Jorjani Biomedicine Journal. 2019; 7(1): P 22-30.

The preventive effects of Chlorhexidine mouth wash on alpha-hemolytic Streptococci and Staphylococcus aureus

Mohammad Hasan Akhavankarbasi[1], Fatemeh Owlia[1], Hengameh Zandi[2], Samira Hajimaghsoodi[1]*

1. Department of Oral Medicine, Faculty of Dentistry, Shahid Sadoughi University of Medical Science, Yazd, Iran.
2. Department of Medical Microbiology, Faculty of Medicine, Shahid Sadoughi University of Medical Science, Yazd, Iran

Article Type:

Original Article

Article History:

Received: 30 Jan. 2019
Revised: 30 Feb. 2019
Accepted: 02 Mar. 2019

Correspondence:

Samira Hajimaghsoodi, Department of Oral Medicine, Faculty of Dentistry, Shahid Sadoughi University of Medical Science, Yazd, Iran.

Dents1390@gmail.com

Abstract

Background and objectives: The association of oral micro-organisms with infectious diseases such as bacterial endocarditis is a concern in dentistry. Bacterial endocarditis is a serious disease with a mortality rate of 40%. The present study was conducted to investigate the effect of using chlorhexidine mouthwash on concentrations of some groups of bacteria in gingival sulcus, which is the main contact point of oral flora with circulating blood during scaling.

Methods: In this Randomized controlled clinical trial, participants were selected among referrals to the department of gingival diseases of Yazd dental school. Participants underwent determining plaque index and after scaling, sampling of the buccal and lingual gingival groove of mandibular central and lateral teeth was performed by paper with walking movement.

Results: participants were 53 patients who divided into two groups. Group A consisted of 27 patients (15 women and 12 men) ranging in age from 21 to 54 years mean of 35) who used 50 mL chlorhexidine mouthwash and Group B consisted of 26 patients (13 females and 13 males) ranging in age from 19 to 51 years old with a mean of 35.76 who used 50 mL normal saline before scaling. Study groups were not significantly different in terms of age, sex and plaque index. (P-values= 0.755, 0.348 and 0.708). Mann Whitney-Wilcoxon test compared the counted values of Streptococcus viridance and Staphylococcus aureus in control group was significantly higher than the study group. (P-value = 0.000).

Conclusion: according to the results of our study chlorhexidine mouthwash prior to scaling teeth significantly reduced Streptococcus viridance and Staphylococcus aureus colony counts and could be recommended as a complementary agent for antibiotic prophylaxis in high risk patients prone to endocarditis, or as the only agent in moderate risk cardiac patients that antibiotic prophylaxis does not been used.

Keywords: Infectious endocarditis; Bacteremia; Streptococcus viridance, Staphylococcus aureus; Chlorhexidine

Introduction

Mouth, is the most contaminated cavity of the body. It has been identified as a source of transmission of various infections and has been an issue should be care about to dentists for transferring bacteria during dental procedures (1).

The association of oral micro-organisms with infectious diseases such as bacterial endocarditis is the major concern should be care about. Bacterial endocarditis is a serious disease with a mortality rate of 40%. It requires simultaneous presence of predisposing cardiac conditions, and high levels of infectious agents in the blood (1). The incidence of this disease ranged from 1.5 to 11.6 per 100000 person years (2). An estimated 106 to 108bacteria per milliliter blood is reported to be able to establish infection in cardiac lesions. A mass result of animal studies have shown that the prevalence of infection in heart valve damage depends on the concentration of inoculated bacteria. Streptococcus and staphylococcus have accounted for approximately 80%ofinfectious endocarditis (IE) cases (3).

Therefore, dental treatments in the presence of certain heart conditions require measures to reduce bacteremia in these patients. According to the revised American Heart Association (AHA) guidelines, antibiotic prophylaxis is recommended in four groups of patients with heart problems by the administration of one dose of antibiotics taken orally or injected 1 hour before dental procedures(3). However, it has been shown that endocarditis prophylaxis cannot completely prevent bacterial endocarditis, therefore supplementary methods such as antimicrobial agents, or topical antibiotics are recommended to be used (4, 5).

The present study was conducted on a group of healthy patients undergoing tooth scaling. The aim was to investigate the effect of using chlorhexidine mouthwash on concentrations of this group of bacteria in gingival sulcus. Gingival sulcus is the main contact point of oral flora with circulating blood during scaling.

Materials and Methods

In this Randomized controlled clinical trial, participants were selected among referrals to the department of gingival diseases of Yazd dental school to be scaling of anterior mandibular teeth. Other inclusion criteria in this survey were maximum 3mm probing depth in the area and not to have history of diabetes, AIDs, pregnancy, consumption of antibiotics over the past 3 months and use of inhaled or oral steroids over the past month. The participants did not require prophylaxis antibiotics and did not have any active dental or oral infections to prevent changing the periodontal pathogens (6-8).

This double-blind Randomized clinical trial registered by code IRCT: 201311015233N1.all participants signed the informed consent. Ethical committee of Yazd Shahid Sadoughi University of medical science (IRSSU187722)

The participants underwent determining plaque index and were classified into one of the following four groups: plaques index of 0 to 25%, 26 to 50%, 51 to 75% and 76 to 100%. By using the random numbers table and considering the plaque index, individuals with survey inclusion criteria entered in one of the study or control group, respectively. It was done in a way that the number of people belonging to each plaque index range was similar between the groups. The exclusion criteria of the study were non-willingness to participate in the study. Ten minutes before scaling, each patient received 50 mL chlorhexidine 0.2% mouthwash or placebo (normal saline) for 1 minute.

Then patients underwent scaling of mandibular anterior teeth with ultrasonic device. After, sampling of the buccal and lingual gingival groove of mandibular central and lateral teeth was performed by paper cone by walking techniques. Each sample was placed into the test tube containing 3 mL TSB transport medium and within an hour after sampling transferred to microbiology laboratories. After mixing the sample with vortex, serial dilutions of 10-1 to 10-3were prepared and each dilution was inoculated in blood agar by 0.01 mL sampler.

plates were kept at temperature of 37°C for 24 hours and colonies suspected Staphylococcus aureus were determined by means of gram staining, catalase, mannitol and coagulase fermentation, and alpha-hemolytic streptococci were detected by gram staining, catalase, alpha-hemolysis production, lack of sensitivity to bacitracin and optochin, and lack of growth in Bile-esculin agar medium. Number of Staphylococcus aureus and alpha hemolytic Streptococcus colonies were counted and the colony counts in each ML of the primary sample volume CFU/ML) were determined, based on the dilution. Mann Whitney-Wilcoxon test has been used to compare the mean values of the two groups.

Considering the meaningful level of 5% and power of 80% and according to the results of similar studies (S=20), by using sample size formula, total number of 25 specimens were evaluated in each group.

Results

In this double-blinded randomized clinical study, participants were 53 patients who divided into two groups. Chlorhexidine group (A)composed of 27 patients (15 women and 12 men) with age range of 21 to 54 years who used 50 mL chlorhexidine mouthwash and normal saline group (B) composed of 26 patients (13 females and 13 males) with age range of 19 to 51 years who used 50 mL normal saline before scaling. The two groups were not statistically different in terms of age and sex (P-values = 0.755, and 0.348, respectively)

Rate of plaque index in group A was 56.89±30.65 with minimum and maximum of 14 and 100 and in group B was 53.80±28.83 with minimum and maximum of 12 and 100 and was not significantly different between the groups. (P-value= 0.708) (table1).

Table1.comparison of plaque index in two groups

Group	Mean ± SD	Min	Max
chlorhexidine	56.89±30.65	14	100
Normal saline	63.80±28.83	12	100

Mean and standard deviation alpha hemolytic Streptococcus colonies in group A was 29.59±12.75 (CI=95%) with minimum and maximum of 0 and 100 and in group B was 67.69±30.33 (CI=95%), with minimum and maximum of 0 and 100. (table2).

Table 2. Descriptive values of the *Streptococcus Viridans* colony count in two groups (CI = 95%)

Group	Mean ± SD	Median	Min	Max
chlorhexidine	29.59±12.75	16	0	100
Normal saline	67.69±30.33	70	0	100

Mean and standard deviation staphylococcus aureus colonies in group A was 2.92±5.96 (CI=95%) with minimum and maximum of 0 and 20 and in group B was 24.26±30.14 (CI=95%), with minimum and maximum of 0 and 100. (table3).

Table 3. Descriptive values of the *Staphylococcus Aureus* count in two groups CI = 95%)

Group	Mean ± SD	Median	Min	Max
chlorhexidine	2.92±5.96	0	0	20
Normal saline	24.26±30.14	14	0	100

This study compared the mean values of Streptococcus viridans and Staphylococcus aureus in groups A and B that showed the significant difference between two groups. (P-value = 0.000) (Table4).

Table4. Comparison of mean S. aureus and S.viridans colony counts between the two groups.

Test	Streptococcus viridians	Staphylococcus aureus
Mann-Whitney	155.5	172
Wilcoxon	533.5	550
Z	-3.501	-3.521
Asymp (Sig2-tailed)	0.000	0.000

Discussion

The purpose of this clinical trial study was to determine the effectiveness of chlorhexidine mouthwash on Streptococcus viridans and Staphylococcus aureus species in gingival sulcus of teeth undergoing scaling. According to the obtained results, the use of chlorhexidine 2%before scaling had a significant impact on the number of Streptococcus viridans and Staphylococcus aureus colonies at gingival sulcus. (P-value = 0.000)

Bacterial endocarditis is a devastating disease with a mortality rate of 40% has been a concern for dentists during dental procedures for high-risk patients. In a case control study by van der Meer et al antibiotic prophylaxis had 75% efficacy rate at best (3). According to epidemiological studies antibiotic prophylaxis appears to be safe and is likely to be cost-effective particularly in those at high risk (9,10). Oliver R et al in a Cochran database systematic review concluded no evidence about whether penicillin prophylaxis is effective (11).

Reports of cases with clear failure of prophylaxis, like those by Hall and colleagues, questioned the adequacy of this method (12). An extensive discussion exists in the literature on the antimicrobial effect of mouthwashes to help the process of prevention and alleviate these concerns, as the American Heart Association has recommended mouth was containing chlorhexidine or povidoneiodine in high risk groups (3).

On the contrary, the European Cardiology Society has expressed no difference in using or not these antiseptics (13).

Since 1970s, several studies have focused on the effect of using some kinds of mouth washes on the prevention of dental bacteremia, the main difference among these studies were related to the type of dental procedure and type of oral mouthwash used. Studies by Lockhart, Tomas, Erverdi, Bartoluzzi and Maharaj used chlorhexidine mouthwash and studies by Rahnand Yamalik compared the effect of chlorhexidine with Povidone-iodine (5, 14-21).

These studies were different from the present study in terms of design of studies and have shown inconsistent results. In the present study, a concentration of 0.2 chlorhexidine mouth wash was used that was the most accessible and common concentration in the market. Studies have reported same efficacy of concentrations of 0.2 and 0.12 of this mouth wash, when appropriate dose is used (22).

Chlorhexidine mouthwash, with its bactericidal function in ph = 5 to 8,is effective against strains of Staphylococcus, Streptococcus, and many gram negative bacterial species and is accepted as the gold standard among antimicrobial mouthwashes (23). Tooth scaling operation was targeted in the present study. Periodontal packets contain a wide variety of micro-organisms and are a big source of the highest amount and a wide diversity of bacteria in the mouth. The most common aerobic species are Streptococcus and Staphylococcus species in periodontal packets (24).

Streptococcus viridans is the most common micro-organism associated with bacterial endocarditis, while S. aureus has a strong relationship with bacterial endocarditis in 30

to 40% of cases) in IV drug users and damaged right cardiac valves.

Since in periodontium area, any disturbance in the natural integrity of the bio film and sub gingival epithelium lead to bacteremia, therefore, this study assessed the bacterial contact surfaces with patient's blood by sampling gingival sulcus (25). Tomas et al and Erverdi et al in their studies concluded that chlorhexidine can significantly reduce oral bacteremia which is consistent with our study (15, 16). Duvall et al in their study found out the effect of chlorhexidine was equivalent to that of antibiotic prophylaxis (26).

Ernst et al have reported that chlorhexidine reduces bacteria in the oral cavity by 90% after using for a few minutes (27). Ruben et al. reported 99% reduction in the number of colonies of Staphylococcus aureus and Streptococcus mutans in saliva by this mouthwash (28). In Arweiler study chlorhexidine mouthwash significantly reduced oral live bacteria count (29). Piovano et al showed that chlorhexidine can diminish streptococcus mutans near to zero (30). And in the study by Azizi and colleagues, chlorhexidine mouthwash reduced mean number of Staphylococcus aureus, Enterococcus and alpha-hemolytic streptococci colonies near to zero (31). Results of all above mentioned literatures are similar to the present study.

In contrast Lockhart et al and Yamalik et al state that mouthwash application does not have any effect on oral bacteremia (14, 21). These studies used blood sample for bacteremia detection so in light of method are different from our study. Maharaj et al concluded that effect of chlorhexidine mouthwash is not comparable with antibiotic prophylaxis (18). We believe this opinion that antibiotic prophylaxis could not be replaced by oral rinse. Mouthwash only as an adjunctive agent could be used beside antibiotic. Rahn et al and Yamalik et al state that povidone iodine mouthwash has greater effect than chlorhexidine on oral bacteremia (20, 21). This impact could be due to higher concentration and viscosity of povidone iodine than chlorhexidine mouthwash. On the other hand chlorhexidine application is more acceptable than the other. One of the most obvious characteristics of this mouthwash is binding to hard and soft tissues of the mouth that could stay effective for 7 to 12 hours (32).

This study has declined confounding factors as much as possible through the mentioned inclusion criteria and plaque index evaluation to match the groups in terms of oral health indices.

Despite the results of colony counting of bacteria in the gingival sulcus and high impact of chlorhexidine mouth wash on oral bacteria, this effect cannot be generalized to the bacteremia during dental procedures. During scaling, depending on the depth of periodontal pockets and depth of mouthwash, a significant percentage of bacteria will be inaccessible by mouthwash in the penetration depth more than 3 mm (4).

Conclusion

Chlorhexidine mouthwash prior to scaling teeth is recommended as a complementary agent for antibiotic prophylaxis in high risk patients prone to endocarditis, or as the only

agent in patients with moderate risk for endocarditis that antibiotic prophylaxis does not be used. Stable effect of this mouthwash until hours after administration can reduce bacteria in contact with patient's blood, beside the reduced dosage of antibiotic. However, it seems that appropriate antimicrobial measures and aseptic conditions that reduce entry of bacteria into the environment of dental procedure have significant importance.

Declarations

Funding source(s)

This study was done under the support of the members in Research Council of Yazd Shahid Sadoughi University of Medical Sciences and as a thesis. The authors would like to thank the council for their helps in carrying out this study.

Ethics approvals and consent to participate

This study was a double-blind Randomized clinical trial registered by code IRCT: 201311015233N1. All participants signed the informed consent. Ethical committee of Yazd Shahid Sadoughi University of medical science (IRSSU187722).

Conflict of interest

There is no conflict of interest

Authors' contributions

Equal contribution.

References

1. Lockhart PB, Brennan MT, Sasser HC, Fox PC, Paster BJ, Bahrani-Mougeot FK. Bacteremia associated with toothbrushing and dental extraction.Circulation 2008; 117: 3118–3125.

2. Thomas L.Holland,Larry M. Baddour, Arnold S Bayer, BranoHoen et al. Infectiveendocarditis. Nat Rev Dis Primers 2016 Sep 1; 2: 16059.

3. Little JW, Falace DA, Miller CS, Rhodus NL. Infective endocarditis. In: Little. JW, ed, dental management of medically compromised patients. 8th Edition. MOSBY: St.louis; 2013: 20-36.

4. Wilson W\, Taubert KA, Gewitz M, Lockhart PB, Baddour LM, et al. Prevention of Infective Endocarditis Guidelines From the American Heart Association: A Guideline From the American Heart Association Rheumatic Fever, Endocarditis, and Kawasaki Disease Committee, Council on Cardiovascular Disease in the Young, and the Council on Clinical Cardiology, Council on Cardiovascular Surgery and Anesthesia, and the Quality of Care and Outcomes Research Interdisciplinary Working Group. Circulation 2007 Jun;1386):739-45, 747-60.

5. Cherry M, Daly CG, Mitchell D, Highfield J. Effect of rinsing with povidone-iodine on bacteraemia due to scaling: a randomized-controlled trial. J ClinPeriodontol 2007;34:148-55

6. Naruse K. Diabetes and periodontal disease: What should we learn next? J Diabetes Investigating 2014;53):249-50.

7. Reznik DA. Oral manifestations of HIV disease. Top HIV Med 2005;13:143-8.

8. Tellapragada C, Eshwara VK, Acharya S, Bhat P, Kamath A et al. Prevalence of Clinical Periodontitis and Putative Periodontal Pathogens among South Indian Pregnant Women. Int. J Microbiol 2014; 2014: 420-149.

9. Franklin M, Wailoo A, Dayer MJ, Jones S et al. The Cost-Effectiveness of Antibiotic Prophylaxis for Patients at Risk of Infective

Endocarditis. Circulation 2016 Nov 15;13420):1568-1578.

10. Thornhill MH, Dayer M, Lockhart PB, Prendergast B. Antibiotic Prophylaxis of Infective Endocarditis. Curr Infect Dis Rep 2017 Feb;192):9.

11. Oliver R, Roberts GJ, Hooper L, Worthington HV. Antibiotics for the prophylaxis of bacterial endocarditis in dentistry. Cochrane Database Syst Rev 2008;4):CD003813.

12. Hall GE, Baddour LM. Apparent failure of endocarditis prophylaxis caused by penicillin-resistant Streptococcus mitis. Am J Med Sci 2002; 324: 51–53.

13. Dayer M.J., Chambers J.B., Prendergast B., Sando J.A., Thornhill M.H. NICE guidance on antibiotic prophylaxis to prevent infective endocarditis: a survey of clinicians' attitudes. QJM 2013; 106: 237–243.

14. Lockhart PB. An analysis of bacteremias during dental extractions: A double-blind, placebo-controlled study of chlorhexidine. Arch Intern Med 1996;156:513-20.

15. Tomás I, Alvarez M, Limeres J, Tomás M, Medina J, Otero JL, Diz P. Effect of a chlorhexidine mouthwash on the risk of postextraction bacteremia. Infect Control HospEpidemiol 2007;28:577-82.

16. Erverdi N, Acar A, Işgüden B, Kadir T. Investigation of bacteremia after orthodontic banding and debanding following chlorhexidine mouth wash application. Angle Orthod 2001;71:190-4.

17. Aguada E, Olona IL, Salazar MB. Gingival degerming by povidone-iodine irrigation: bacteremia reduction in extraction procedures.J Philipp Dent Assoc 1997;49:42-50.

18. Maharaj B, Coovadia Y, Vayej AC.A comparative study of amoxicillin, clindamycin and chlorhexidine in the prevention of post-extraction bacteraemia.Cardiovasc J Afr 2012 Oct;239):491-4.

19. BortoluzziMC, Santos FA. Amoxicillin and 0.12% chlorhexidine mouthwash may not be better than placebo for reducing bacteremia in third molar extractions. J Evid Based Dent Pract 2014 Mar;141):34-5.

20. Rahn R, Schneider S, Diehl O, Schäfer V, Shah PM. Preventing post-treatment bacteremia: comparing topical povidone-iodine and chlorhexidine. J Am Dent Assoc 1995;126:1145-9.

21. Yamalik MK, Yücetas S, Abbasoğlu U. Effects of various antiseptics on bacteremia following tooth extraction. J Nihon UnivSch Dent 1992; 34:28-33.

22. García-Caballero L, Carmona IT, González MC, Posse JL, Taboada JL. Evaluation of the substantivity in saliva of different forms of application of chlorhexidine. Quintessence Int 2009;40:141–144.

23. Minguez-Serra MP, Salort-Llorca C, Silvestre-Donat FJ. Chlorhexidine in the prevention of dry socket: effectiveness of different dosage forms and regimens. Med oral Patol Oral Cir Bucal 2009;14:e445-9.

24. Lockhart PB, Brennan MT, Thornhill M, et al. Poor oral hygiene as a risk factor for infective endocarditis-related bacteremia. J Am Dent Assoc 2009;14010):1238–1244.

25. Parahitiyawa NB, Jin LJ, Leung WK, Yam WC, Samaranayake LP. Microbiology of odontogenic bacteremia: beyond endocarditis. ClinMicrobiol Rev 2009; 22:46-64.

26. . Duvall NB, Fisher TD, Hensley D, Hancock RH and Vandewalle KS. The Comparative Efficacy of 0.12% Chlorhexidine and Amoxicillin to Reduce the Incidence and Magnitude of Bacteremia during Third Molar Extractions: A Prospective, Blind, Randomized Clinical Trial. Oral Surg Oral Med Oral Pathol Oral Radiol 2013 Jun;115(6):752-63.

27. Ernst CP, Canbek K, Dillenburger A, Willershausen B. Clinical study on the Effectiveness and side effects of hexetidine and chlorhexidine mouthrinses versus a negative control. Quintessence Int 2005;36:641–52.

28. de Albuquerque RF Jr, Head TW, Mian H, Rodrigo A, Müller K, Sanches K, Ito IY.

Reduction of salivary S. aureus and mutans group streptococci by a preprocedural chlorhexidine rinse and maximal inhibitory dilutions of chlorhexidine and cetylpyridinium. Quintessence Int 2004;35:635-40.

29. Arweiler NB, Netuschil L, Reich E. Alcohol-free mouthrinse solutions to reduce supragingivalplaqueregrowth and vitality: A controlled clinical study. J ClinPeriodontol 2001; 28: 168-174.

30. Piovano S, Marcantoni M, Dono R, Bellagamba H. Effect of a chlorhexidine varnish on Streptococcus mutans in saliva. ActaOdontolLatinoam 2005; 18: 7-13.

31. AAzizi, B Fatholahzadeh, P Maleknejad. Evaluation of the Effects of Chlorhexidine 0.12% Mouthwash on Mouth Pathogen Streptococcus and Normal Microflora. Journal of Dentistry 2008; 93): 299-303.

32. Nascimento AP, Tanomaru JM, Matoba-Júnior F, Watanabe E, Tanomaru-Filho M, Ito IY. Maximum inhibitory dilution of mouthwashes containing chlorhexidine and polyhexamethylenebiguanide against salivary Staphylococcus aureus. J Appl Oral Sci 2008;16:336-9.

How to cite:

Akhavan Karbasi M. H., Owlia F., Zandi H., Hajimaghsoodi S. The preventive effect of Chlorhexidine mouthwash on alpha-hemolytic Streptococci and Staphylococcus aureus. Jorjani Biomedicine Journal. 2019; 7(1): 22-30.