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FREQUENCY OF TOOTH BRUSHING AND ORAL NEUTROPHIL COUNT"- A PILOT STUDY

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ABSTRACT

Introduction: For maintaining good oral health, efficiency and frequency of tooth brushing is important. Accumulation of the microbial plaque on the tooth surface is a direct cause of gingivitis and that gingivitis may precede periodontitis.[10] Before the clinical signs of gingivitis become evident, neutrophils start appearing, acting as a first line of defense against invading microbes. By tooth brushing, though the percentage of gingivitis reduces, it is only minimal. This study was done to check the amount of oral neutrophil count in people who brush once and twice a day. **Aim:** To check the effectiveness of frequency of tooth brushing on gingival status and oral neutrophil count. **Materials and Methods:** 30 subjects who gave the informed consent participated in the study. At the baseline visit they were randomly assigned to (1) once a day tooth brushing group, (2) twice a day tooth brushing group, according to baseline gingival scores Followed by estimation of salivary neutrophil count. All were asked to use the same toothbrush and toothpaste and were recalled on the 16th day for re-evaluation. **Results:** Baseline GI scores and oral neutrophil count demonstrated no statistical differences between the two groups (P>0.05). At the 2-week examination, even though statistically there was no significant difference but clinically, both oral neutrophil count and gingivitis reduction were more in twice a day toothbrushing group. **Conclusions:** Although there was no statistically significant difference in gingival scores and neutrophil count between the two groups, the result of this pilot study showed clinically greater reduction in neutrophil count in twice a day tooth brushing group. So this study shows some direction for conducting future studies in this area.

KEYWORDS: Toothbrushing, Gingivitis, Salivary Neutrophil Count.

INTRODUCTION

Periodontal disease and dental caries are the most prevalent oral diseases worldwide. These diseases in their advanced stage impair the normal oral as well as general function of the body.[6] Generally periodontal disease begins as gingivitis and its prevalence and severity increases with age.[7]

Dental plaque, a bacterial biofilm on the tooth surface, is commonly associated with dental caries and periodontal diseases. The presence of plaque on the tooth surface results in gingivitis which if left untreated may ultimately lead to periodontitis.[1]

Maintaining good oral hygiene is essential for preventing and controlling of periodontal disease along with regular dental visit. Tooth brushing is the most efficient oral hygiene aid for the removal of dental plaque, which if not done properly causes gingival inflammation.

Many studies suggest that toothbrushing, which is an effective measure for plaque control which in turn helps in the prevention of gingivitis and periodontal disease.[2,25] According to Goldman et al. brushing five times in a day is ideal for proper plaque control. But due to impracticality of this regimen, they suggested that, at least twice a day toothbrushing should be performed.[14] According to Greene and Arnim, once a day toothbrushing is adequate for plaque control and gingival health maintenance.[15] But according to Loe, tooth brushing every second day may be sufficient for proper plaque control.[16] According to Lilienthal et al. the periodontal health of 600 individuals significantly improved by increasing the frequency of brushing.[17] According to Stanmeyer, there was significant increase in gingival health, when the teeth were brushed twice daily.[18] Although there are many views related to frequency of toothbrushing, but keeping in mind the practicality, twice a day toothbrushing is an effective measure for plaque control.

Neutrophils are White Blood Cells that play an important role in our immune system. They circulate in the blood stream and when they sense signals (infection), they are the first cells to migrate to the site of the infection to kill the invading microbes. Similarly neutrophils are also present in the healthy mouth. These cells migrate through the gingival crevices into the oral cavity and act as a first line of defense against the invading microbes, before the clinical signs start appearing. Neutrophil recruitment requires adhesion to and transmigration through blood-vessel walls at sites where the vascular endothelium is activated by pro-inflammatory mediators. Even with optimal plaque control, the junctional epithelium is never sterile and neutrophils exit the gingival microvasculature and enter into the periodontal tissue.[19] Neutrophil count in the gingival exudates reflects its degree of inflammation. Thus neutrophil count increases in the gingival sulcus during the course of gingivitis.[20] Neutrophils present in the saliva are also called "Salivary Corpuscles". These salivary corpuscles are part of local defense mechanism in the oral cavity against many oral diseases including periodontal diseases.[13]

By twice a day tooth brushing (morning and at night after meals), though the percentage of gingivitis reduces, but people fail to brush at night and usually brush only once i.e., in the morning. Although studies have been done on relationship between gingivitis and toothbrushing but it takes longer duration to show the changes in signs and symptoms of gingivitis. As neutrophils appear in the pre clinical stage of gingivitis and also changes in the neutrophil count can be detected in lesser time through any intervention and it also shows greater percentage change as compared to gingivitis percentage change which can be a better way to motivate the public regarding twice a day toothbrushing. Therefore this study was attempted to check the neutrophil count change in once and twice a day toothbrushing group for a smaller time period (15 days). Our null hypothesis was, there will be no difference in oral neutrophil count in people who brush once and people who brush twice a day and our research hypothesis was that oral neutrophil count in people who brush twice a day will be less as compared to people who brush once a day, so early detection of gingivitis can be done through oral neutrophil count.

AIM

To check the effectiveness of frequency of toothbrushing on gingival status and oral neutrophil count

OBJECTIVES

- To determine the baseline mean gingival scores and oral neutrophil count of the two study groups (i.e., Group 1: Once a day toothbrushing group and Group 2: Twice a day tooth brushing group).
- To determine the mean gingival scores and oral neutrophil count among the two study groups at 15 days follow up interval.
- To compare the mean gingival scores and oral neutrophil count among the two groups after 15 days follow up visit.

MATERIALS AND METHOD

The present study was a randomized, 2-cell parallel design study, conducted in the Department of Public health Dentistry, SDM College of Dental Sciences and Hospital, Dharwad. Prior to the start of the study Ethical clearance was obtained by the Ethical Review Committee, S.D.M. College of Dental Sciences and Hospital, Dharwad.

Sample size estimation

According to Steven A Julious's Rule of Thumb a sample size of 12 per group is sufficient for pilot study (for clinical trial).[26] Therefore in this pilot study 15 subjects were taken in each group.

Source and number of subjects

30 healthy subjects, belonging to both the genders, aged above 18 years, who met the selection criteria were taken into the study.

Inclusion criteria

1. Subjects brushing once a day
2. Scorable facial and lingual surfaces of a minimum of 20 sound natural teeth
3. People who are capable to read, understand and sign the informed consent form
4. 18 years and above aged male and female subjects in good general health
5. Subjects with not more than 4 pockets and pockets <6 mm.
6. A Gingival Index score of ≥1.0
7. Availability of subjects for the entire study duration

Exclusion criteria

1. Destructive periodontal disease
2. Significant soft tissue pathology, severe gingivitis/systemically related gingival enlargement
3. History of diabetes, hepatic, renal disease or other serious medical condition and transmissible disease
4. Orthodontic appliance or any kind of fixed or removable appliances
5. History of allergies to dental products or their ingredients
6. Pregnant and breast feeding women
7. History of adverse habits like smoking and tobacco chewing
8. Patients under antibiotics, steroid therapy or any anti inflammatory drugs in the previous month.
9. Oral prophylaxis in the preceding month or periodontal treatment in the preceding 3 months or participation in any other plaque and gingivitis clinical study involving oral products within the last 30 days
10. Treatment with any drugs that might alter Polymorphonuclear neutrophil number or function.

Method

Initial visit: Fourty adult subjects who visited the dental clinic for the purpose of study were screened. Soft and hard tissues evaluations of all the subjects were done in the initial visit. This examination included an evaluation of the soft and hard palate, gingival mucosa, buccal mucosa, mucogingival fold areas, tongue, sublingual, submandibular areas, tonsilar, pharyngeal areas and teeth. 30 of them were selected, since 10 of them did not meet the inclusion criteria. For the standardization purpose all the subjects who were selected underwent a washout period for 7 days. All were given similar washout toothpaste and a toothbrush (Colgate MaxFresh toothpaste and a medium bristled Colgate toothbrush). All were asked to brush once a day (in the morning) with it for 7 days and were recalled on the 8th day for the baseline visit.

Baseline visit: The subjects visited the clinic having refrained from all the oral hygiene procedures. The washout toothpastes and toothbrushes were taken back and the toothpaste was weighed. The weight of the toothpaste was noted in the subject demographic form. The visit forms of the subjects were filled, and then the subjects were given 10 ml of saline and were asked to rinse with it for 30 seconds and expectorate it into a 50 ml falcon tube. This oral rinse was collected for the purpose of oral neutrophil estimation, which was examined using fluorescent microscope under blue light. Subjects then underwent the baseline gingivitis examination, their gingival scores were recorded using Loe and Silness gingival index[21] (Talbott et al modification[22]). Subjects were randomized into two study groups (Group 1: Once a day toothbrushing group and Group 2: Twice a day toothbrushing group) based on their baseline gingival scores. They were asked to continue use the same toothpaste and toothbrush for 15 more days. The subjects in the group 1 were instructed to brush once a day and that in group 2 were instructed to brush twice a day. To check the compliance of the subjects, they were asked to get their toothpastes during their future visit. Subjects were recalled after 15 days for the final visit.

Final visit: The subjects visited the clinic without brushing and rinsing. Gingival scores and oral neutrophil count were recorded using the above mentioned procedure and also to check for the product usage the toothpastes were weighed.

Compliance: During the study period, all the subjects were given a reminder regarding the usage of their products and their visits through a phone call and through text messages at certain interval. Subjects were also asked to get their respective products during each visit and the products were weighed. It is compared with actual weight of the product, so that we get to know the amount of product the subject has used. If it was found that the subject has not used the product in required amount or has not followed the instructions then again he will be instructed regarding the usage, so that he will follow it in future. The investigator considered the subjects as drop out if they did not follow the instructions.

Study duration

The present study was conducted over a period of 1 month (study was of 15 days but since it was not possible to examine all the subjects in 1 day it took almost 1 month to complete the study) in September 2016 in Dharwad city.

Study Products

All the subjects received the same toothpastes and toothbrushes throughout the study. Toothpaste used was Colgate MaxFresh and the toothbrush used was a medium bristled Colgate toothbrush.

Prohibited / allowable medications or precautions

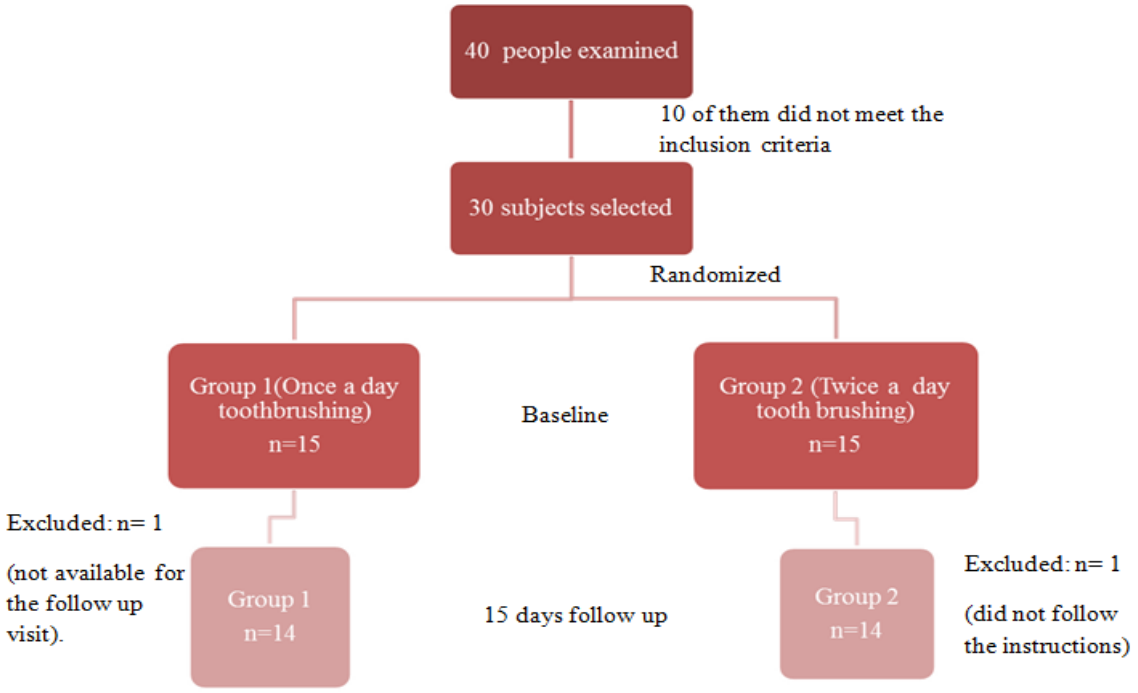
If subjects during the study period due to some health issues, were under medication then they were not forced to stop the medication, but they have to inform the study investigator regarding the usage, so that the investigator can drop out the subject from the study, or else the usage of medicines may interfere with the study results

Subject progress and discontinuation

Subjects were considered to have completed the study if they were followed up throughout the duration of the study. Subjects were considered lost to follow up if no contact had been established by the time the study was completed such that there was insufficient information to determine the subject's status. A genuine effort was made to determine the reasons of dropout. Subjects could be dropped out if any of the following occurred.

Subjects failed to substantially comply with the protocol requirement.
Subject failed to report for a scheduled examination.
Subjects received emergency dental or medical treatment or any medication that may interfere with the parameters under study.
Subject developed serious adverse reactions.
Subject chose to terminate participation in the study.
Subject discontinued treatment or relocated.

Consort flow Diagram Showing the Distribution of Study Subjects



Statistical Analysis

The gingival scores and the oral neutrophil count of the subjects at each visit were entered into the computer (MS-Office 2007, Excel data sheet). The data was subjected to statistical analysis using the statistical package (SPSS version 20). Shapiro-Wilk test was done to assess Normality. Since the majority of the variables followed normal distribution, parametric test was applied. Comparison of the gingival scores and the oral neutrophil count at baseline and final visit, within the group was done using paired t test and between the 2 groups were done using unpaired t test. Statistical significance was recorded if the P-value was 0.05 or less.

RESULTS

A total of 30 subjects were included in the present study and were randomly allocated into two groups of 15 subjects each. There were **7** males and **8** females in group 1 and **9** males and **6** females in group 2. The mean age of the study subjects in the group 1 was **22.06±1.98 years** and that in the group 2 was **25.26±5.13. Table 1** shows the distribution of study subjects among the two groups, i.e., Group 1: Once a day tooth brushing group and Group 2: Twice a day tooth brushing group.

Table. 1: Distribution of study subjects by age and gender.

GROUPS	GENDER		TOTAL	MEAN AGE (YEARS)
	Male	Female		
Group 1 (Once a day toothbrushing)	7	8	15	22.06±1.98
Group 2 (Twice a day toothbrushing)	9	6	15	25.26±5.13

Baseline data

Table 2 shows the mean baseline gingival scores and the neutrophil count among once a day and twice a day toothbrushing group. Independent sample t test was applied which showed that there was no statistically significant difference in the baseline gingival scores and neutrophil count between the two groups (***P>0.05***) i.e., at baseline there was not much difference in the gingival scores and neutrophil count in both the groups.

Two weeks versus baseline

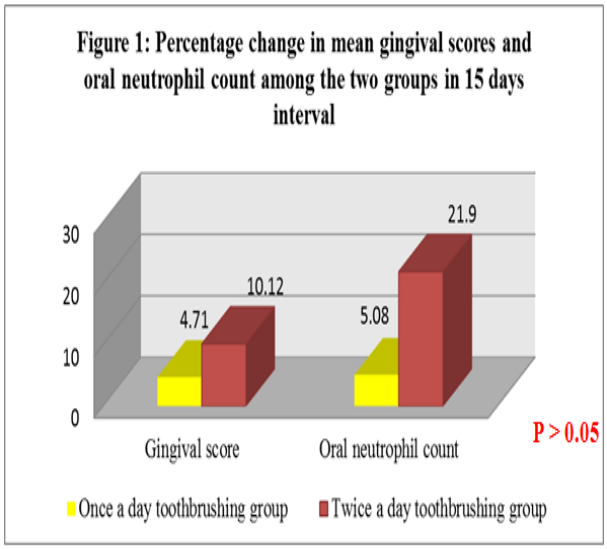
Paired t test was applied which showed that within the group, there was a significant difference in the gingival score in both the groups (***P<0.05***). But no significant difference in the neutrophil count in once a day brushing group (***P>0.05***) whereas in twice a day toothbrushing group there was a significant difference (***P<0.05***). Independent sample t test was applied which showed that there was no significant difference in the mean gingival score and neutrophil count among the two groups at 15 days follow up (***P>0.05***). [Table 2].

Table. 2: Mean gingival score and oral neutrophil count among the two groups at baseline and after 15 days follow up (final visit).

Variables	Once a day toothbrushing	Twice a day toothbrushing	*P value**
	Mean ± S.D	Mean ± S.D	
Baseline Gingival score	0.9914± 0.1429	0.9914±0.179	1
Final visit Gingival score	0.947±0.1675	0.895±0.213	0.478
P value	0.030	0.003	
Baseline oral Neutrophil count in lakhs	3.89±2.18	3.42±1.57	0.524
Final visit oral Neutrophil count in lakhs	3.2±1.9	2.74±1.5	0.487
P value	0.304	0.002	

SD- Standard Deviation, p≤0.05

Percentage change in mean gingival scores and oral neutrophil count among the two groups in 15 days interval: Statistically there was no significant difference in the percentage change in mean gingival scores and oral neutrophil count among the two groups in 15 days interval [Figure 1].



DISCUSSSION

Toothbrushing, which is the basis of oral hygiene measures worldwide, but in spite of the widespread use of both toothbrush and toothpaste, the majority of the population do not brush their teeth thoroughly to prevent accumulation of plaque. This is accredited to be a result of lack of understanding of the disease process.[10] As the frequency of daily tooth brushing increases there is a decrease in the plaque and gingival scores, indicating better gingival health.[2] Epidemiological studies and reviews of data indicate that twice daily tooth brushing improves gingival health.

It is understood that salivary neutrophils are the part of local immunological mechanisms in oral cavity involved in the defence against microbes which lead to various oral diseases including periodontal diseases.[13] Level of oral neutrophils are a good indicator of oral inflammatory load and periodontal diseases status and they become evident before the clinical signs of gingivitis becomes evident.[4]

Even though many studies have been done related to toothbrushing and gingivitis, but there is no any study reported, related to toothbrushing and salivary neutrophil count which is an inflammatory biomarker for many oral diseases including gingivitis.

This study was done to check the amount of neutrophil count in once and twice a day toothbrushing group for a smaller time period i.e., for 15 days (as it is proved that neutrophils start appearing before the clinical signs of gingivitis become evident).

The result of the study showed that, at baseline, the mean gingival score in both the group were 0.99, even though we considered gingival score **>1** as an inclusion criteria. During the allocation time the mean gingival score was >1, due to dropouts during the study period, the gingival score has become <1.

By the end of 15 days it was noticed that within the group significant reduction in the gingivitis were seen in both the groups. A study which was done by Lorraine B et al [23] also showed a significant reduction in the gingivitis in 15 days in the manual toothbrush using group, who were asked to brush twice daily. In the present study oral neutrophil count reduction in once day toothbrushing group was not significant whereas in twice a day toothbruhsing group even oral neutrophil count reduction was significant in 15 days.

In this pilot study, between once and twice a day toothbrushing group there was no significant difference in the gingivitis score and oral neutrophil count by the end of 15 days. Possible reason could be that the study was done for 15 days. So, if the study will be done on a larger group of people and for a longer duration then might be we can appreciate the difference even between the groups. Long term studies may provide a more accurate appraisal of the effectiveness of twice a day toothbrushing.

Even though statistically there was no significant difference, but clinically this study demonstrates that both oral neutrophil count and gingivitis reduction were more in twice a day toothbrushing group as compared to once and in 15 days follow up, the oral neutrophil count reduction was greater **(21.9%)** than gingivitis reduction

(10.12%) in twice a day tootbrushing group. On the basis of this percentage reduction in oral neutrophil count in people who brushed twice a day, if similar study will be done on a larger group of people and for a longer duration and if the result of that study shows a positive result then we can motivate the public to adopt twice a day tooth brushing for maintaining better oral hygiene.

CONCLUSION

The results of this pilot study demonstrated that, clinically Oral neutrophil count reduction was greater than gingivitis reduction in twice a day tootbrushing group as compared to once a day toothbrushing. Whereas statistically there was no significant difference in the gingival scores and oral neutrophil count among people who brush once a day and people who brush twice a day. So if this study will be done on a larger group of people and for a longer duration of time then it might show a positive result.

This study shows some direction for conducting further studies in this area.

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Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Mirza BA, Hussain VM. The effect of frequency of tooth brushing on the gingival status among 10-13 years old school children in Dohuk. J Bagh Coll Dentistry, 2006; 18(3): 48-50.
2. Barenie JT, Leske GS, Ripa LW. The relationship of frequency of toothbrushing, oral hygiene, gingival health and caries-experience in school children. J Public Health Dent., 1973; 33(3): 160-71.
3. Amunulla A, Venkatesan R, Ramakrishnan H, Arun KV, Sudarshan S, Talwar A. Lymphocyte subpopulation in healthy and diseased gingival tissue. J Indian Soc Periodontol, May-Aug., 2008; 12(2): 45-50.
4. Landzberg M, Doering H, Aboodi GM, Tenenbauem HC, Glogauer M. Quantifying oral inflammatory load: oral neutrophil counts in periodontal health and disease. J Periodontal Res., Jun, 2015; 50(3): 330-6.
5. Nair KS, Ebenezer S, Jalaluddin MD, Faizuddin M, Jayanthi D. Leucocyte Count: A Window to Periodontal Disease? A Pilot Study. IOSR –JDMS. Aug 2014; 13(8): 54-57.
6. Khursheed DA, Zardawi FM, Shamall OA, Hamagharib DS, Hawzhen MM, Azheen JA, Ranjdar MT. Effects of tooth brushing maneur on gingival health status. IOSR-JDMS. Sep., 2015; 14(9): 90-96.
7. May Gh Al–Ajrab. Oral hygiene and gingival health status among teenagers population lived in Al–Rashidiya, Ninevah. Al–Rafidain Dent J., 2005; 5(2).
8. Van Dyke TE, Vaikuntam J. Neutrophil function and dysfunction in periodontal disease. Curr Opin Periodontol, 1994; 28: 19-27.
9. Vibhute, Akshay, and K. L. Vandana. "The effectiveness of manual versus powered toothbrushes for plaque removal and gingival health: a meta-analysis." Journal of Indian Society of Periodontology, 2012; 16(2): 156.
10. Claydon NC. Current concepts in toothbrushing and interdental cleaning. Periodontol, 2000; 2008; 48: 10-22.
11. Prasad K.V.V, Anupama I, Rao N, Sreenivasan PK, Subramanyam R, Kulkarni RD. The effect of the dentifrice on gingivitis and dental plaque: A 6-week clinical study in India. JIAPHD. Jan-march, 2015; 13(1): 4-10.
12. Lo¨e H, Theilade E, Jensen S. Experimental gingivitis in man. I. J Periodontol, 1965: 36: 177–187.
13. Lukac J, Mravak-Stipetić M, Knezević M, Vrcek J, Sistig S, Ledinsky M, Kusić Z. Phagocytic functions of salivary neutrophils in oral mucous membrane diseases. J Oral Pathol Med., May 2003; 32(5): 271-4.
14. Goldman, Henry, Schlugler, Saul, and Fox, Lewis. Periodontal therapy, St. Louis. Mosby, 1956; 565 p. (p. 518).
15. Ariaudo, A. A., Arnini, S. S., Greene, J. C., and Loe, Harald. In our opinion: How frequently must patients carry out effective oral hygiene procedures in order to maintain gingival health? J. Periodont, May 1971; 42: 309-13.
16. Loe, Harald. A review of the prevention and control of plaque. p. 259-70; (In McHugh, W. D., ed. Dental plaque. London, Livingstone, 1970. 298 p.).
17. Lilienthal, B., Anierena, V., and Gregory, G. An epidemiological study of chronic periodontal disease. Arch. Oral Biol., 10: 553-66, July-Aug. 1965.
18. Stanmeyer, W. R. A measure of tissue response to frequency of toothbrushing. J. Periodont, Jan. 1957; 28: 17-22.
19. Scott DA, Krauss J. Neutrophils in periodontal inflammation. Front Oral Biol., 2012; 15: 56-83.
20. Tempel TR, Snyderman R, Jordan HV, Mergenhagen SE. Factors from saliva and oral bacteria, chemotactic for polymorphonuclear leukocytes: their possible role in gingival inflammation. J Periodontol. 1970 Feb; 41(2): 71-80.
21. Löe H, Silness J: Periodontal disease in pregnancy. I. Prevalence and severity. Acta Odont Scand, 1963; 21: 533–551.

22. Talbott K, Mandel ID, Chilton NW. Reduction of baseline gingivitis scores with repeated prophylaxes. J Prev Dent., Nov-Dec 1977; 4(6): 28-9.
23. Forgas-Brockmann LB, Carter-Hanson C, Killoy WJ. The effects of an ultrasonic toothbrush on plaque accumulation and gingival inflammation. J Clin Periodontol, May, 1998; 25(5): 375-9.
24. Lang NP, Sander L, Barlow A, Brennan K, White DJ, Bacca L, Bartizek RD, McClanahan SF. Experimental gingivitis studies: effects of triclosan and triclosan-containing dentifrices on dental plaque and gingivitis in three-week randomized controlled clinical trials. J Clin Dent., 2002; 13(4): 158-66.
25. Sharda S, Prasad K. V. V, Shetty P. J. Effectiveness of herbal and 0.12% chlorhexidine mouthwash on gingivitis and salivary neutrophil counts. ejpmr, 2018; 5(2): 506-513.
26. Julious S A. Sample size of 12 per group rule of thumb for a pilot study. Pharmaceut. Statist, 2005; 4: 287–291.