ORIGINAL ARTICLE

EFFECT OF ELASTOMERIC SEPARATOR ON MICROBIAL COUNT IN GINGIVAL CREVICULAR FLUID

Samia Sattar Khan[1], Saad Asad[2], Umar Hussain[3]

[1]Freelancer, [2]Rawal College of Dentistry, Lahore, [3]Saidu College of Dentistry, Swat-Pakistan

Background: The separators are a preliminary step for band insertion, but there is a potential risk of bacteraemia during their placement, particularly in susceptible patients. The objective of the study is to determine the effect of separators on the bacterial count in gingival crevicular fluid (GCF) and to assess the efficacy of chlorhexidine mouth rinse and saline irrigation in the reduction of the bacterial count. **Methods:** This randomized controlled trial was conducted on 51 participants who were divided into three equal groups randomly (brushing only/control, saline irrigation, and 2% chlorhexidine mouthwash rinse). The inclusion criteria were age between 18–25 years, good oral hygiene, gingival and plaque index <1, no previous orthodontic treatment, and healthy individuals. The bacterial count was obtained from GCF samples after two hours, on the third day, and on the seventh day. Kruskal Wallis test was used to compare the bacterial count among the three groups, and post hoc analysis was done using Dunn's test. Friedman test was applied to see the difference at three-time points in each group. **Results:** In both saline and chlorhexidine groups the mean bacterial count decreased significantly from baseline to 3rd day and 7th day after separator placement (p<0.001). For the third day, a significant difference was found in control versus saline and control versus chlorhexidine. No significant difference was found between saline and chlorhexidine on the third day. Similar results were found on the 7th day. For controls, the bacterial count increased with time and for both saline and chlorhexidine groups the bacterial count decreased. The highest decrease in the bacterial count was found for the chlorhexidine group. **Conclusion:** After the placement of separators, there was an increase in the bacterial count in GCF. Notably, chlorhexidine was found to be more effective than saline irrigation in reducing the bacterial count.

Keywords: Bacterial count; Orthodontic separator; Gingival crevicular fluid; Chlorhexidine

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INTRODUCTION

Orthodontic appliances are designed to move dentition to a desired position for optimal facial aesthetics and balance, and require adherence to the enamel surface.[1] While bondable attachments like molar tubes are considered the gold standard of care, strict indications for banding molars still exist, such as large restorations, heavy forces from extra-oral traction, and the need for lingual auxiliaries.[2,3] Separators are preliminary step to create space for these bands insertion.[4] They are used to separate the teeth before banding and left in place long enough for initial tooth movement to occur. The main advantage of separators is that they are retained well and may be left in position for a somewhat longer time.[5]

Bacteraemia resulting from dental procedures such as separator placement in susceptible individuals, including those with congenital or acquired heart defects, prosthetic heart valves, and rheumatic fever, may result in infective endocarditis (IE).[6] Therefore, modifications should be made to these procedures to prevent IE, or antibiotic prophylaxis should be administered. However, it is important to note that antibiotic prophylaxis may lead to adverse effects, such as anaphylactic shock and death.[7]

Separators placement can results in bacteraemia reported by previous studies.[8,9] Erverdi *et al*.[8] in study on 40 healthy orthodontic cases with good oral hygiene reported 7.5% bacteria after separator use. An investigation conducted by Lucas *et al*.[9] on four commonly used procedures in orthodontics which were; impression taking, separators placement, band insertion, and fixed appliance adjustment and reported the separator placement is the only procedure causing bacteremia.

Chlorhexidine is effective antimicrobial agents commonly prescribe to orthodontic

patients to prevent plaque stagnation and bacteraemia. Dua *et al.*[10] conducted a trial on 27 orthodontic patients using chlorhexidine mouth after separator placement. Their results showed that there was no bacteraemia before (control) after separator placement with use of chlorhexidine mouth.

Though the assessment of bacteraemia can be conducted on venous sample easily but the analysis of GCF can be a non-invasive way.[11] Various methods have been employed to record bacterial count from GCF like lavage, suction or absorption strip. Lavage involves irrigating the crevice with sterile solution and aspirating the fluid, suction involves inserting a sterile tube to aspirate the fluid, and absorption strip method involves placing a sterile paper strip to absorb the fluid for analysis.[12] Among these GCF methods the absorption strips are least invasive.[13] This is because a single absorption strip can collect a small volume of GCF, which corresponds to 1.2 μL. This is a relatively small amount compared to other GCF collection methods, such as lavage or suction, which can be more invasive. The use of absorption strips has advantages, such as minimal discomfort to the patient, ease of use, and cost-effectiveness. Furthermore, the detection of bacterial count in GCF using absorption strips may serve as a novel diagnostic tool that circumvents the invasive procedures associated with other GCF collection methods.[14] Another study assessed the effect of 0.2% chlorhexidine (CHX) on bacteraemia associated with banding and de-banding and their showed that though CHX reduced bacteria but the difference was not statistically significant.

This primary objective of this trial was to determine the effect of separator on bacterial count of gingival crevicular fluids. The secondary objective was to know whether chlorhexidine mouth rinse is more effective than saline irrigation and control group (no active intervention) or not.

MATERIAL AND METHODS

This three-parallel arms randomized clinical trial was conducted according to CONSORT (Consolidated Standards of Reporting Trials) guidelines 2010.[15] Ethical approval was obtained from concerned hospital, department of Orthodontics, University of Lahore, Pakistan (Ref No. 1047) and study was conducted from 1st January 2022 to 1st July 2022. A written consent was obtained from all participants. This trial was not registered.

A total 51 participants were selected by permuted block randomization technique at department of Orthodontics, University of Lahore, Pakistan. Among these 35 were patients coming for orthodontic treatment and 16 were volunteer (final years BDS students). The inclusion criteria were participants with age from 18 to 25 years, good oral hygiene, gingival and plaque index <1, with no previous orthodontic treatment and no previous dental treatment during last six months. Patients with chronic medical disorders affecting periodontal health, and those who have taken antibiotics during last six months were excluded from this study.

The power analysis in STATA 14.0 showed that the inclusion of a total of 51 participants (17 per group) at α=0.05 and three mean bacterial counts in the control, saline, and chlorhexidine groups (148, 117, and 99, respectively), and using within-group variance of 1601 yielded an estimated power of 88.98%. The sample size calculation was not performed prior to the commencement of the research study. In order to assess the adequacy of the included sample size, a power analysis was conducted. The results of the power analysis indicated that the sample size was sufficient and justifiable for the study.[16]

Elastomeric separators were place in first molars mesial and distal sides of all quadrants in subjects fulfilling inclusion criteria. The participants were divided into three equal groups (each having 17 participants). Group A were instructed to follow a regular oral hygiene regimen of tooth brushing twice a day. Group B was asked to add saline rinse use once at night after brushing. Group C were asked to use 2% chlorhexidine mouthwash rinse once at night after brushing.

Gingival crevicular fluid samples were taken from mesiobuccal surface of maxillary right first molar using an absorbent paper, two hours, at third day and seventh day after passing separator. The GCF samples were placed immediately in a sterile test tube, transported immediately to the laboratory in icepack and saved in freezer at a temperature of 8 °C until further processing. Once all (n=153) samples were collected, laboratory procedure was started.

Nutrient broth and blood medium was used for the growth of microorganisms. Test tubes, petri dishes and media were sterilized. The agar plates were prepared using aseptic technique. Nutrient broth (2 ml) and the paper point with GCF samples were put in test tube. These test tubes were placed in the incubator to confirm the bacterial growth. Inoculum (1 ml) was taken from test tube and spread over the agar plate. After incubation for 24 hours at 37 °C, colony counting was done over quarter of the agar plate using colony counter. This gave the most probable number of viable bacteria (cfu/ml). (Figure-1)

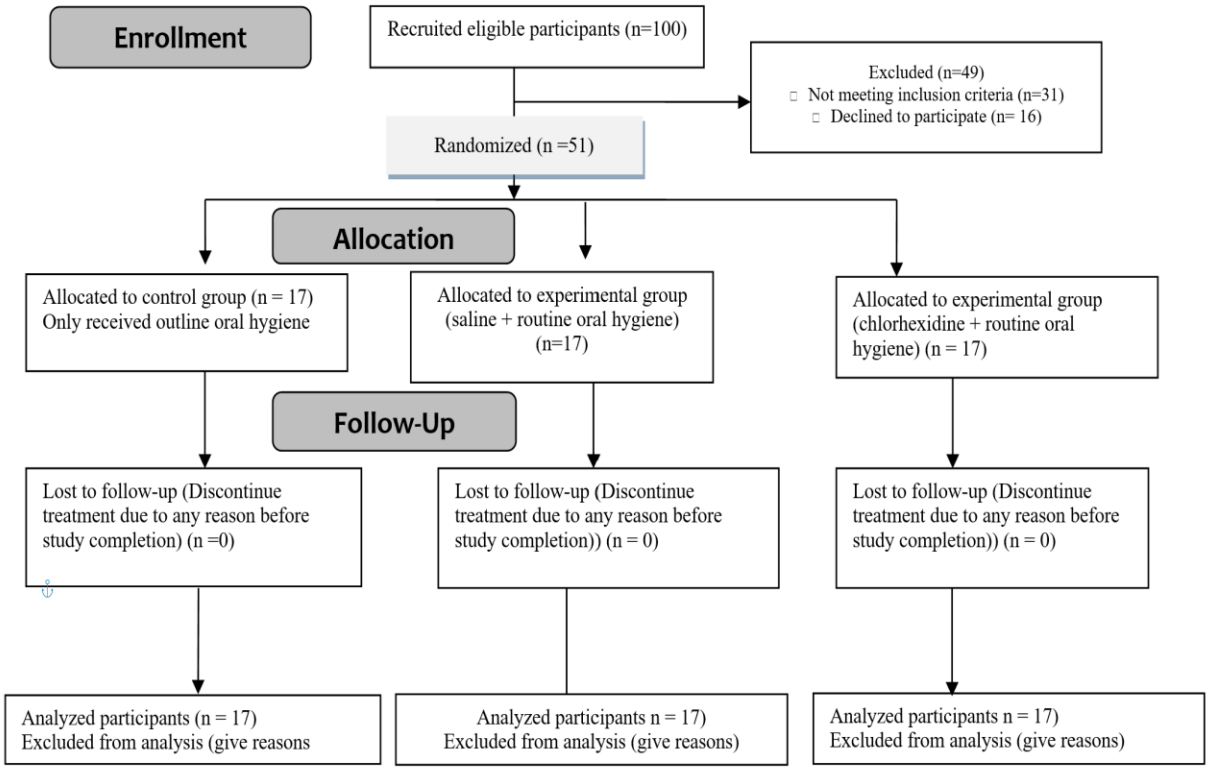

Figure-1: A CONSORT diagram showing the flow of participants through each stage of the trial

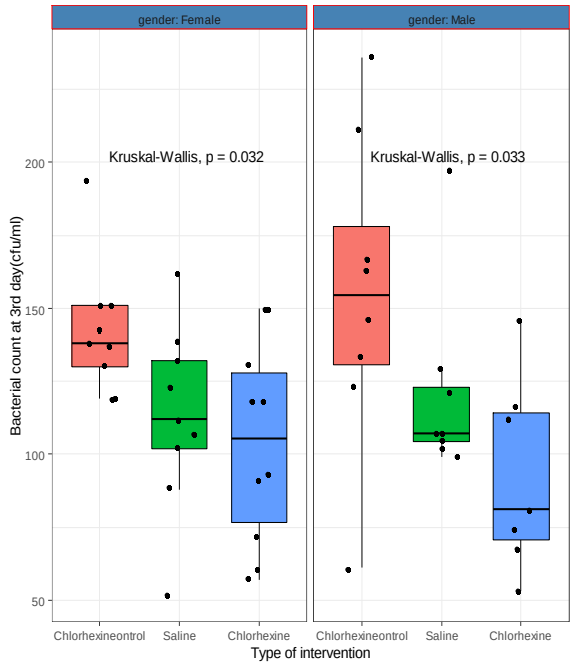

Figure-2: Bacterial count for three groups for both males and females at third day

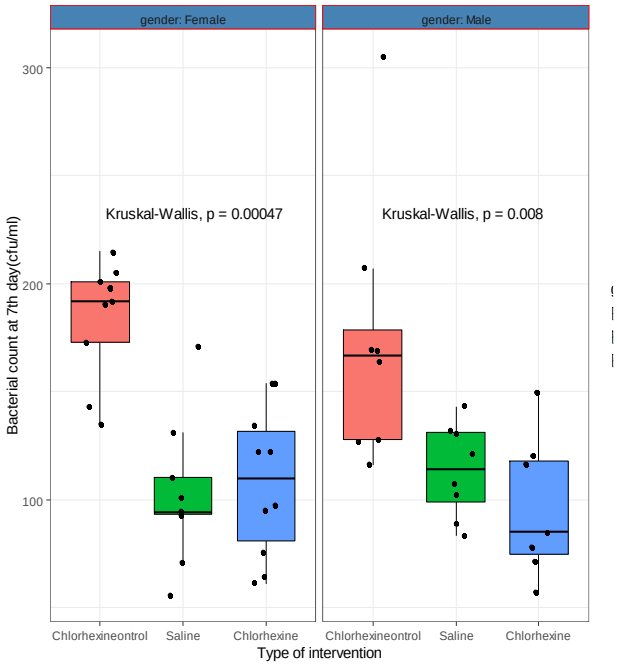

Figure-3: Bacterial count for three groups for both males and females at 7th day

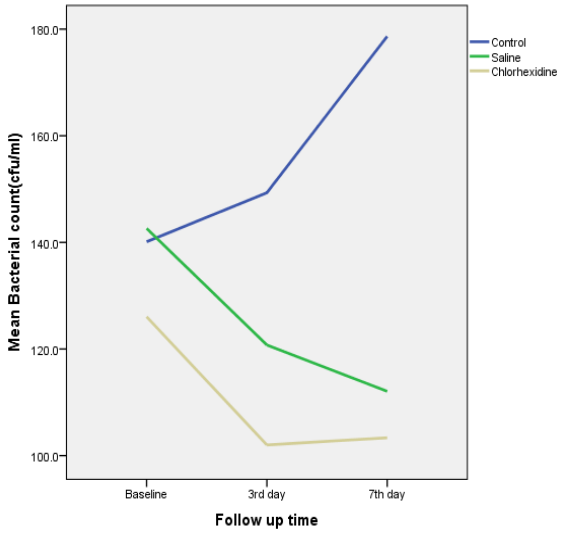

Figure-4: Bacterial count with respect to time in three groups

The collected were analyzed in R package version 4.1.2. Normality of the data was assessed with Shapiro-Wilk test. The data were not normally distributed (p<0.05). Kruskal Wallis test was run to compare bacterial count among three groups at two hours (T1), 3rd day (T2) and 7th day (T3) after separators placement. Post hoc analysis was done for multiple comparisons using Dunn'a test. Friedman test was applied to see difference at three time points (T1, T2, and T3) in each group. The level of significance was kept at p≤0.05.

RESULTS

A total of 153 samples of 51 patients were taken and studied. The females were 28(53.8%) and males were 23(44.2%). The mean age was 24.27±3.96 years. Table-1 shows the comparison of bacterial count among control, saline and chlorhexidine group. There was no statistical difference for control group at baseline. In both saline and chlorhexidine group the average number of bacterial counts decreased significantly form baseline to 3rd day and 7th day after separator placement (p<0.001). For third day the significant different was found control versus saline and control versus chlorhexidine. No significant difference was found between saline and chlorhexidine at third day. Similar results were found at 7th day. Figure-2 shows that the bacterial count at 3rd day were almost similar in both genders in each group. The p-value shows lack of significant difference among genders. Figure-3 shows similar results for 7th day. For control group the bacterial colonies increased significantly from baseline to 7th day after separator placement (p=0.05). For saline group the bacterial count show decreases but was not statistically (p=.08). For chlorhexidine group the mean bacterial count show statistically significant decrease (p=0.005). (Table-2)

For controls the bacterial count increase with time and for both saline and chlorhexidine group the bacterial decreases. The highest decrease in bacterial count was found for chlorhexidine group. (Figure-4)

Table-1: Comparison of bacterial count among control, saline and chlorhexidine group

Characteristic	Control group (A)*	Saline irrigation (B)*	Chlorhexidine (C)*	p-value**	Post hoc analysis p-value***
Bacterial count at baseline (cfu/ml)	141 (23)	143 (52)	130 (53)	0.063	--
Bacterial count at 3rd day(cfu/ml)	148 (40)	117 (31)	99 (33)	<0.001	A vs B; p=0.013 A vs C; p<0.001 B vs C; p=0.31
Bacterial count at 7th day(cfu/ml)	179 (45)	108 (28)	103 (33)	<0.001	A vs B; p<0.001 A vs C; p<0.001 B vs C; p=0.081

* Mean (SD), ** Kruskal-Wallis rank sum test, *** Dunn'a test

Table-2: Comparison of bacterial count in control, saline and chlorhexidine groups at various time points

Group	Bacterial count (cfu/ml)	Mean ± SD	Mean Rank	p-Value*
Control	**Baseline**	140.52±23.01	1.71	.05
	3rd day	148.35±39.62	1.82	
	7th day	178.64±45.21	2.47	
Saline	**Baseline**	143.23±51.56	2.41	.080
	3rd day	116.70±31.26	1.94	
	7th day	107.58±28.24	1.65	
Chlorhexidine	**Baseline**	130.11±52.62	2.29	.005
	3rd day	99.35±33.36	1.35	
	7th day	103.35±33.36	2.35	

*Friedman test

DISCUSSION

This randomized clinical trial was conducted to determine the effect of separator on bacterial count of gingival crevicular fluids to compare antibacterial property of chlorhexidine with saline irrigation. Our findings showed that bacterial count increase from day 1 to day 3 and 7 in control group in which no intervention was given. In both saline and chlorhexidine group the number of bacteria decrease from day 1 to day 3 and 7. Only significant decrease in bacterial count was found for chlorhexidine.

Dental procedures leading to bacteraemia have been recognized by many researchers. It is highly imperative for every dental practitioner to be conscious of all the procedures that might cause disturbance of oral ecology. This change of microbial environment may in turn lead to pathology elsewhere in the body.[17] The oral environment directly affects the resident microflora. The proximal region of dentition is protected from the oral hygiene practices and cleansing effects of mastication and salivary movement. It has a low redox potential therefore supporting a more diverse bacterial community and a major cause of diseases.[18]

Gingival crevicular fluid is most favourable for analysis of periodontal diseases and the microbiota involved in the process. GCF can be collected by a range of methods including suction, lavage or absorption. In our study we used paper point for collection of GCF sample. It was minimally invasive and easy for the patient to tolerate.

The incubation period for bacteria is 72 hours which is the minimum time required after which replication is identifiable. Considering this fact, we decided to plan our sampling technique accordingly. We collected GCF sample at two hours, 72 hours, 3rd day and 7th day after passing of separator.

In this study we used chlorhexidine to test its efficacy in reduction of bacterial count in GCF after separator placement. Our results showed that lowest bacterial count was found in chlorhexidine group. Previous studies show that significant reduction in bacterial count occur after use of chlorhexidine mouth rinse.[19,20] Chlorhexidine is an antiseptic agent used in dentistry extensively.[21,22] The positive difference came from the use of mouthwash. Chlorhexidine in 2 % concentration controlled the increase in bacterial count to a significant level. This shows that the routine protocol (tooth brushing twice a day) of oral hygiene techniques are not enough to avoid increment in bacterial count. Our study results suggest that leaving the separator in oral cavity for 7 days will lead to increase in the number of microbiota in GCF which in future might cause higher levels of bacteraemia.

The bacterial count increases in the control group from two hours to day 3 and 7 while in saline and cholorhexine groups it is decreased. The separator is made from elastomeric materials which rapidly degrade in oral environment and provide area for bacterial growth. Our findings showed that saline irrigation can also reduce bacterial count after separator placement. So regular rinsing of mouth after separators placement is suggested to reduce bacterial count. Previous literature also reported that elastomeric material can increase bacterial count.[23]

The initial count after two hours of insertion of separator (baseline) shows that bacterial count was less in chlorhexidine and was also similar in saline irrigation and control group but there was no statistical difference. **(**Figure-3**).** This can be due to high antibacterial property of chlorhexidine and quick action.

This study has some limitations like it only focus on number of bacterial counts without investigating the type of bacteria in GCF. Other limitation this is single center research and multicentre can better explained this area.

CONCLUSION

The placement of separators resulted in an increase in bacterial count in GCF. Nevertheless, the application of saline irrigation and chlorhexidine mouth rinse demonstrated significant reduction in bacterial count. It is noteworthy that chlorhexidine was found to be more effective in reducing bacterial count in GCF after separator placement, compared to saline irrigation. These findings suggest the potential of chlorhexidine as a promising technique or material in enhancing oral health outcomes during placement of orthodontic separators.

AUTHORS' CONTRIBUTION

SSK: Conception of topics, Data acquisition, and contribution to writing. SA: Research supervisor, writing. UH: Literature review, statistical analysis and result reporting

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Address for Correspondence:

Umar Hussain, Lecturer, Saidu College of Dentistry, Swat-Pakistan
Cell: +92 345 450 0578
Email: drumarhussain@gmail.com