Comparative evaluation of plaque removal efficacy and quantitative analysis of *Streptococcus mutans* in saliva after using nylon and charcoal bristles toothbrushes in children: A clinico-microbiological study

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Abstract

Background: Dental biofilms are the primary aetiologic factors in the 2 most prevalent oral diseases, dental caries and periodontal disease. Among all oral hygiene methods available, mechanical plaque removal with a toothbrush is the primary mode of maintaining good oral hygiene.

Objectives: Comparative evaluation of plaque removal efficacy and quantitative analysis of *Streptococcus mutans* in saliva after using nylon and charcoal bristles toothbrushes in children.

Method: Forty healthy children aged 8-10 years having decayed-missing-filled-tooth (DMFT) score less than 5 were randomly allocated into two groups, one using charcoal bristles and the other using nylon bristles toothbrush. Loe & Silness Plaque Index was recorded along with saliva sample collection for *Streptococcus mutans* count before and after using the respective toothbrushes for 1 week.

Results: A significant reduction in plaque index was observed in each group with a non-significant reduction in *Streptococcus mutans* CFU. However, the comparison between the two groups was not statistically significant.

Conclusions: This randomised, parallel, single-blinded clinical study demonstrated that both nylon and charcoal bristles toothbrushes significantly enhanced plaque control but there was no significant difference in the two methods. There was no significant reduction in *Streptococcus mutans* CFU.

(Key words: Toothbrush, Microbiological, Dental plaque, Comparative study, *Streptococcus mutans*).

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Introduction

Dental biofilm is the main causative factor for the two common diseases of the oral cavity, dental caries and periodontal disease[1]. Dental caries affects 60% of children aged 5 to 17 years worldwide[2]. Children contracting the disease at a young age were more likely to develop caries and periodontal disease later in life[3]. The chemo-parasitic theory by WD Miller, suggesting that the acids produced by bacteria on the tooth initially demineralize the tooth enamel, is largely acknowledged[4]. *Streptococcus mutans* is a potent cariogenic bacterium, according to various studies[5]. Salivary levels of *S. mutans* were positively associated with their proportion in dental plaque and number of colonised tooth-sites[6]. Dental plaque was defined by World Health Organisation in 1961 as "a specific but highly variable structural entity, resulting from the sequential colonization of microorganisms on tooth surfaces, restorations and other parts of the oral cavity and composed of salivary components like mucin, desquamated epithelial cells, debris and microorganisms, all embedded in an extracellular gelatinous matrix"[7]. It has also been understood that dental plaque can lower the pH at the enamel surface that can dissolve the hydroxyapatite crystals and induce caries[8]. The principal way to maintain good oral hygiene is mechanical plaque removal by toothbrush because it is readily available, simple to use, and inexpensive[9].

The toothbrush was invented in China around 1000 AD and toothbrush technology has been improving in a beneficial way to enhance oral hygiene[10]. Recently launched in the market are charcoal toothbrushes, developed in Southeast Asian nations. Their black nylon bristles are infused with binchotan charcoal[11]. Activated charcoal absorbs and binds bacteria at the microscopic level and lessens halitosis. It is suggested that the activated charcoal in the toothbrush's bristles will absorb the

tannins that cause teeth staining[12]. Thus, the producers of the toothbrush assert that adding charcoal to nylon bristles can lessen halitosis, plaque, stains and also kill bacteria by raising the pH level[11,13]. Many studies have compared the efficacy of designs and composition of various toothbrushes on plaque removal and antimicrobial properties. Yet there is scanty literature regarding evaluation of the plaque removal efficacy of charcoal and its specific effect on salivary *Streptococcus mutans*.

Objectives

To assess plaque removal efficacy using Loe & Silness Plaque index and reduction of the salivary *Streptococcus mutans* count after using nylon and charcoal bristle toothbrushes in two different groups of children and finally compare the results for both groups.

Method

The study design was prospective, interventional, with single blinding. The trial period was from 28/09/2022 – 19/12/2022.

Sample size was estimated using the mean and standard deviation values based on the literature by Durhan et al., 2018 to be 13 per group using the following formula[14]:

$$n = \frac{2\,(Z_\alpha + Z_\beta)^2\ [s]^2}{d^2}$$

where Zα is the z variant of alpha error i.e., a constant with value 1.96 and Zβ is the z variant of beta error i.e., a constant with value 0.84. However, to avoid loss by attrition, the study was finally conducted on 40 healthy students of a residential school in Sangli, staying in a common accommodation and aged 8-10 years with decayed-missing-filled-tooth (DMFT) score less than 5. Figure 1 shows the CONSORT flowchart.

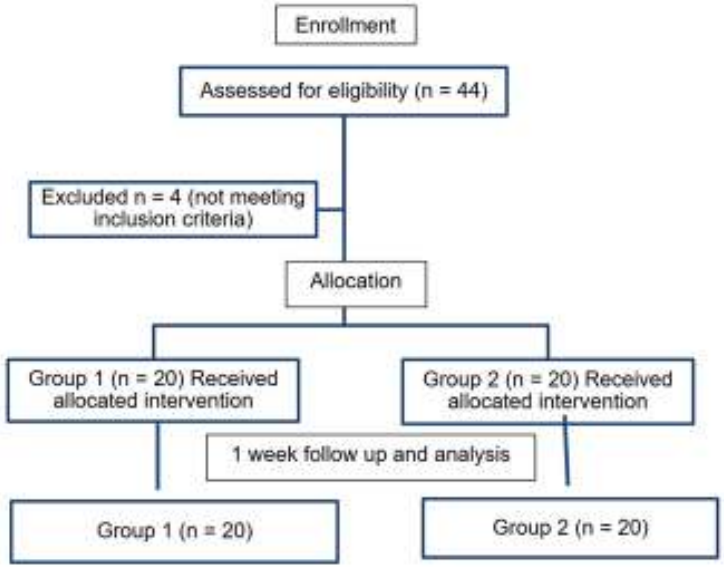


Figure 1: ***CONSORT Flowchart for the present study***

The selected children were then randomly allocated into two groups by tossing a coin –

- Group 1: 20 children (10 boys and 10 girls), mean age 102.8±16.2 months using nylon bristles toothbrush.
- Group 2: 20 children (10 boys and 10 girls), mean age 103.2±15.7 months using charcoal bristles toothbrush.

The investigator was blinded and Loe & Silness Plaque Index was recorded on the index teeth - 16, 52, 64, 36, 32, 84 (Table 1).

Table 1: Loe & Silness plaque index scoring criteria[15]

Grade	Inference
0	No plaque
1	Thin plaque layer at the gingival margin, only detectable by scraping with an explorer.
2	Moderate layer of plaque along the gingival margin; interdental spaces free from plaque, but plaque is visible to the naked eye.
3	Abundant plaque along the gingival margin; interdental spaces filled with plaque.

Whole saliva was collected for *Streptococcus mutans* count before intervention. Child was asked to spit saliva into an empty sterile conical flask; 0.5 mL of saliva sample was transferred in reduced transport fluid. Each group was instructed about modified bass brushing technique and instructed to brush with similar looking nylon bristles and charcoal bristles toothbrushes (Bamboo India, India) respectively twice a day for one week by the assistant. Recording of Loe & Sillness Plaque Index along with whole saliva sample collection was done again at the end of one week. All the children were provided with the same toothpaste by the investigator along with the toothbrushes for the study.

Saliva sample processing: Saliva sample was sent to Central Research Laboratory, Maratha Mandal's Nathajirao G. Halgekar Institute of Dental Sciences and Research Centre, Belagavi, where after centrifugation and removal of the supernatant, culturing was done on blood agar which is an enriched medium and Mitis salivarius agar which is a selective medium for *Streptococcus mutans*. The plates were incubated in carbon dioxide jar for 2-3 days at 37°C and checked for the growth of *Streptococcus mutans* and subsequently colony counts were done.

Ethical issues: Study was approved by the Institutional Ethics Committee of Bharathi Vidyapeeth (Deemed to be University) Medical College and Hospital, Sangli [No. BV(DU) MC&H/Sangli/IEC/Dissertation2020-21/ D-40] on 02/03/2021. The study was registered with Clinical Trials Registry, India (No. CTRI/ 2022/ 04/ 042279). Informed written consent was obtained from the guardians and informed assent from the children.

Statistical analysis: Data obtained were analysed using Statistical Package for Social Sciences version 26. Normality of numerical data was checked using Shapiro-Wilk test; as the data did not follow a normal curve; non-parametric tests were used for comparisons. Inter-group comparison was done using Mann Whitney U test. Intra-group comparison was done using Wilcoxon Signed rank test. For all statistical tests, $p < 0.05$ was considered significant, keeping α error at 5%, β error at 20% and power of the study as 80%.

Results

Table 2 shows the comparison of data collected regarding plaque index and *Streptococcus mutans* count pre- and post-intervention in the group using nylon bristle toothbrush. The mean plaque index reduced from 1.307 to 0.898 which was statistically significant ($p<0.01$). A reduction from 71.15 to 69.75 in colony forming units (CFU) of *Streptococcus mutans* was also seen but this difference was not statistically significant ($p>0.05$).

Table 2: ***Comparison of pre- versus post-intervention values in group 1 (Nylon bristle toothbrush) for plaque index (PI) and S. mutans colony forming units (CFU)***

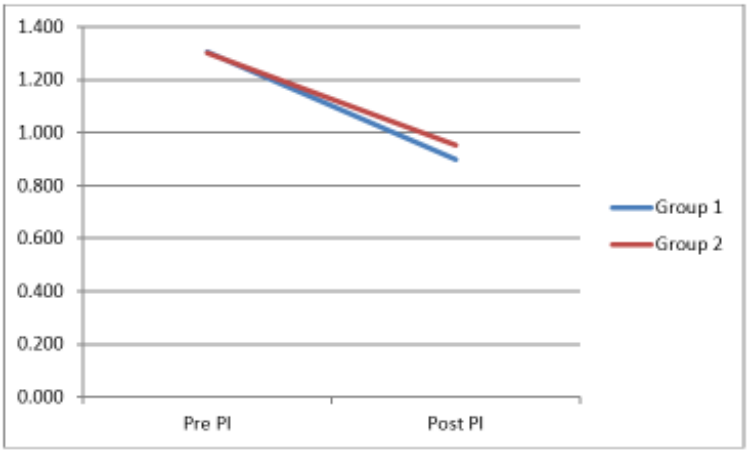
Group 1	Mean	Standard deviation	Mean difference	Standard deviation of difference	Z-value	p-value Wilcoxon signed-rank test
Pre-PI	1.307	0.14	0.4090000	0.2745504	-3.923	0.000
Post-PI	0.898	0.28				
Pre-CFU	71.15	39.39	1.400	17.569	-0.026	0.979
Post-CFU	69.75	34.51				

Table 3 shows the comparison of data collected regarding plaque index and *Streptococcus mutans* count pre- and post-intervention in the group using charcoal bristle toothbrush. The mean plaque index declined from 1.300 to 0.954 which was statistically significant ($p<0.01$). A reduction from 93.15 to 83.15 in colony forming units of *Streptococcus mutans* was also seen but this difference was not statistically significant ($p> 0.05$).

Table 3: ***Comparison of pre- versus post-intervention values in group 2 (Charcoal bristle toothbrush) for plaque index (PI) and S. mutans colony forming units (CFU)***

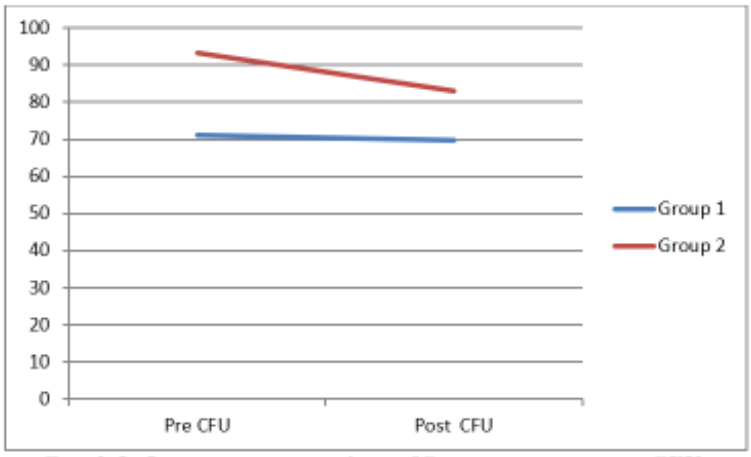
Group 2	Mean	Standard deviation	Mean difference	Standard deviation of difference	Z-value	p-value Wilcoxon signed-rank test
Pre-PI	1.300	0.1373049	0.3465000	0.1967706	-3.921	0.000
Post-PI	0.954	0.1663628				
Pre-CFU	93.15	64.721	10.000	31.389	-1.416	0.157
Post-CFU	83.15	55.596				

Graph 1 shows the reduction of plaque index (PI) after using both toothbrushes. Group 1 (Nylon bristle toothbrush) shows higher degree of reduction as compared to group 2 (Charcoal bristle toothbrush) which is represented by the steeper slope of the line depicting the difference of pre- and post-intervention PI for group 1.



Graph 1: ***Intra group comparison of Plaque Index***

Graph 2 shows the decline in *Streptococcus mutans* CFU after using both the toothbrushes. Group 2 (Charcoal bristle toothbrush) shows higher degree of reduction as shown by its steeper slope of the line depicting the difference of pre and post intervention plaque index when compared to Group 1 (Nylon bristle toothbrush).



Graph 2: ***Intra group comparison of Streptococcus mutans CFU***

Table 4 shows the comparisons between both groups in relation to plaque index and *Streptococcus mutans* CFU and also their differences. The table shows non-significant difference between both groups for all criteria as 'p'-value for all the comparisons is more than 0.05.

Table 4: ***Inter group comparison of the plaque index (PI) and S. mutans colony forming units (CFU)***

	Group	Sample size	Mean	Standard Deviation	Mann-Whitney U value	'Z' value	'p' value of Mann-Whitney U test
Pre -PI	1 2	20 20	1.307000 1.300000	0.1385679 0.1373049	195.000	-0.137	0.891
Post- PI	1 2	20 20	0.898000 0.953500	0.2767785 0.1663628	181.500	-0.505	0.614
PI difference	1 2	20 20	0.409000 0.346500	0.2745504 0.1967706	179.500	-0.555	0.579
Pre-CFU	1 2	20 20	71.15 93.15	39.392 64.721	169.000	-0.839	0.401
Post -CFU	1 2	20 20	69.75 83.15	34.513 55.596	181.000	-0.515	0.606
CFU difference	1 2	20 20	11.60 15.30	13.004 29.054	198.000	-0.054	0.957

Discussion

Plaque is the primary aetiologic agent in the initiation and mediation of dental caries and periodontal infections as it harbours the causative microorganisms[16]. As a means of plaque removal, the toothbrush is extensively used by the public thus helping to reduce the microbial load[17]. Since the introduction of simple toothbrushes with nylon bristles and straight plastic handles in the 1930s, the modern toothbrush has evolved to include new materials, different bristle shapes and stiffness, tuft arrangements and novel handle designs, all aiming at improving plaque removal and oral health[18]. Charcoal toothbrush, a newer variety of toothbrush, is currently being assessed for its efficiency in plaque removal, due to addition of charcoal which may provide additional benefits[18].

The present study was conducted to compare the effect of nylon and charcoal bristle toothbrushes on removal of dental plaque and reducing *Streptococcus mutans* count. Patil SP, *et al*[19] had shown significantly higher plaque removal efficacy with the modified Bass technique in 6-8-year-old children[19]. Regarding toothbrushing programmes, it has been suggested that once a habit is established, classmates could supervise each other to brush[20]. It has also been shown that without re-instruction and positive reinforcement, the novelty of the instrument and the compliance to the brushing instructions and protocol diminish rapidly[16]. Hence, residential school setup was found appropriate for the conduction of the study so that a healthy oral habit could also be inculcated in children through it. The cost of toothbrushes and toothpaste may also be sometimes a potential barrier to regular toothbrushing habits[20]. Hence, it was more practical to provide fluoridated toothpaste along with the toothbrushes under study at school through the programme directly. This added to the standardization of the study protocol.

Dental caries is a complex process and microbes play a vital role. Conventional culture has long been employed for detection and quantification of microbes. Strong positive numerical association was noted between caries experience and *Streptococci* levels in Indian populations[5]. Pannu P, *et al*[6] reported a positive correlation between the concentration of *S. mutans* in saliva and dental caries. Hence, in the present study, caries experience was assessed using the DMFT index to fulfill the inclusion criteria as a standardization of the caries experience of the participating subjects and saliva samples were used to evaluate *Streptococcus mutans* CFUs. For the purpose of evaluating the reduction of the *S. mutans* counts in respective groups for intergroup comparisons, the mean reduction scores were calculated for each group. A reduction from pre interventional CFU count of 71.15×10^3/ml to 69.75×10^3/ml of *Streptococcus mutans* was seen in the group using nylon bristle toothbrush while a reduction from pre interventional CFU count of 93.15×10^3/ml to 83.15×10^3/ml for the same was seen in the group using charcoal bristle toothbrush. Although a reduction in *Streptococcus mutans* CFUs was observed in both groups, the difference was not statistically significant, neither was the comparison between both groups.

The results of the present study agree with the study by Sharma M, *et al*[4] which showed the minimal effect of toothbrushing with a dentifrice on *Streptococcus mutans* count in plaque samples. However, in contrast, another study found a statistically significant difference in the number of CFU count assessed before and after brushing using active ionic toothbrush[16]. The minimum reduction in *Streptococcus mutans* CFUs following toothbrushing in the present study could be due to the factor studied by Svanberg (1978) according to which sometimes toothbrushes are shown to be heavily infected by *S. mutans*, which may lead to the spread of these and other organisms from one site to another[4]. This is in support of a greater reduction observed in the charcoal bristle toothbrush as compared to nylon bristle toothbrush due to the antimicrobial property of charcoal bristles as shown by Lee J *et al*[21]. The study by Bhat DS, *et al*[22] also concluded that there was a substantial reduction in the number of CFUs in charcoal toothbrushes when compared to non-charcoal toothbrushes after one week of usage.

Our one week single-blind study also focused on the comparative efficacy of nylon and charcoal toothbrushes in plaque control[18]. The amount of dental plaque at baseline and one week was assessed using Loe and Silness plaque index as recorded by Agarwal A, *et al*[15] in mixed dentition. Findings of our study showed a statistically significant reduction of plaque scores over a one-week period for both groups. The mean plaque index reduced from 1.307 to 0.898 in group 1 (Nylon bristle toothbrush) and from 1.300 to 0.954 in group 2 (Charcoal bristle toothbrush). However, the comparative analysis for plaque index reduction between the two groups was not statistically significant which agrees with the study by Prusty AK, *et al*[16] who concluded in their 12-week clinical trial on 19–21-year-old subjects that manual, powered and charcoal toothbrushes were all clinically effective in removing plaque, no toothbrush being superior to the other. However, a study by Kaur S[17] on 17-25-year-old subjects concluded after 15 days that charcoal toothbrush was more effective in plaque removal. On the other hand, a study by Bhat DS *et al*[22] on 23-25-year-old subjects to compare the efficacy of charcoal and

conventional toothbrushes to control plaque and maintain periodontal health, concluded that while charcoal toothbrushes are useful in improving periodontal health, conventional toothbrushes are more effective in removing plaque than charcoal toothbrushes. Kini V, *et al*[23] showed increased plaque removal efficacy of nylon bristle toothbrush over charcoal bristle toothbrush on subjects aged 18-25 years from baseline to 3 weeks. However, from 3 weeks to 6 weeks, the plaque removal efficacy of nylon bristle reduced as compared to charcoal bristle toothbrush due to increased wear index of nylon bristle toothbrush. The plaque reduction in our study can be attributed to the mechanism of action of brushes and the natural cleansing effect of charcoal. This non-significant difference in plaque reduction between nylon and charcoal brushes could be due to the synergistic effect of charcoal added to the bristles of the straight handle brush which had a natural cleaning effect along with mechanical cleaning to pull away plaque from tooth surfaces. The reason for the same could be familiarisation and equal motivation of all the children with the brushing techniques and the brushes they were using in this study[16]. The decrease in plaque score is attributed to the fact that blending charcoal into nylon bristles can reduce plaque, halitosis (as charcoal has adsorptive and non-toxic properties). It also kills bacteria developing in the bristles during storage, reducing bacterial contamination of toothbrushes, according to the manufacturers of these toothbrushes[11].

One limitation of the study is the relatively small sample size. A larger sample size and longer duration is recommended for more accurate clinical results.

Conclusions

This randomised, parallel, single-blinded clinical study demonstrated that both nylon and charcoal bristles toothbrushes significantly enhanced plaque control but there was no significant difference in the two methods. There was no significant reduction in *Streptococcus mutans* CFU.

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