

# Antimicrobial Efficacy of Charcoal vs. Non-charcoal Toothbrushes: A Randomized Controlled Study

Reema Zaid AlDhawi<sup>1</sup>, Najla Hamad AlNaqa<sup>1</sup>, Oula Esam Tashkandi<sup>1</sup>, Ahmed Tawfiq Gamal<sup>2</sup>, Haifa Fahad AlShammery<sup>3</sup>, Samar Mohammad Eltom<sup>4</sup>

<sup>1</sup>Dental Intern at Riyadh Elm University, <sup>2</sup>Assistant Professor, Preventive Dentistry Department at Riyadh Elm University, <sup>3</sup>Lecturer in Microbiology at Riyadh Elm University, <sup>4</sup>Laboratory Specialist in Microbiology at Riyadh Elm University, Riyadh, Saudi Arabia

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ABSTRACT

**Aim:** To assess the efficacy of the antimicrobial properties of charcoal vs. non-charcoal toothbrushes and the level of bacterial contamination in the oral cavity using a charcoal toothbrush. **Materials and Methods:** This was a randomized, double-blind controlled study in which both male and female subjects aged from 18 to 35 were included ( $n = 30$ ; 15 males and 15 females). The subjects were selected from (students) of Riyadh Elm University in Riyadh, Saudi Arabia. Subjects were informed about the study and signed the consent form before participation. From January to April 2019, subjects were given charcoal and non-charcoal toothbrushes. **Results:** The Wilcoxon signed rank test showed that there was a significant difference in bacterial counts between non-charcoal and charcoal toothbrushes ( $P = 0.000$ ). Of the subjects, 70% showed a decrease in the number of bacterial counts while 30% showed no increase in bacterial counts. There was a statistically significant decrease in the number of bacteria in the gingival crevicular fluid (GCF) with charcoal treatment ( $P < 0.001$ ). Of the subjects, 96.6% showed a decrease in the count of bacteria in GCF after using a charcoal toothbrush. Only 3.3% of the subjects had the similar counts of bacteria in GCF after using the charcoal toothbrush. **Conclusion:** The study demonstrates that charcoal toothbrushes reduce bacterial contamination and the poor effects on oral health after 1 week of use.

**KEYWORDS:** Antibacterial, charcoal, contamination, oral health, toothbrush

## INTRODUCTION

Dental plaque is a gentle tissue that accumulates on tooth surface, and it is the most common etiological factor in developing various oral diseases.<sup>[1,2]</sup>

Maintaining good oral hygiene is an important act by cleaning the oral cavity; it can be achieved with various ways, of which toothbrush is commonly used.<sup>[3]</sup>

Toothbrushes are essential to keeping the oral cavity clean and healthy. However, they can increase the risk of infection and disease transmission if not cleaned and disinfected correctly.<sup>[4]</sup> Contamination of toothbrushes is inevitable due to the presence of plaque and microorganisms in the oral cavity or the presence of bacteria in the environment and other factors.<sup>[5]</sup> Toothbrushes can be contaminated

by bacteria, yeasts, viruses, and fungi after even a single use for 30s to 4min. These bacteria are present in the oral cavity and the external environment.<sup>[6]</sup> They can accumulate and will survive on the toothbrush causing disease when transmitted to an individual.<sup>[4]</sup>

There are many ways to reduce bacterial contamination whether by disinfecting toothbrushes in mouthwashes such as chlorhexidine or covering the toothbrush to isolate it from the surrounding environment.<sup>[7]</sup> Most studies show a degree of effectiveness when immersing the toothbrush in some kind of disinfectant material.<sup>[8]</sup>

**Address for correspondence:** Dr. Najla Hamad Alnaqa, Riyadh Elm university, An Namuthajiyah campus, Riyadh, Saudi Arabia.  
E-mail: Najlanhs@gmail.com

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Instead of the standard nylon/synthetic bristles, which simply sweeps and captures debris and bacteria, the bristles are infused with charcoal.<sup>[8]</sup> When the charcoal is embedded in these new brushes, the manufacturers claim that the blending of charcoal into the nylon bristles can prevent or reduce the amount of contamination, reduce halitosis, and kill bacteria due to antimicrobial properties.<sup>[8]</sup> However, no scientific evidence has yet been provided to back or support these claims. Our study aims to assess the efficacy of the antimicrobial properties and the level of bacterial contamination in the oral cavity using charcoal toothbrushes in comparison with non-charcoal toothbrushes. Microorganisms can adhere to and survive on toothbrushes, especially if the toothbrush is misused.<sup>[9]</sup> These microbes can be transmitted to the user and lead to diseases or infections.<sup>[9]</sup>

## MATERIALS AND METHODS

The study was conducted in Riyadh Elm University (REU) in Riyadh, Saudi Arabia, and was registered in the research center and received ethical approval from the institutional review board of REU number (RC/IRB/2018/1632).

The study included 30 male and female subjects who brushed manually using a modified bass technique. The subjects ranged between 18 and 35 years of age. They were selected randomly and were informed about the study. A signed consent form was obtained before participation from January to April 2019. The goal was to study the antimicrobial efficacy of charcoal vs. non-charcoal toothbrushes and to assess whether less contaminated toothbrushes enhance oral health.

We excluded patients with active carious lesions, plaque index scores more than 2, gingival index score more than 2, periodontitis cases, throat infections, irregular brushing frequency, those unwilling to use a charcoal toothbrush, those who use mouthwash and/or antibacterial toothpastes, pregnant women, smokers, and subjects who received any periodontal treatment in the last 3 months or those medically compromised including those taking medication that effects the oral cavity health.

Sixty charcoal toothbrushes were obtained with superfine <0.01 mm slim tips and black bristles infused with charcoal (manufactured in India). Non-charcoal controls were similarly obtained and were distributed in two separate envelopes. Each envelope was enumerated as group 1 and group 2 with the investigators blinded to the brush type. The envelopes were distributed randomly by a participant who was not involved in the experiment. The samples were then collected from the participating subjects and were allocated to produce a 1:1 ratio between the test group and the control group. Gingival

crevicular fluid (GCF) was collected from the subjects prior to starting the experiment to establish the bacterial count baseline. The subjects were then asked to choose a toothbrush between group 1 or group 2 and use it for 1 week [Figure 1]. The used toothbrushes were returned in sterile pouches, and gingival crevicular fluid was collected. The subjects were asked to use the other type of the toothbrush for 1 week. Finally, all subjects returned the used toothbrushes after the second week, and GCF was collected again. A total of 150 samples were obtained. Each investigator was responsible for taking samples from the same 10 subjects every week. The GCF samples were collected from the sulcus of the Ramfjord teeth using subgingival paper points size 25, 30, and 35 (10s) for each tooth. Samples were placed in nutrient broth immediately [Figure 2]; 0.1 mL of GCF was then inoculated using a micropipette and spread with sterilized cotton swabs on blood agar plates. Colony-forming units (CFU) were measured after 24h of incubation.

Two-thirds of the bristles from the used toothbrushes were sectioned using a number 11 blade and placed in 9 mL of saline [Figure 3]. Next, 0.1 mL of saline was inoculated using a micropipette and spread with sterilized cotton swabs on blood agar plates. CFU were measured after 24h of incubation. A turbidity test was done for the GCF samples before inoculating the samples into the cultures, and there was a significant difference between using charcoal and non-charcoal toothbrushes visually after 1 week of use. No side effects were noted after completion of the study.

## STATISTICAL ANALYSIS

A Shapiro test evaluated the normality of the data. The results showed that the data were not normally distributed ( $P < 0.001$ ). A nonparametric test was used in this analysis. The Wilcoxon signed rank test was then conducted to test the change in numbers of bacteria in a toothbrush and differences in bacteria numbers between toothbrushes.

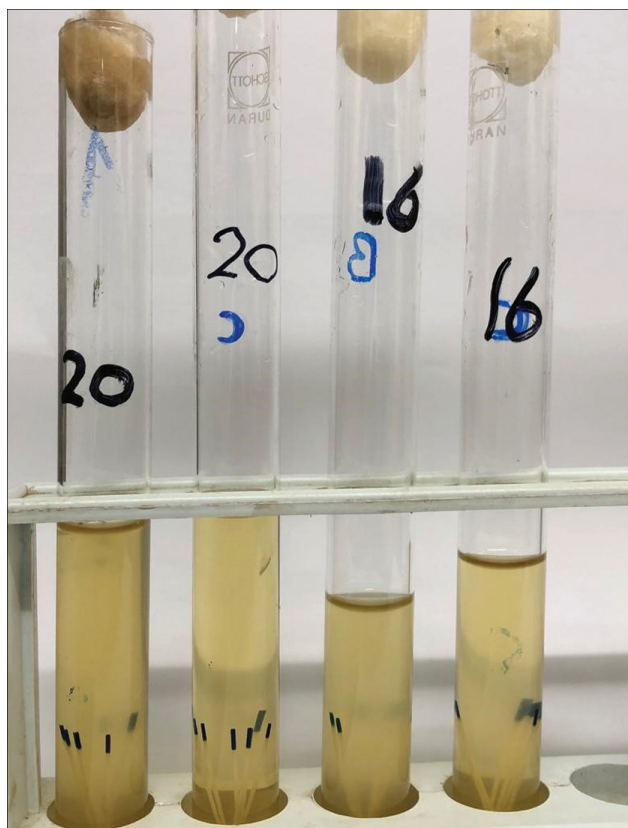


**Figure 1:** Charcoal and non-charcoal toothbrushes in sterile pouches

## RESULTS

### TOOTH BRUSH ANALYSIS

The Wilcoxon signed rank test showed that there was a significant difference in numbers of bacteria between non-charcoal and charcoal toothbrushes [ $P < 0.001$ ; Figure 4].



**Figure 2:** Paper points containing gingival crevicular fluid submerged in nutrient broth



**Figure 3:** Two-thirds of the bristles from each of the used toothbrushes were sectioned using a number 11 blade, collected in a sterile petri dish, and placed in 9mL of saline; 0.1 mL was inoculated on blood agar plates using a micropipette and sterilized cotton swabs

Negative rank means that the bacteria counts decreased in charcoal toothbrushes vs. non-charcoal toothbrushes for the same subject. These results were achieved for 21 participants.

Positive rank means no increase in bacteria counts after using charcoal toothbrushes (no subjects showed an increase in bacteria in his/her toothbrush after using charcoal vs. non-charcoal toothbrushes). Ties mean that nine subjects had the same bacteria count in each type of toothbrush (non-charcoal)/(charcoal).

### GCF ANALYSIS

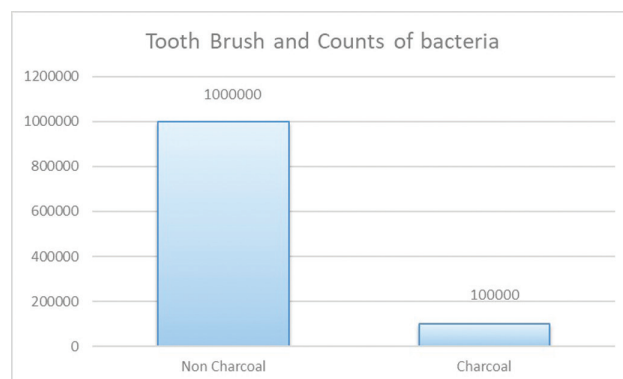
The Wilcoxon signed rank test showed that there was a statistically significant decrease in the bacteria present in GCF from charcoal brushes ( $P < 0.001$ ) [Figure 5]

Here, negative rank means that bacteria counts decreased in GCF after using charcoal toothbrushes for the same subject. These results were seen in 29 subjects. Positive rank means no increase in counts of bacteria after using charcoal toothbrushes (no subjects showed an increase in bacteria in GCF after using charcoal toothbrushes). Ties mean that only one subject had the same bacteria count in GCF after using charcoal toothbrushes.

### COMPARING BETWEEN GENDER

An independent sample median test was used to test the median of bacterial counts in charcoal toothbrushes between genders. The results shown in [Figure 6] showed no significant difference in bacterial counts in charcoal toothbrushes between male and female subjects ( $P = 0.33$ ).

An independent sample median test was used to test the median of bacterial counts in GCF after using charcoal toothbrushes between male and female subjects. The results showed that there was a significant difference in bacterial counts in GCF after using the charcoal toothbrushes between male and female subjects ( $P = 0.002$ ).



**Figure 4:** Toothbrush analysis



## DISCUSSION

Bacterial contamination of toothbrushes can be avoided by properly disinfecting them. Although toothbrush disinfection should be done regularly, studies show patient noncompliance seeing as they require extra time and effort.<sup>[10]</sup> An extensive search of the relevant literature was conducted and yielded few published research projects on this subject. In our study, we managed to confirm the antimicrobial efficacy of charcoal toothbrushes in comparison with non-charcoal brushes.

The charcoal and non-charcoal toothbrushes were compared here for bacterial contamination after 7 days of use.<sup>[11]</sup> Toothbrushes are usually stored in the bathroom where they can be exposed to enteric bacteria dispersed by aerosols. Aerosols from the toilet can release millions of bacteria in the atmosphere.<sup>[11,12]</sup> Here, subjects were included and instructed to keep the toothbrushes 2 feet away from the toilet.

Toothbrush contamination has been considered a means of microbial transport, growth, and retention. This may be because the person's reinfection with pathogenic bacteria can be an environmental reservoir of microorganisms.<sup>[13]</sup> Tooth brushing with a contaminated bristle introduces new organisms while

concurrently reducing existing normal flora. The area of the toothbrush in which the tufts are attached is prone to contamination. Food debris and fluids can accumulate in the space between the tufts by capillary action, which leads to bacterial growth.<sup>[13]</sup> Prevention of toothbrush contamination is achieved by not sharing them and not storing them covered in a damp environment. The toothbrush must be in an upright position to allow it to dry. Finally, the toothbrush should be replaced every 3–4 months or sooner if the bristles appear worn or damaged.

Karibasappa recommended ways to preserve the toothbrush, including antimicrobial solutions, toothbrush sanitizer, or natural air drying. Commercially available antimicrobial solutions include 1% sodium hypochlorite, 0.2% chlorhexidine, 3% hydrogen peroxide, dettolin, and 2% triclosan.<sup>[14]</sup> Many studies confirm that chlorhexidine solution is considered the “gold standard antimicrobial agent” in comparison to other solutions.<sup>[15-17]</sup>

The results revealed that substantially lower CFU counts in the blood agar plates were found in charcoal bristles compared with non-charcoal bristles. This is compatible with Mitali Vilas Thamke study which showed the number of CFUs in charcoal toothbrushes was considerably less when comparing with non-charcoal toothbrushes after 1 week of usage.<sup>[10]</sup> The default null-hypothesis of no difference in the CFU between the charcoal toothbrush and non-charcoal toothbrush was rejected as the findings of this study showed significantly lower CFU counts in blood agar plates in the GCF with a charcoal toothbrush compared to the non-charcoal toothbrush.

As a consequence, charcoal-infused toothbrushes have less bacterial contamination which promotes oral

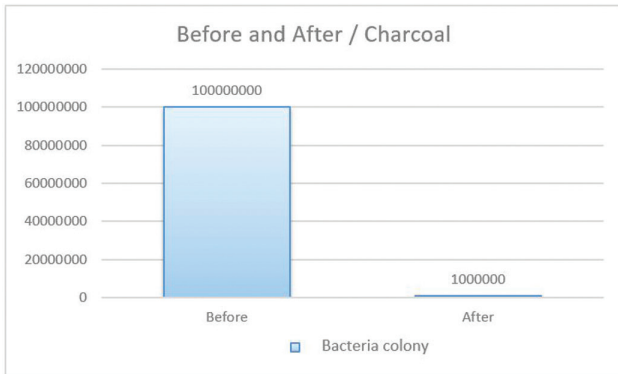


Figure 5: GCF analysis

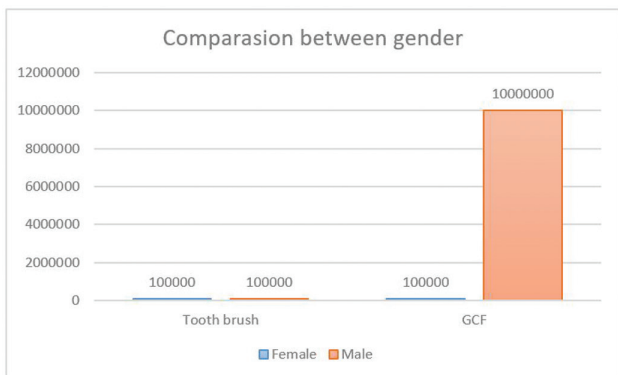


Figure 6: Comparing between gender

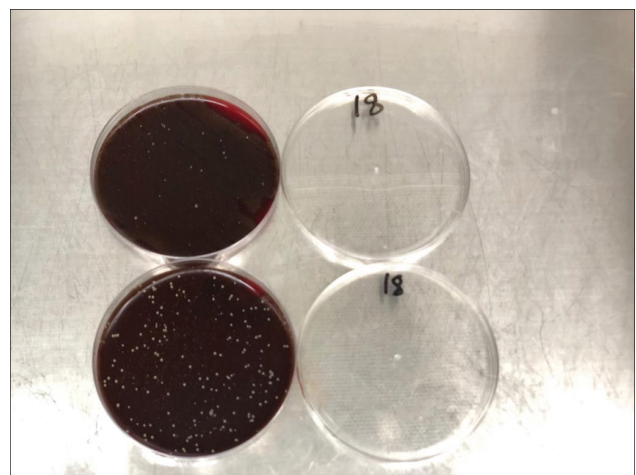


Figure 7: Comparison of bacterial growth after using charcoal and non-charcoal toothbrushes for 1 week

health. So, it is recommended to decontaminate non-charcoal toothbrushes using disinfecting agents such as chlorohexidine routinely.<sup>[18]</sup>

#### LIMITATIONS

The antimicrobial property of charcoal toothbrushes was compared with non-charcoal toothbrushes on 30 subjects in 1 week. Further studies can be carried out in comparing charcoal toothbrush with non-charcoal toothbrush with a larger sample size, longer duration, and analysis of the specific type of bacteria (aerobic/anaerobic). In addition, the manufacturers of charcoal toothbrushes have not provided sufficient information regarding the percentage of charcoal-infused in the bristles of the toothbrush.

#### CONCLUSION

Our results confirmed the antimicrobial properties of charcoal toothbrushes in reducing bacterial contamination for better oral health. After 1 week of charcoal brush usage, the number of CFUs in GCF was substantially less than non-charcoal toothbrushes [Figure 7]. Charcoal-infused tooth bristles are a new product to prevent bacterial contamination.

#### ACKNOWLEDGEMENT

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#### FINANCIAL SUPPORT AND SPONSORSHIP

No institutional funding was provided.

#### CONFLICT OF INTEREST

There are no conflicts of interest connected to the authors in regard to this article

#### AUTHORS CONTRIBUTIONS

Not applicable.

#### ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

Ethical approval from the institutional review board of REU number (RC/IRB/2018/1632).

#### PATIENT DECLARATION OF CONSENT

A signed consent form was obtained before participation from January to April 2019.

#### DATA AVAILABILITY STATEMENT

Not applicable.

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