International Journal of Pharmaceutical Science Invention
ISSN (Online): 2319 – 6718, ISSN (Print): 2319 – 670X
www.ijpsi.org Volume 5 Issue 3 ǁ June 2016 ǁ PP.18-24

Assessment of Microbial Contamination of the Tooth Brush Head Used On Orthodontic Appliances-A Randomized Control Study

Padma. K. Bhat[1], Komalraj.M.R[2] ,Rajkumar S Alle[3],Shivaprasad.R.K[4]
[1]*Professor and Head, Department Of Public Health Dentistry, Rajarajeswari Dental College and Hospital, Bengaluru, Karnataka, India*
[2]*Postgraduate Student, Department Of Public Health Dentistry, Rajarajeswari Dental College and Hospital, Bengaluru, Karnataka, India*
[3]*Professor and Head, Department Of Orthodontics and Dentofacial-orthopedics, Rajarajeswari Dental College andHospital, Bengaluru, Karnataka, India*
[4]*Reader, Department Of Periodontics, M.R.Ambedkar Dental College and Hospital, Bengaluru, Karnataka, India*

ABSTRACT:
Introduction: *Oral diseases can be greatly controlled by reducing the microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene.Tooth brushes are the most commonly used oral hygiene aid to promote oral health and prevent dental diseases. The insertion of fixed appliances alters the oral microbiological profile, thus increasing the risk for caries and gingivitis considerably.*
Aim: *To assess the microbial growth of S.Mutans and Lactobacillus between and among the brushes.*
Setting and Study Design: *A Hospital setting and Randomized Control study design*
Methods:*A total of 56 (MB) patients aged 16-26 years received a toothbrush [Regular soft bristle design (group-A) and Orthodontic bristle design (group B)],A sterile gamma radiated pouch and checklist was distributed to each participant. After 2 weeks period the brushes were collected and placed in 5ml saline solution (0.05g Sodium Chloride). The suspension was incubated on selective agar plates and the amount of Streptococcus mutans and lactobacilli for each brush head was assessed.*
Results:*The retention of S.Mutanswas found to be higher in group A, as compared to group B and was found to be statistically more significant between the two groups (P<0.001). The retention of Lactobacillus was also found to be higher in group A, as compared to group B and was found to be statistically significant between the groups (P= 0.001). However, there was no significant difference (P= 0.101) observedamong the microbial growth of S.Mutans and Lactobacillus in two bristle designs.*
Conclusions: *Regular soft bristle design had a higher microbial load than those of subjects using orthodontic bristle design, a more frequent replacement of toothbrushes during t treatment may be advisable. Due to significant differences between the two bristle designs, the orthodontic toothbrush is recommended for patients undergoing orthodontic t appliances.*
Keywords: *Microbial contamination, Toothbrush design, appliance, Stretococcusmutans, Lactobacillus*

I. Introduction

Oral cavity is free of micro-organisms at birth because the fetus develops in a well-protected environment, but soon after it is habituated by numerous micro-organisms. [1]Oral diseases can be greatly controlled by reducing microbial load in the oral cavity and this can be achieved by maintaining proper oral hygiene.Tooth brushes are the most commonly used oral hygiene aid to promote oral health and prevent dental diseases.Prolonged use of the toothbrush facilitates contamination by various micro-organisms such as Streptococcus, Staphylococcus, and lactobacilli. These micro-organisms are implicated to cause dental caries, gingivitis, stomatitis, infective endocarditis in an individual, affecting both oral and general health.[2]

As a result of increasing health consciousness and demand for an aesthetic dentition,patients undergoing orthodontic treatment have increased during the last years. According to the guidelines of health care systems over one third of adolescents require orthodontic therapy nowadays [3] and the majority of these treatments is performed with multibracket (MB) appliances. The insertion of fixed appliances alters the oral microbiological profile, thus increasing the risk for caries and gingivitis considerably.[4-6]The 6th to 12th week of orthodontic therapy is the period of the most intensive intraoral growth of S mutans and Lactobacillus and a time of very intensive salivary functions and physiologic response.[7] Streptococci of the mutans-group have been considered essential bacteria for inducing caries, but lactobacilli and Candida albicans are also held responsible for the initiation and progress of dental decay. These germs favour a high-carbohydrate diet and increase in numbers depending on the presence of retentive areas in the mouth; it would be desirable to recommend toothbrushes to

orthodontic patients, which retain as little caries-associated microorganisms on the brush heads as possible. An ideal design for toothbrushes used during orthodontic treatment is not yet agreed on [8], so it remains mainly up to the patient what kind of toothbrush he or she prefers and is able to use effectively. Therefore, the present study was to evaluate:The retention of two caries-associated microorganisms (S. mutans and lactobacilli) on two manual toothbrushes differing in their filament design (Regular soft bristle design vs. Orthodontic bristle design), to assess the influence of a multibracket appliance on the microbial contamination of the brush head,to assess the microbial growth of S.Mutans and Lactobacillus between and among the brushes

II. Materials And Methods

a) Materials:

Materials used in the present study includes toothbrushes-Regular soft bristle toothbrushes, Orthodontic toothbrushes, different agar media, agar plates, resealable gamma radiated pouch, 5ml saline solution, Disposable Gloves, Disposable Mouth masks, Sterile Mouth mirrors, Sterile Tweezers, Sterilized Cotton rolls, Cotton holder, Sterile Kidney trays, and compound microscope

The medias were prepared for the isolation of Lactobacillus (deManRogosa Sharpe Agar) and Streptococcus mutants (Trypticase-soy broth media's) for required amount.

To the 5ml saline solution(0.05g Sodium Chloride) given toothbrush samples were mixed properly and by pour plate method, 0.1ml of the solution was added and media was poured upon it. And plates were incubated for 24 to 48hrs and observe for the results.Colony-forming unit *(*CFU*)* was calculated after the incubation period in each plates.Standardization was done with respect to materials, instruments, methodology and calibration for the microbial analysis.

MediaComposition:

Lactobacillus MRS Agar- (deManRogosa Sharpe)

Ingredients	**Gms / Litre**
Proteose peptone	10.000
Beef extract	10.000
Yeast extract	5.000
Dextrose	20.000
Ammonium Nitrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium phosphate	2.000
Agar	12.000
Final pH	**6.5±0.2**

b) Streptococcus mutants TYS Composition*: (Trypticase-soy broth)*

Ingredients	**Gms / Litre**
Enzymatic Digest of Casein	17.0 g
Enzymatic Digest of Soybean Meal(Peptone)	3.0 g
Sodium Chloride	5.0 g
Dipotassium Phosphate	2.5 g
Dextrose	2.5 g
Yeast Extract	10g
Sucrose	20% w/v
Bacitracin	10mg per ml of DMSO
Agar	12.000
Final pH	**7.3 ± 0.2**

Methods:

1) Inclusion Criteria

- Subjects aged 16-26 years old
- Subject with informed/written consent (if subjects are under 18years of age, an additional parental consent required)
- Subjects having DMFT score= 0 to 3 score
- Orthodontic treatment with the attachments on ≥20 teeth.

2) Exclusion Criteria

- Subjects who do not give informed consent
- Subjects with any oral and systemic disease.
- Subjects using antibiotic medications, mouthwashes at any time of the study or 15 days prior to it.

3) ***Ethical Clearance:***Proposed study protocol was prepared and submitted to Institutional Review Board (of the dental institution, Bangalore) for its approval, after review from the IRB committee the study protocol was approved.

4) ***Informed Consent:***The study participants were selected according to the eligibility criteria and included in the clinical trial only after obtaining a written informed consent from them. The study purpose objectives and procedures were explained to the subjects before obtaining their consent.

5) ***Selection Of Study Subjects:*** A total of 56 multibracket (MB) patients aged 16-26 years with attachments on ≥20 teeth were selected using Simple Random sampling by an anonymous person not involved in the study undergoing treatment at the Department of Orthodontics from reputed Dental Institution in Bangalore.

Clinical examination was carried out by the calibrated examiner to exclude participants with oral diseases, using mouth mirror, explorer and probe according to WHO type IIunder aseptic conditions. Medical history of the participants was obtained from the participants to rule out systemic diseases. DMFT indices (In1987 by Henry Klein, Carrole E Palmer, John W Knutson)was carried out on enrolled subjects using WHO criteria as per the proposed protocol.

Group A:Regular soft bristle tooth brush (n=28)and ***Group B:***Orthodontic toothbrush (n= 28).After 2 weeks, total 51 toothbrushes were collected in a resealable gamma radiated pouchalong with completed checklist adhering to strict protocol and 05 subjects were dropouts (02-use of antibiotics, 03-return of brush ≥24h late) (Table I). The toothbrushes were sent for further processing to Azyme Biosciences, Bangalore.

6) Blinding Procedure

It was a double blind clinical trial. Both participants and microbiologist were blinded.

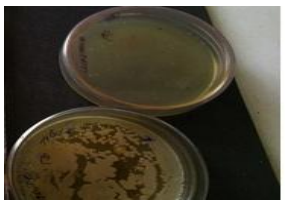
III. Instructions To The Subjects Before The Study Procedure

At the start of the study, each participant was given either Regular soft bristle toothbrush or Orthodontic toothbrush respectively based on random allocation.

To help follow the instructions the checklist with dichotomous questions ("yes" or "no") was also provided.

The following oral hygiene instructions were given during the study period:The subjects wereasked to use the dentrifice they had been routinely using, to Brush twice daily (morning and night) and the time required- 2-3 minutes with the allocated toothbrush only for 14 consecutive days. The toothbrushes should be exclusively used by the participant and not to be shared with anyone.After brushing, the brush head had to be cleaned with running tap water for about 5 seconds. The toothbrush was stored head up until the next brushing sequence.The use of mouthwash and interdental brushes was not allowed.The additional use of dental floss will be permitted.

Fig 1: Growth of Lactobacillus (MRS) and Streptococcus mutants (TYS) between Regular soft bristle design and orthodontic bristle design

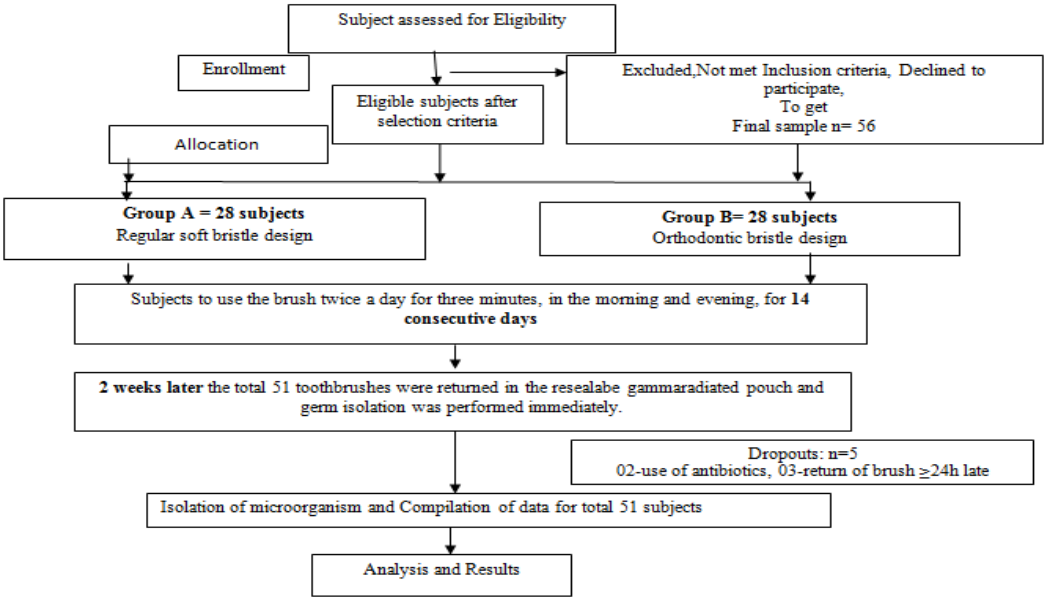


Growth on Regular soft bristle	**Media**	**Incubation time**
	MRS Agar	48hrs
	TYS Agar	48hrs

Growth on Orthodontic bristle	**Media**	**Incubation time**
	MRS Agar	48hrs
	TYS Agar	48hrs

Fig 2: Resealable gamma radiated pouch

IV. Schematic Representation Of Study Design



Statistical Analysis

As a normal distribution of values could not be assumed, hence non-parametric methods (Mann-Whitney U test) were applied. The statistical software SPSS 21(Statistical Package of Social Science)was used for the analysis of data.Statistical significance was fixed at $p<0.05$.

V. Results

A total of 56 participants were enrolled into the study according to the eligibility criteria. However after 2 weeks, 51 subjects completed the study belonged to 16-26 year age group out of which 19 were males and 32 were females, resulting in a dropout-rate of 8.9%. The reasons for the dropouts are listed in Table II. Thus microbial analyses were conducted for 51 brushes. No gender-related difference was observed with respect to microbial colonisation of the brushes ($p=0.778$). In total the Regular soft bristle design (Group A) was used by 25 patients, Orthodontic bristle design (Group B) by 26 participants (Table III).

Figure 3: Design of toothbrush heads of brand-new Regular soft bristle design (Group A) and Orthodontic bristle design (Group B) (right).

Group a Group B

CFU of S. Mutans and Lactobacillus between the brushes:

Colony-forming unit (CFU) of Streptococcus mutants was found to be higher in group A with the median value of 104, as compared to group B with the median value of 3. The microbial growth of S.Mutans was found to be statistically more significant between the two bristle designs (P<0.001).
Colony-forming unit (CFU) of Lactobacillus was found to be higher in group A with the median value of 328, as compared to group B with the median value of 19 .The microbial growth of Lactobacillus was found to be statistically significant between the two bristle designs (P= 0.001) (Table III).

CFU of S.Mutans and Lactobacillus among brushes:
In group A (Regular bristle), colony-forming unit (CFU) of Lactobacillus was found to be higher with the median value of 328, as compared to Streptococcus mutants with the median value of 104. However, there was no significant difference (P= 0.101) among the microbial growth of S.Mutans and Lactobacillus.
In group B (Orthodontic bristle), colony-forming unit (CFU) of Lactobacillus was found to be higher with the median value of 19, as compared to Streptococcus mutants with the median value of 3. However, there was no significant difference (P= 0.385) among the microbial growth of S.Mutans and Lactobacillus (Table IV).
The highest contaminated bristles was found in Group A (Regular soft bristle design), the lowest in group B (Orthodontic bristle design) (Table IV). Since, due to significant differences between the two bristle designs, the orthodontic toothbrush is recommended for patients undergoing orthodontic multibracket appliances.

VI. Discussion

The present study was carried out to evaluate the retention of microorganisms (S.mutans and Lactobacilli) on two different manual toothbrushes (Regular soft bristle design vs. Orthodontic bristle design) differing in their filament design, to assess the microbial contamination of the brush head used on multibracket appliances (MB), the microbial growth of S.Mutans and Lactobacillus between the brushes and among the brushes.
The micro-organisms like S.mutans and Lactobacillus isolated in this study cause different diseases, e.g., Str. mutans causes initiation of dental caries in human beings; Lactobacilli cause the progression of the dental caries.
In the present study there was no gender-related difference in the microbial load of the brushes, as possible influence of this factor seems negligible. Wheeler TT et al [9] in 1994 reported that gender distribution was not homogenous, which does not seem unusual bearing in mind that the request for orthodontic treatment, is much higher in females.
The association between orthodontic treatment and changes in oral microflora and salivary functions are neither numerous nor unambiguous. Because saliva provides a general protective effect, clinically significant changes in salivary functions may be considered an etiologic factor that contributes to the development or prevention of dental caries, a significant increase in cariogenic microorganisms S mutans and Lactobacillus in saliva was found after commencing fixed orthodontic therapy. [7]
Storage conditions of toothbrushes are an important factor for bacterial survival. In the present study, to prevent reduction of germs due to drying after the brushes last use, resealable sterile gamma radiated pouch were distributed, so brushes were under standardized conditions. Dayoub et al. in 1977 [10]reported that the number of microorganisms in the toothbrushes kept in aerated conditions was lower than in toothbrushes stored in plastic bags. Several authors have reported that bacterial contamination can be reduced by washing toothbrushes after use, and drying in aerated conditions.[14]Caudry et al.in 1995 [12]and Malmberg E et al in 1994[13]reported that wet environment increases bacterial and cross contamination. Therefore, as time increases between one toothbrushing and another, more microorganism development can occur in the toothbrushes stored in a wet/moisture environment. The toothbrushes from the participants were collected after two weeks similar to other study. [14]
In this study colonisation of S. mutans and Lactobacillus was found more in Group A with CFU of 104 and CFU of 328 than compared to Group B with CFU of 3and CFU of 19, revealing the greatest variance between the groups. The bristle design seemed to have an impact on the number of germs retained on the brush head. However, a significant difference became obvious when comparing brushes from subjects with Group A to those of Group B, indicating that Group A brushes used by orthodontic patients with fixed appliances tend to harbor more microorganisms. On review of literature using pubmed search engine, there was no similar studies found as all microbial studies on toothbrushes were performed in vitro or in vivo on subjects without MB, so that only the amounts of microorganisms of the nMB groups are suitable for comparison.
The study revealed the increase incidence of Lactobacilli bacteria with CFUs 328 in group A brushes and CFUs 19 in group B brushes, regarding the fact that during MB-treatment the amount of lactobacilli are reported to increase considerably, which is mainly associated with the many retention sites a multibracket appliance offers. Similar finding results were found in the study conducted by KupietzkyA et al[11] in 2005where there was a higher number of CFUs of LB associated with the group wearing orthodontic appliances which play a role in the increased levels of plaque seen in many orthodontic patients.

In the current study for assessing individual perceptions, such asbrushing comfort, cleaning efficacy of the brushes, frequency of brushing, a checklist was used, which is considered superior to verbal methods.
In the present study the Group B brush is likely to convey a soft and pleasant feeling, although few subjects described no difference concerning the cleaning efficacy of both brushes. Eichenauer J et al in 2014[15] proved that conical and fine filaments are considered to a better cleaning efficacy at the gingival margin and in proximal regions, which seems to be correlated to the flexibility of the filaments that may easily access the areas around the brackets and below the arch wire.

VII. Conclusion

Bacterial contamination of the toothbrushes is the major cause of concern. Among toothbrushes of multibracketpatients, Regular soft bristle design had a higher microbial load than those of subjects using orthodontic bristle design, a more frequent replacement of toothbrushes during Multibracket treatment may be advisable. Due to significant differences between the two bristle designs, the orthodontic toothbrush is recommended for patients undergoing orthodontic multibracket appliances. This study shows that use of orthodontic toothbrushes are preferred than compared to regular soft bristle toothbrushes in reducing the microbial contamination in the patient undergoing orthodontic multibracket appliances.

Acknowledgement

The authors would like to thank all the patients who participated in this study.

Competing Interest

The authors declare that they have no competing interests

Funding

It was a Self-funded study.

References

[1]. McCarthy C, Synder ML, Parker RP. The indigenous oral flora of man. The newborn to the 1 year old infant. Arch Oral Biol 1965;10;61-70
[2]. Wetzel WE, Schaumburg C, Ansari F, Kroeger T, Sziegoleit A. Microbial contamination of toothbrushes with different principles of filament anchoring.J Am Dent Assoc 2005;136:758-64.
[3]. Glasl B, Ludwig B, Schopf P: Prevalence and development of KIG-relevant symptoms in primary school students from Frankfurt am Main. J OrofacOrthop 2006, 67:414–423.
[4]. Arslan SG, Akpolat N, Kama JD, Özer T, Hamamci O. One-year follow-up of the effect of fixed orthodontic treatment on colonization by oral Candida. J Oral Pathol Med 2008, 37:26–9.
[5]. Hägg U, Kaveewatcharanont P, Samaranayake YH, Samaranayake LP: The effect of fixed orthodontic appliances on the oral carriage of Candida species and Enterobacteriaceae. Eur J Orthod 2004, 26:623–9.
[6]. Petti S, Barbato E, SimonettiD´Arca A: Effect of orthodontic therapy with fixed and removable appliances on oral microbiota: a six-month longitudinal study. New Microbiol 1997, 20:55–62.
[7]. Peros K, Mestrovic S, Anic-Milosevic S, Slaj M: Salivary microbial and nonmicrobial parameters in children with fixed orthodontic appliances. Angle Orthod 2011; 81; 901–6.
[8]. Thienpont V, Dermaut LR, van Maele G: Comparative study of 2 electric and 2 manual toothbrushes in patients with fixed orthodontic appliances. Am J OrthodDentofacialOrthop 2001; 120; 353–60.
[9]. Wheeler TT, McGorray SP, Yurkiewicz L, Keeling SD, King GJOrthodontic treatment demand and need in third and fourth grade schoolchildren:Am J OrthodDentofacialOrthop. 1994 Jul; 106(1):22-33.
[10]. Dayoub MB, Rusilko D, Gross A. Microbial contamination of toothbrushes. J Dent Res 1977;56:706
[11]. Kupietzky A, Majumdar AK, Shey Z, Binder R, Matheson PB: Colony forming unit levels of salivary Lactobacilli and Streptococcus mutans in orthodontic patients. J ClinPediatr Dent 2005, 30:51–53.
[12]. Caudry SD, Klitorinos A, Chan ECS. Contaminated toothbrushes and their disinfection. J Can Dent Assoc 1995; 61:511-16.
[13]. Malmberg E, Birkhed D, Norvenious G, Noren JG, Dahlen G:Microorganisms on toothbrushes at day-care centers.ActaOdontolScand, 1994 Apr; 52(2); 93-8.
[14]. Bhat PK, Badiyani BK, Sarkar S, Sandhya,Bhaskar NN. Effectiveness of antimicrobial solution on streptococcus mutans in used toothbrushes. World journal of Dentistry, 2012; 3(1): 6-10.
[15]. Johanna Eichenauer, Julia von Bremen and Sabine Ruf.Microbial contamination of toothbrushes during treatment with multibracket appliances. licenseeBioMed Central Ltd, Head & Face Medicine 2014; 10; 43; 1-7.

Tables/Graphs:

Table I: Dropout Reasons

Reason	**N**
Use of antibiotics	2
Return of brush ≥24h late	3
Total	**5**

Table II: Number and gender of subjects in two experimental groups

Gender	**Male**	**Female**	**Total**
	n	N	**n**
Regular soft bristle design (Group A)	10	15	**25**
Orthodontic bristle design (Group B)	9	17	**26**
Total	**19(37%)**	**32 (63%)**	**51**

Table III:CFU of S.Mutans and Lactobacillus between the brushes

Design		N	Median	Min.	Max.	Mann-Whitney U	'p' value
Streptococcus mutants TYS	Orthodontic bristle (Group B)	26	3	2	552	132	***<0.001***
	Regular soft bristle(Group A)	25	104	4	604		
Lactobacillus MRS Agar	Orthodontic bristle (Group B)	26	19	2	480	156	***0.001***
	Regular soft bristle(Group A)	25	328	1	520		

Table IV:CFU of S.Mutans and Lactobacillus among brushes

Design		N	Median	Min.	Max.	Mann-Whitney U	'p' value
Orthodontic bristle(Grp B)	Streptococcus mutants TYS	26	3	2	552	291	0.385
	Lactobacillus MRS Agar	26	19	2	480		
Regular soft bristle (Grp A)	Streptococcus mutants TYS	25	104	4	604	228	0.101
	Lactobacillus MRS Agar	25	328	1	520		