflected in the oral hygiene of the patient. It is a paradoxical statement. It is usually seen that if the count of bacteria is higher, more the caries would be. Also, more the oral hygiene indices scores are, worser the caries risk is. But this study has shown that there is no direct association. Hence, previous results for bacterial adhesion estimation, when done with molecular techniques, can show a different result.

Various techniques have been used by investigators to arrive at the proper bacterial adhesion properties of materials. Velazquez-Enriquez et al., (2012)[34] used radioactive marker to codify the bacteria (^{3}H) for quantitative analysis. Passariello and Gigola (2013)[35] have used quantitative real time PCR. Baka et al., (2013)[36] used real-time polymerase chain

reaction. Condò et al., (2013)[37] and Jacobo et al., (2014)[40] used scanning electron microscopy to observe the biofilm.

It should be understood that, not much can be offered by materials for discouraging plaque accumulation. Those materials are made to perform specific bio-mechanical function. Modifying them to discourage plaque adhesion would only result in added expenditure and limited success. In order to counter this problem of floral adhesion to surfaces, there should be a way that has high efficacy, low side effects and affordable. In this direction, currently mouth washes are used extensively, but results in limited control only. Several disadvantages of mouthwashes have been felt in orthodontics, like reliance on patient compliance, spectrum of activity against the wide flora of oral cavity etc.

Since the dentition with brackets and wires are an extremely difficult task for salivary self-cleaning and mechanical debridement, use of probiotics can potentially change the microbiota of the patient, leading to growth of healthier flora which does not allow growth of cariogenic or periodontal flora. It is already observed that biological methods of plaque control only suppress the infection but not eliminate it. Probiotics were defined by the Food Agricultural Organization/World Health Organization as live microorganisms which when administered in adequate amounts (in food or as a dietary supplement) confer a health benefit on the host (improving microbiological balance in the intestinal tract). In oral cavity, probiotic species can create a biofilm, which is a protective layer for oral tissues against pathogens.

Ghasempour et al., (2014)[38] have shown that the acidity and the count of streptococci were inhibited by Kefir. Gizani et al., (2016)[14] have shown the antagonistic effect of lozenges containing probiotic bacteria on white spot lesion (WSL) formation as well as on salivary lactobacilli (LB) and mutans streptococci (MS) counts. This study was conducted on orthodontic patients for a period of 17 months. They recorded white spot lesions and bacterial count. Within the limitations of the study, they concluded that daily intake of probiotic lozenges did not influence the WSL during orthodontic treatment with fixed appliances. However, due to change in perspective with molecular techniques are discussed earlier, more studies with different methodologies are warranted.

Sajedinejad et al., (2018)[16] have suggested that probiotic mouthwash is healthy for daily use as an alternative for maintaining dental and periodontal health. Their study duration was for 4 weeks, similar to current study. They used a mouthwash containing L. salivarius NK02 at a dose level of 10^8 (CFU/ml). But the study was not conducted on orthodontic patients. Goyal et al., (2019)[50] have reported that levels of P. gingivalis reduced with use of probiotic mouth washes in orthodontic patients. Shah et al., (2019)[51] have shown that probiotics and chlorhexidine oral rinses in orthodontic patients had equal efficacy.

By observing these results, it is not completely clear whether these probiotics can have a positive influence on reducing cariogenic flora. This has necessitated further studies,

which are critically designed and executed. In current study it is seen that the study was designed to evaluate the change in streptococcal count after 30 days of use of probiotics. It is found that there was no statistically significant reduction in count. Similar results have been reported with other modalities of probiotic administration like lozenges Gizani et al., (2016).[14]

This study has assessed the effect with minimum duration of intervention for such therapeutic modalities. This study has confirmed that though there was reduction in s.mutans count in the experimental probiotic group among those 15 patients observed in 30 days period , it was not proved to be stastically significant . Though Gizani et al., (2016)[14] have reported similar scenario after 17 months with lozenges and studies on probiotic mouthwash in orthodontic patients, is hitherto unreported. Hence, this study has thrown valuable light on time period of evaluation.

Since no changes are seen within a month and previous reports have shown promising results in non-orthodontic patients, it gives rise to two types of inferences. Primarily, its use may not have clinical benefit in orthodontic patients. Secondarily, since it changes the oral flora effectively, it might be recommended to start with probiotics before commencement of orthodontic treatment. The duration of such pre-orthodontic probiotic prophylaxis as it can be called, can vary according to the patient's needs.

The reason for persistant perseverance on using probiotics is that it has no side-effects and has permanent positive impact on oral cavity. The question that has to be answered now is that why this is not being effective in orthodontic patients. The difference between non-orthodontic patients and orthodontic patients are manifold. Primary difference is that amount of available adhesion surface in orthodontic patients, which is much higher than their counterparts. As said before self-cleansing action of saliva is lost here. Macro-sized food particles stay in the oral cavity for longer periods of time. Due to these differences, probiotics may not be equally efficous in the same modality. However, if the modality of delivery can change this scenario is bound to change.

SUMMARY

Placement of orthodontic appliance is known to cause an increase in plaque accumulation due to various reasons. The consequence of such adhesion and plaque formation is the dissolution of enamel and compromise in periodontal health. While mechanical cleansing has superior cleansing effect, it may not be always feasible to perform by the patient. Other modalities have been tried with limited success. In search of a technique to work on carious flora and inhibit them without any adverse effect, focus has been shifted to the concept of increasing the number of favourable flora in the oral cavity that shifts the balance towards healthy tissues. While success has been demonstrated by using such probiotics in non-orthodontic patients, its application in orthodontics has not been adequately explored.

This study aimed to comparing the minimal adhesion of Streptococcus mutans over the stainless steel brackets between probiotic and chlorhexidine mouth washes. The study population was selected from the outpatient section of the Department of orthodontics and dentofacial orthopaedics Tamilnadu Government Dental College & Hospital, Chennai, Tamilnadu, India. This clinical case control study was performed using 30 subjects divided into two Study groups. One group of patients was given the probiotic mouth wash, and the other group was given the chlorhexidine mouth wash. Regular oral hygiene instructions were given. Patients undergoing Orthodontic treatment with the straight wire appliance with good periodontal conditions were considered for the study. On the day of commencement, the plaque sample was obtained from the subjects. After 30 days of use of

the mouthwashes, the study subjects called and plaque was collected and submitted for RT-PCR reaction to determine bacterial count of Streptococcus mutans.

Both the groups had no significant difference preoperatively, implying that there was no significant bias in the patient selection. The difference between pre and post use of the mouthwash in chlorhexidine was statistically significant. In probiotics group, there was statistically no difference between pre and post use of probiotic mouth wash. In addition, comparison of post test values between the groups did not differ either. That implied that the results are inconclusive about the use of probiotics in place of mouthwashes.

By observing these results, it is not completely clear whether these probiotics can have a positive influence on reducing cariogenic flora. The purpose of short term study was to assess the minimum duration of action for such therapeutic modalities. This study has confirmed that no significant changes can be observed in 30 days period. Hence, this study has thrown valuable light on time period of evaluation.

Its use may not have clinical benefit in orthodontic patients. Since it changes the oral flora effectively, it might be recommended to start with probiotics before commencement of orthodontic treatment.

CONCLUSION

Within the limitations of the study, it can be concluded that the use of probiotic mouthwash may not have clinical benefit in orthodontic patients. Since it changes the oral flora effectively, it might be recommended to start with probiotics before commencement of orthodontic treatment. In future such modalities can be tried to enhance the plaque or caries control during orthodontic therapy.

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PARTICIPANT INFORMATION SHEET IN LOCAL LANGUAGE

ANNEXURE – I

ஆராய்ச்சிபற்றியதகவல் படிவம்

மரு. மு. ரம்யா ஆகிய நான் மரு. பாலசண்முகம் MDS, அவர்களின் வழி நடத்துதலின் கீழ **"பல்சீரமைப்பு சிக்ச்சைக்காக ஒட்டப்படும் உலோகபொத்தான்களின் மீது படரகூடிய நுண்ணுயிர் கிருமிகளின் வளர்ச்சியை தடுக்கும் இரு வகையான வாய்கழுவிகள் (probiotic மற்றும் chlorhexidine) செயல்திறனை ஒப்பீடு செய்தல்"** தொடர்பாக ஆய்வு செய்யஉள்ளேன்.

ஆய்வின் நோக்கம்:

இருவகையான வாய்கழுவிகள் செயல்திறனை ஒப்பீடு செய்தல்

செய்முறை:

ஆராய்ச்சிக்காக தேர்ந்தெடுக்கப்பட்டவர்களுக்கு வழக்கம் போலவே பல்சீரமைப்பு சிகிச்சை மேற்கொள்ளப்படும். பின்னர் அவர்களுக்கு தேர்தெடுக்கப்பட்ட பல்லின் உலோக பொத்தான்களில் இருந்து சோதனை மாதிரி எடுக்கப்படும். அவர்களை இருபிரிவுகளாக பிரித்து, இருவகையான வாய்க்கழுவிகள் முறையே பயன்படுத்த அறிவுறுத்தப்படும். ஒருமாதம் கழித்து முன்னர்போன்று அதே பல்லில் சோதனை மாதிரி எடுக்கப்படும். இந்த இரண்டு சோதனை மாதிரிகளும் உள்ள நுண்ணுயிர் கிருமிகளின் எண்ணிக்கை அளவிடப்படும்.

நன்மைகள்:

probiotic மற்றும் chlorhexidine ஆகிய வாய்கழுவிகள், வாய் நோய் பரவும் தீய நுண்ணுயிர்கள் பரவுவதைதடுக்கும்.

இரகசியதன்மை:

நோயாளிகள் பற்றியகுறிப்புகள் ஆராய்ச்சி முடியும் வரை ரகசியமாக பாதுகாக்கப்படும் . இந்த ஆராய்ச்சியைவெளயிடும்போது நோயாளிகளின் தனிப்பட்ட விவரங்கள் எதுவும் பாதிக்கப்படமாட்டாது.

பங்குபெறுவோரின் உரிமை:

இந்த ஆராய்ச்சியில் பங்குபெறுவதுநோயாளிகளின் தனிப்பட்டவிருப்பம். மேலும், நோயாளிகள் இந்த ஆராய்ச்சியிலிருந்து எப்போது வேண்டுமென்றாலும் விலகிக் கொள்ளலாம். நோயளிகளின் இந்தமுடிவினால் அவருக்கோ அல்லது ஆராய்ச்சியாளருக்கோ எவ்விதபாதிப்பும் கிடையாது.

இந்த ஆராய்ச்சியின் முடிவுகள் நோயாளிகளுக்கு ஆராய்ச்சி முடியும் தருவாயிலோ அல்லது இடையிலோ தெரிவிக்கப்படும். ஆராய்ச்சியின் பொழுது ஏதும் பின் விளைவுகள் ஏற்பட்டால் அதை சரிசெய்யதகுந்த உதவிகள் அல்லது தேவையான சிகிச்சைகள் உடனடியாக மேற்கொள்ளப்படும்.

இழப்பீடு: எதுவும் வழங்கப்படமாட்டாது.

ஆய்வு பற்றியதகவலைபெற
மரு. மு. ரம்யா
மூன்றாம் ஆண்டு MDS,
முதுநிலைமாணவி,
தமிழ்நாடுபல் மருத்துவகல்லூரி மற்றும் மருத்துவமனை,
சென்னை-600 003.

செல்பேசி: ----------

நோயாளியின் பெயர் கையொப்பம்/கைரேகை
தேதி

ஆராய்ச்சியாளரின் பெயர் கையொப்பம்/கைரேகை
தேதி

ANNEXURE-II

PARTICIPANT INFORMATION SHEET

TITLE OF THE STUDY : "Comparative evaluation of probiotic and chlorhexidine mouthwashes effect on the adhesion of streptococcus mutans on stainless steel brackets-A Clinical trial"

Name of the research institution: Tamilnadu government dental College & hospital

Purpose and procedure of the study:

To compare the adhesion of streptococcus mutans on the stainless steel by using two mouth washes(probiotic and chlorhexidine mouth wash)

One group of patients (Group 1) was given the chlorhexidine mouthwash and the other group (Group 2) was given the probiotic mouthwash. The patients were instructed to use mouthwash, after brushing their teeth with a prescribed toothpaste

The patients were asked to brush twice daily for 2 min; this was demonstrated by the operator. They were instructed to restrict intake of any food or beverage 30 min to 1 h, before and after using the mouthwash and avoid chewing gums, lozenges and antibiotics during the study. The mouthwash is to be administered to the patients from day 1 after the first plaque sample had been assessed and continued until day 30. Plaque samples were to be again taken and evaluated at the end of day 30. At each appointment, the elastomeric modules were carefully removed, and archwires were disengaged. Plaque samples were collected from the labial surfaces surrounding the orthodontic brackets of the maxillary lateral incisors with a scaler] using a 4-pass technique .

The plaque samples were suspended in 1 ml of sterile phosphate buffer saline (0.12 M NaCl, 0.01 M Na_2 HPO_4, 5 mM KH_2 PO_4 pH 7.5) and sealed for transport for Real time-polymerase chain reaction (RT-PCR). The PCR values were obtained as CFU/ml. The values were tabulated, and the statistical analysis was performed.

Risk of participation:

Discomfort during orthodontic treatment

Benefits of participation :

- Patients get orthodontic treatment
- The result of the study will help us to identify which of the two mouthwash is more efficient to reduce the adhesion of S.mutans over orthodontic brackets.

1. **Confidentiality:**

The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

2. Participant's rights:

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time. Your decision will not result in any loss of benefits to which you are otherwise entitled.

3. Compensation: NIL

Contacts:

For queries related to the study:

PRIMARY INVESTIGATOR: DR. M. RAMYA

CONTACT DETAILS: PG SECTION, DEPT OF ORTHODONTICS AND DENTOFACIAL ORTHOPEDICS,

TAMILNADU GOVT DENTAL COLLEGE & HOSPITAL,

FRAZER BRIDGE ROAD, Chennai-600003**.**

Ph : 9094005740

For queries related to the rights as a study participant, please write to:

The Chairperson,

TAMILNADU GOVT DENTAL COLLEGE & HOSPITAL,

FRAZER BRIDGE ROAD, CHENNAI-600003

PATIENT CONSENT FORM IN LOCAL LANGUAGE

ANNEXURE – III

சுய ஒப்புதல் படிவம்

பெயர்: ஆராய்ச்சிசேர்க்கை எண்:

வயது: பால்:

ஆராய்ச்சிசெய்யட்டப்படும் தலைப்பு

"பல்சீரமைப்பு சிக்ச்சைக்காக ஒட்டப்படும் உலோகபொத்தான்களின் மீது படரகூடிய நுண்ணுயிர் கிருமிகளின் வளர்ச்சியை தடுக்கும் இரு வகையான வாய்கழுவிகள் (probiotic மற்றும் chlorhexidine) செயல்திறனை ஒப்பீடு செய்தல்".

ஆராய்ச்சிநிலையம்: அரசுபல் மருத்துக் கல்லூரி, சென்னை – 600 003.

பங்குபெறுபவரின் பிறந்ததேதி: தேதி_____ மாதம்_____/வருடம்_____

இந்துஆய்வு சம்பந்தமாகநான் மேலேகூறப்பட்டதகவல் படிவத்தை முழுமையாக படித்துப் பார்த்தேன் என்று உறுதிகூறுகிறேன்.

நான் இது தொடர்பாக அனைத்து கேள்விகளுக்கும் நிறைவான பதில்கள் பெறப்பட்டேன்.

இந்த ஆய்வில் எனது பங்கு தன்னிச்சையானது என்றும் எந்தநேரத்திலும் இந்த ஆய்வில் இருந்து சட்டஉரிமைகள் பாதிக்கப்படாமல் விலகிக் கொள்ளசம்மதிக்கிறேன்.

மருத்துவ ஆய்வு அதிகாரிகள், எனது சிகிச்சை தொடர்பான பதிவேடுகளை பார்வையிடவும், எந்த நேரத்திலும், ஆய்வில் இருந்துநான் விலகினாலும் பார்வையிட சம்மதிக்கிறேன். எனது அடையாளகுறிப்புகள் மூன்றாவது நபருக்கு தெரிவிக்கப்படமாட்டாது என்று புரிந்துகொண்டேன்.

இந்த ஆய்வு அறிக்கைகளை பயன்படுத்தவும், வெளியிடவும் நான் சம்மதிக்கிறேன். ஆய்வாளர் எனது மருத்துவகுறிப்புகளை வெளியிட தடையாக இருக்கமாட்டேன் என உண்மையாகசம்மதிக்கிறேன்.

பங்கேற்பவரின் கையொப்பம்:_____ இடம்_____ தேதி

கட்டைவிரல் ரேகை

பங்கேற்பவர் பெயர் மற்றும் விலாசம்

ஆய்வாளரின் பெயர்:

ஆய்வாளரின் கையொப்பம்:

ANNEXURE- IV

INFORMED CONSENT FORM

"Comparative evaluation of probiotic and chlorhexidine mouthwashes effect on the adhesion of streptococcus mutans on stainless steel brackets- A Clinical trial"

"I have read the foregoing information sheet given to me about the methods and procedures to be followed for the study, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to participate as a participant in this study and understand that I have the right to withdraw from the study at any time without in any way it affecting my further medical care."

| --- | --- | --- |
| Date | Name of the participant | Sign/ Thumb impression of participant |

[The literate witness selected by the participant must sign the informed consent form. The witness should not have any relationship with the research team; If the participant doesn't want to disclose his / her participation details to others, in view of respecting the wishes of the participan t, he / she can be allowed to waive from the witness procedure (This is applicable to literate participant ONLY). This should be documented by the study staff by getting signature from the prospective participant]

"I have witnessed the accurate reading of the consent form to the potential participant and the individual has had opportunity to ask questions. I confirm that the individual has given consent freely"

| --- | --- | --- |
| Date | Name of the witness | Signature of the witness |

| --- | --- | --- |
| Date | Name of the interviewer | Signature of the interviewer |

CHART 1

PARTICIPANT MONITORING SHEET-Mouthwash

DAY	PARTICIPANT		PARENT
	MORNING	NIGHT	
1			
2			
3			
4			
5			
6			
7			
8			
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10			
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CHART-2

PARTICIPANT MONITORING SHEET -VEGETARIAN DIET

DAY	PARTICIPANT	PARENT
1		
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