Clinical Efficacy of Probiotics as An Adjunct to Scaling and Root Planning in The Treatment Of Chronic Periodontitis

Sana Ikram[1], Muhammad Arsalan Raffat[2] , Saeeda Baig[3], Shazia Akbar Ansari[4], Kevin Joseph Jerome Borges[2], Nuzhat Hassan[5]

Abstract

Objective: The objective of this study was to assess the clinical efficacy of scaling and root planing (SRP) alone and SRP along with adjunctive probiotic containing Lactobacillus reuteri (L.reuteri) in the management of chronic periodontitis (CP) and comparing the efficacy of two treatment modalities.

Methods: Twenty-eight systemically healthy participants, clinically diagnosed with CP on the bases of pocket depth were enrolled from the outpatient department of Periodontology, Ziauddin College of Dentistry, Ziauddin University, Clifton, Karachi, Pakistan. After complete periodontal examination all clinical periodontal parameter i.e., Probing Pocket depth (PPD), Clinical attachment level (CAL), percentage sites for plaque accumulation (PI) and bleeding on probing (BOP) were measured and recorded at baseline, 6 weeks and 12 weeks after therapy. Patients meeting the inclusion criteria underwent non-surgical periodontal therapy i.e. SRP both with hand instruments and with ultrasonic scalers. After SRP oral hygiene measures were reassured and then participants were randomly allocated to one of two groups i.e., Probiotics (N=14) or Placebo (N=14). Both L. reuteri and placebo were given twice daily for 12 weeks and patients were recalled for assessment.

Results: At baseline both groups were similar clinically. Intra-group comparison of clinical periodontal parameters showed improvement in the participants of both groups. Inter-group comparison showed greater reduction in PPD and BOP and more gain in CAL in probiotic group on each follow up visits and statistically significant difference was observed in two groups. Whereas for PI the difference between two groups at follow-up visit was insignificant i.e. both treatment strategies were equal in reducing percentage sites of plaque accumulation.

Conclusion: The results of our study concludes L.reuteri containing probiotic along with SRP is superior when compared to SRP alone in terms of resolving inflammation and improving periodontal health. Probiotics can be used as a promising alternative adjunctive therapy with minimal or no side effects in the treatment of chronic periodontitis.

Keywords: Chronic Periodontitis, probiotics, lactobacillus reuteri, root planing.

IRB: Approved by the Ethical Review Committee of Ziauddin University, Ref Code: 0220817SIOB. Dated: 1st October 2017.

Citation: Ikram S, Hassan N, Baig S, Ansari SA, Borges KJJ, Raffat MA. Clinical Efficacy of Probiotics as An Adjunct to Scaling and Root Planing in The Treatment of Chronic Periodontitis [online]. Annals ASH KMDC 2019;24:.

(ASH & KMDC 24(1):31;2019)

Introduction

Inside the oral cavity a balance exists between the resident commensals and pathogenic microbes in healthy condition. An inflammatory response develops whenever this balance is disturbed. Chronic periodontitis (CP) is one such inflammatory response of oral mucosa due to increase in pathogenic organism residing under a shelter of calculus and is the second most common cause of tooth loss after dental caries[1]. To overcome inflammation there is a need to reduce these pathogenic bacteria[2] to restore the microbial balance. Scaling and root planing (SRP) is an effective non-surgical treatment option as it removes calculus from crown and root surfaces of the teeth and results in reduction of bacterial load[3]. This reduction in bacterial count is

[1] Department of Oral Biology,
Ziauddin Medical College, Ziauddin University.
[2] Department of Oral and Maxillofacial Pathology,
Ziauddin College of Dentistry, Ziauddin University.
[3] Department of Biochemistry,
Ziauddin Medical College, Ziauddin University.
[4] Department of Oral Pathology,
Dow Dental College, Dow University.
[5] Department of Anatomy,
Ziauddin Medical College, Ziauddin University.

Correspondence: Dr. Sana Ikram
Department of Oral Biology,
Ziauddin College of Dentistry, Ziauddin University.
E-mail: drsanaikram@gmail.com
Date of Submission: 22nd October 2018
Date of Acceptance: 23rd January 2019

regardless of their pathogenicity i.e., whether those bacteria are commensal or pathogenic in nature. Considering the physical limitations[4] encountered by SRP in complete removal of microbial deposits i.e. inaccessibility to pocket depth, furcation and interproximal areas, adjunctive therapies have effectively improved clinical periodontal outcomes[4]. After removal of calculus through SRP, harbouring oral mucosal surface with commensals, good or beneficial bacteria i.e., probiotics can be of greater interest as they not only resolve inflammation but also help in the shift of microbial environment from pathological to commensal[5]. According to the World Health Organization (WHO) probiotics are the live microorganisms which when administered in adequate amount they confers health benefits to the host[6].

During the last decade use of beneficial bacteria "probiotics" have proven to be effective in management and prevention of various systemic conditions/pathologies[7]. These include respiratory infections, rheumatoid arthritis, cardiovascular disease, renal disease, obesity, diabetes and gastric disturbance including diarrhoea and inflammatory bowel disease[7,8]. In the field of dentistry probiotics are found to be effective in the prevention of tooth decay (dental caries)[9], halitosis and oral infections[10,11]. Currently the conventional non-surgical periodontal treatment in Pakistan comprises of SRP with systemic antibiotics as an adjunctive[12]. Systemic antibiotics have various adverse effects such as antimicrobial resistance, destruction of the normal flora, gastro-intestinal problems and disturbances, and in few cases occurrence of superinfection[13]. Considering the above-mentioned detrimental effects of systemic antibiotics an alternative treatment approach with less or no side effects could be of great interest. Probiotics can be that alternative but its role in the field of periodontology is still debatable.

Combining the antibacterial properties of these beneficial probiotics with SRP could be efficacious in overcoming the limitations offered by SRP with minimal or less side effects. Therefore, the research question of this study was whether the combination of probiotic and SRP will resolve periodontal inflammation and improve clinical periodontal outcome more efficiently than SRP alone.

Subjects and Methods

This double-blind placebo controlled clinical trial (RCT) was performed in the setting of Ziauddin College of Dentistry, Ziauddin University, Clifton, Karachi, Pakistan. This RCT was designed to evaluate the clinical impact of SRP alone and SRP combined with Probiotic containing Lactobacillus reuteri (L.reuteri), and to compare these two treatment modalities in the patients with chronic periodontitis.

A total of 67 patient were screened in the department of periodontology for the diagnosis of CP. Subjects from both genders, age $\geq$ 30 years, who were systemically healthy with clinically diagnosed CP, having pocket depth of $\geq$4mm were included in the study. Subjects with systemic or local illness or having the history of antibiotic use 2 months prior to the commencement of study, smoking or alcohol were excluded. Pregnant and lactating women and subjects with history of any periodontal treatment during past 6 months were excluded. Subjects who were unable to maintain their oral hygiene and failed to give written consent were also excluded from the study. After explaining the study procedure and pros and cons of the therapy, verbal and written informed consent was taken from the patients and total 28 participants were enrolled in the study (Fig 1).

All study participants meeting the inclusion criteria of the study were allocated to one of two groups (Group 1= SRP + placebo and Group 2 = SRP + probiotics) through randomization. For the purpose of randomization sealed opaque envelopes were used. Participants were asked by the appointed research assistant to pick one envelope containing the name of the therapy which was provided to them after the baseline periodontal examination and recording the clinical periodontal parameters. Throughout the study period full blinding was maintained with the help of research assistant who held all the details related to the study groups and treatment strategy till the completion of the trial and complete analysis of the research data.

Sample size was calculated to be 14 in each group through the formula of clinical superiority trials from the website www.sealedenvelope.com, setting

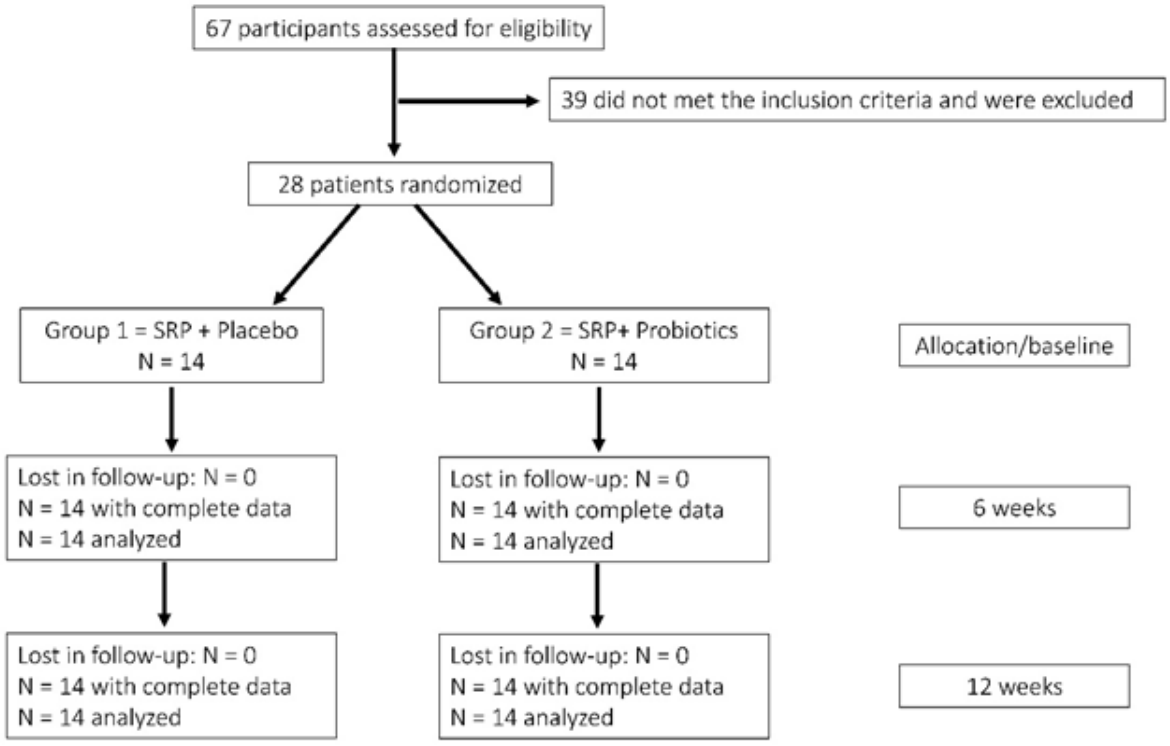

Fig 1. Patient selection criteria.

the power of the study at 95%, taking into account the mean study outcomes of L. reuteri and p-value ≥ 0.05 was taken as significant.

The study was conducted after approval of Ethics Review Committee (ERC) of Ziauddin University, Karachi, Pakistan (Reference code: 0220817SIOB). Study protocols were followed as per guideline of Consolidated Standards of Reporting Trials (CONSORT). Clinical trial was registered in the website www.Clinicaltrial.gov priorto initiating of the study (Reference code: NCT03499184).

Plaque index (PI), bleeding on probing (BoP), probing pocket depth (PPD) and clinical attachment loss (CAL) were the clinical periodontal parameters which were taken into account as a measure of periodontal health[14]. These clinical periodontal parameters were recorded with the help of community periodontal index probe at the baseline (day 0), 6 weeks and 12 weeks intervals from all the teeth excluding 3rd molars. All participants were taught brushing technique (modified bass technique) and were instructed to use similar tooth paste (Colgate Total) during study period, twice daily. After complete periodontal examination baseline recordings of all periodontal parameters were taken and then all participants underwent SRP through both hand and ultrasonic scaler (Woodpacker). After SRP participant according to their allocated groups were given adjunctive therapy for 12 weeks i.e. Group 1 was given placebo powder containing sachets and Group 2 was given probiotics containing sachets. Both probiotic and placebo powder were similar in colour and texture.

All participants were advised to apply the powder of sachets over the tooth surface around the gingival margin after mixing it with small quantity of normal tap water for 2 minutes, with the help of toothbrush after regular brushing. Participants were recalled for follow-up visits at 6 and 12 weeks intervals.

All collected data were analysed using Statistical Package for the Social Sciences (SPSS) (version 23 for Windows, IBM, Chicago, IL). Results were expressed in mean ± SD and proportion as percentages. Intra-group comparison was performed

using repeated measured ANOVA and inter-group comparison at each interval was performed using independent sample t-test. P-value of ≥ 0.05 was considered to be statistically significant.

Results

Out of 28 participants 17 (60.7%) were males, whereas, 11 (39.28%) were females. Mean age of the participants in Group 1 was 40.14 ± 2.64 years and mean age in Group 2 was 41.78 ± 3.58 years (Table 1). Intra-group difference in the clinical parameters for Group 1 and Group 2 were analysed at baseline-6 week, 6 week-12 week, baseline-12week intervals. Intra-group difference in both study groups at each interval found to be statistically significant (Table 2). Inter-group comparison was made at baseline-6 week, 6 week-12week, baseline-12 week intervals and it revealed a statistically significant difference between Group 1 and Group 2 in PPD, CAL and BOP at three study intervals. Whereas, for PI inter-group comparison was found to be insignificant at 6-12 week and overall 0-12 weeks intervals (Table 3).

There was no dropout during the study period and no side effects were reported by participants with the use of probiotics and placebo.

Discussion

To the best of our knowledge, this was the first study conducted in Pakistan, to evaluate the clinical impact of L.reuteri containing probiotics as an adjunctive therapy along with SRP in improving the clinical periodontal parameters in comparison to SRP alone.

This was double-blind randomized control clinical trial of 12 week. CAL, PPD, BOP and PI were taken as measures of periodontal health and were recorded at baseline and each follow-up visit. The results of this trial suggested that although intra-group improvement in periodontal heath was observed in both groups but additional use of probiotics with SRP yielded greater improvement in the clinical periodontal outcomes. At baseline, all clinical periodontal parameters in both groups were similar. Significantly, larger reduction in PPD and BOP was observed in probiotic group at follow-up visits (Table 3). Patients using local probiotics containing L.reuteri gained significantly more attachment than the patients taking placebo (Table 3). Inter-group comparison of PI was statistically insignificant that indicated the two groups 1 and 2 were similar in reducing percentage sites of plaque accumulation.

In intra-group analysis of our study, Group 1 showed significant reduction in pocket depth, bleeding sites and percentage sites of plaque accumulation and gain in attachment level at baseline-6 week, 6 week-12 week, baseline-12 week intervals (Table 2). Previous literature suggests similar results showing SRP as an effective non-surgical therapeutic mean in improving periodontal health[15,16]. A possible reason could be as SRP removes soft and hard microbial deposits it reduces bacterial load as well as the secretion of inflammatory cytokines[17]. These two etiological factors elicit inflammatory response leads to periodontal destruction. Reducing bacterial count and cytokine secretion could provide support to the surrounding tooth structures to overcome inflammation.

Throughout the study period, intra group comparison of group 2 showed significantly improved periodontal outcomes (Table 2). PPD reduced and CAL gained significantly. Significantly, less sites with bleeding and plaque accumulation were also observed in the group. This result is in accordance with previous study[18]. The possible reason could be that introduction of L. reuteri into oral microbiome will shift the microbiota from pathological to commensal[14] making the oral environment favourable for periodontal healing. Another possible reason could be the effect of L. reuteri on bone growth as this bacteria is able to induce bone growth[19]. Gain in clinical attachment level could be due to the bone growth promoting properties of L. reuteri.

Table 1. Demographic data

Variables	Treatment groups	
	Group 1 SRP + Placebo	Group 2 SRP + Probiotics
Number of patients (n)	14	14
Gender (M/F)	8/6	9/5
Age (mean in years ± SD)	40.14 ± 2.64	41.78 ± 3.58

n; sample size, SD; Standard deviation, SRP; Scaling and root planing.

Table 2. Intra-group difference

Variable	Treatment Group 1 Baseline	Day 42	Day 84	p-value	Treatment Group 2 Baseline	Day 42	Day 84	p-value
Over all percentage of the site with plaque (PI)	84.58 ± 8.06	54.38 ± 8.13	33.67 ± 9.47	.00	85.23 ± 8.23	43.46 ± 9.17	26.28 ± 4.12	.00
Overall % of site with BOP	71.94 ± 23.13	58.23 ± 12.77	46.24 ± 11.40	.00	70.47 ± 11.8	34.25 ± 6.32	13.89 ± 3.25	.00
PPD Overall	4.25 ± 1.12	4.08 ± 0.76	3.95 ± 0.78	.00	4.32 ±0.91	3.44 ± 0.64	2.54 ± 0.52	.00
CAL Overall	4.12 ± 0.74	3.99 ± 0.89	3.86 ± 0.59	.00	4.08 ± 0.66	3.69 ± 0.67	3.24 ± 0.47	.00

PI; plaque index, BOP; bleeding on probing, PPD; probing pocket depth, CAL; clinical attachment loss.

Table 3. Inter-group comparison

Variables	Treatment		Intervals Baseline to 6 weeks	6week to12 weeks	Baseline to 12 weeks
PPD	Group 1	Group 2	.01	.00	.00
CAL	Group 1	Group 2	.00	.00	.00
Over all percentage of the site with plaque (PI)	Group 1	Group 2	.00	.13	.18
Over all percentage of the site with BOP	Group 1	Group 2	.00	.00	.00

PPD; probing pocket depth, CAL; clinical attachment loss, PI; plaque index, BOP; bleeding on probing.

During inter-group comparison, Group 2 was found to be significantly more effective than placebo group as an adjunct to SRP. The possible reason could be that although SRP effectively eliminates calculus but in certain situations such as in cases of increased pocket depth and crowding of teeth and malocclusion, access to the pocket depth becomes difficult. These circumstance makes calculus and plaque removal[20] difficult, the chances of bacterial recolonization[14] becomes higher. Studies show that these limitations of SRP alone could be overcome by addition of adjunctive therapies[15,21]. One of the possible reason for the significant improvement in test group might be due to antibacterial effects of L. reuteri. L. reuteri causes increased mucin secretion[22] from epithelium surface which further enhances the epithelial barrier creating difficulties in bacterial adhesion to the epithelium. In addition, this probiotic and pathogenic microbe share the same binding sites over mucosal surface. Competition for these binding sites by L. reuteri over mucosal surface[23] results in competitive exclusion of pathogenic microbes[24]. L. reuteri also produces reuterin[25,26] which is a bacteriocin that is bactericidal in nature. All of these properties of L.reuteri make it a favourable natural antibacterial agent. Apart from overcoming deficiencies of SRP, this treatment strategy prevents recolonization of pathogens[14], which is one of the foremost reason of relapse of non-surgical periodontal treatment. The results of inter-group comparison of our study for PPD,CAL and BOP is similar to previously published data[14,18,27]. However, our data for percentage sites of plaque accumulation differs from previous studies[13,17,26].

Although intra-group improvements were observed for percentage sites for plaque accumulation but both groups showed similar reduction in plaque accumulation. This may be due to the fact that plaque accumulation can be reduced with proper oral hygiene maintenance[28,29] and does not entirely depend on SRP. Previous studies reported reduction in plaque accumulation with the use of probiotic[18] and antibiotics[30] in comparison to SRP alone but over study showed insignificant difference between two groups.

Although probiotics complement the clinical impact of SRP further studies are warranted to

evaluate the effect of SRP and probiotics at radiological and microbiological level.

Conclusion

The results of our study concludes L.reuteri containing probiotic along with SRP is superior when compared to SRP alone in terms of resolving inflammation and improving periodontal health. Probiotics can be used as a promising alternative adjunctive therapy with minimal or no side effects in the treatment of chronic periodontitis.

Acknowledgement

The authors would like to acknowledge Ziauddin University for funding this study.

Conflict of interest

The authors declare no conflict of interest, and all authors have studied and approved the final manuscript.

References

1. Petersen PE, Ogawa H. The global burden of periodontal disease: towards integration with chronic disease prevention and control. Periodontol 2000 2012;60:15-39. [DOI: 10.1111/j.1600-0757.2011.00425.x.].
2. Lamont RJ, Hajishengallis G. Polymicrobial synergy and dysbiosis in inflammatory disease. Trends Mol Med 2015;21:172-83. [DOI: 10.1016/j.molmed.2014.11.004.].
3. Akram Z, Abduljabbar T, Kellesarian SV, Hassan A, Ibrahim M, Javed F, et al. Efficacy of bisphosphonate as an adjunct to nonsurgical periodontal therapy in the management of periodontal disease: a systematic review. Br J Clin Pharmacol 2017;83:444-54. [DOI: 10.1111/bcp.13147.].
4. Vohra F, Akram Z, Safii SH, Vaithilingam RD, Ghanem A, Sergis K, et al. Role of antimicrobial photodynamic therapy in the treatment of aggressive periodontitis: a systematic review. Photodiagnosis Photodyn Ther 2016;13:139-47. [DOI: 10.1016/j.pdpdt.2015.06.010.].
5. Gupta N, Sharma S, Sharma VK. Probiotic-An emerging therapy in recolonizing periodontal pocket. J Oral Biol Craniofac Res 2017;7:72-3. [DOI: 10.1016/j.jobcr.2016.09.002.].
6. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506. [DOI: 10.1038/nrgastro.2014.66].
7. Singh VP, Sharma J, Babu S, Singla A. Role of probiotics in health and disease: a review. J Pak Med Assoc 2013;63:253-7.
8. Carlson J, Slavin JL. Health benefits of fibre, prebiotics and probiotics: A review of intestinal health and related health claims [Online]. Quality Assurance and Safety of Crops & Foods 2016;8:539-54. Available from: https://www.wageningenacademic.com/doi/10.3920/QAS2015.0791. Accessed on: 19th November 2018. [DOI: https://doi.org/10.3920/QAS2015.0791].
9. Lin T-H, Lin C-H, Pan T-M. The implication of probiotics in the prevention of dental caries. Appl Microbiol Biotechnol 2018;102:577-86. [DOI: 10.1007/s00253-017-8664-z.].
10. Yoo J-I, Shin I-S, Jeon J-G, Yang Y-M, Kim J-G, Lee D-W. The Effect of Probiotics on Halitosis: a Systematic Review and Meta-analysis. Probiotics Antimicrob Proteins 2017:1-8. [DOI: 10.1007/s12602-017-9351-1.].
11. Ohshima T, Kojima Y, Seneviratne CJ, Maeda N. Therapeutic application of synbiotics, a fusion of probiotics and prebiotics, and biogenics as a new concept for oral candida infections: a mini review. Front Microbiol. 2016;7:10. [DOI: 10.3389/fmicb.2016.00010.].
12. Moideen S, Siddiq A, Habib A. Antimicrobial Intervention in Periodontal Therapy [Online]. EC Dental Science. 2018;17:1219-23. Available from: https://www.ecronicon.com/ecde/pdf/ECDE-17-00692.pdf. Accessed on: 20th November 2018.
13. Friedman ND, Temkin E, Carmeli Y. The negative impact of antibiotic resistance. Clin Microbiol Infect 2016;22:416-22. [DOI: 10.1016/j.cmi.2015.12.002.].
14. Teughels W, Durukan A, Ozcelik O, Pauwels M, Quirynen M, Haytac MC. Clinical and microbiological effects of Lactobacillus reuteri probiotics in the treatment of chronic periodontitis: a randomized placebo controlled study. J Clin Periodontol 2013;40:1025-35. [DOI: 10.1111/jcpe.12155].
15. Smiley CJ, Tracy SL, Abt E, Michalowicz BS, John MT, Gunsolley J, et al. Systematic review and meta-analysis on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. J Am Dent Assoc 2015;146:508-24. e5. [DOI: 10.1016/j.adaj.2015.01.028.].
16. Drisko CL. Periodontal debridement: still the treatment of choice. J Evid Based Dent Pract

2014;14:33-41. e1. [DOI: 10.1016/j.jebdp.2014.02.007.].

17. Prakasam S, Srinivasan M. Evaluation of salivary biomarker profiles following non?surgical management of chronic periodontitis. Oral Dis 2014;20:171-7. [DOI: 10.1111/odi.12085.].
18. Vivekananda M, Vandana K, Bhat K. Effect of the probiotic Lactobacilli reuteri (Prodentis) in the management of periodontal disease: a preliminary randomized clinical trial [Online]. J Oral Microbiol 2010;2:5344. Available from: https://www.tandfonline.com/doi/full/10.3402/jom.v2i0.5344. Accessed on: 19th November 2019. [DOI: 10.3402/jom.v2i0.5344].
19. McCabe LR, Parameswaran N. Advances in probiotic regulation of bone and mineral metabolism. Calcif Tissue Int 2018;102:480-488. [DOI: 10.1007/s00223-018-0403-7.]
20. Heitz, Mayfield LJ, Lang NP. Surgical and nonsurgical periodontal therapy. Learned and unlearned concepts. Periodontol 2000 2013;62:218-31. [DOI: 10.1111/prd.12008.].
21. Ikram S, Hassan N, Raffat MA, Mirza S, Akram Z. Systematic review and meta analysis of double blind, placebo controlled, randomized clinical trials using probiotics in chronic periodontitis. J Investig Clin Dent 2018:e12338. [DOI: 10.1111/jicd.12338].
22. Linden S, Sutton P, Karlsson N, Korolik V, McGuckin M. Mucins in the mucosal barrier to infection. Mucosal Immunol 2008;1:183. [DI: 10.1038/mi.2008.5.].
23. Meurman J, Stamatova I. Probiotics: contributions to oral health. Oral Dis 2007;13:443-51.
24. Mukai T, Asasaka T, Sato E, Mori K, Matsumoto M, Ohori H. Inhibition of binding of Helicobacter pylori to the glycolipid receptors by probiotic Lactobacillus reuteri. FEMS Immunol Med Microbiol 2002;32:105-10.
25. Gómez-Torres N, Ávila M, Delgado D, Garde S. Effect of reuterin-producing Lactobacillus reuteri coupled with glycerol on the volatile fraction, odour and aroma of semi-hard ewe milk cheese. Int J Food Microbiol 2016;232:103-10. [DOI: 10.1016/j.ijfoodmicro.2016.05.031.].
26. Dobson A, Cotter PD, Ross RP, Hill C. Bacteriocin production: a probiotic trait? Appl Environ Microbiol 2012;78:1-6. [DOI: 10.1128/AEM.05576-11.].
27. Tekce M, Ince G, Gursoy H, Dirikan Ipci S, Cakar G, Kadir T, et al. Clinical and microbiological effects of probiotic lozenges in the treatment of chronic periodontitis: a 1 year-follow-up study. J Clin Periodontol 2015;42:363-72. [DOI: 10.1111/jcpe.12387.].
28. Slot D, Wiggelinkhuizen L, Rosema N, Van der Weijden G. The efficacy of manual toothbrushes following a brushing exercise: a systematic review. Int J Dent Hyg 2012;10:187-97. [DOI: 10.1111/j.1601-5037.2012.00557.x.].
29. Van der Weijden G, Hioe K. A systematic review of the effectiveness of self performed mechanical plaque removal in adults with gingivitis using a manual toothbrush. J Clin Periodontol 2005;32:214-28.
30. Cosgarea R, Heumann C, Juncar R, Tristiu R, Lascu L, Salvi GE, et al. One year results of a randomized controlled clinical study evaluating the effects of non-surgical periodontal therapy of chronic periodontitis in conjunction with three or seven days systemic administration of amoxicillin/metronidazole [Online]. PloS One 2017;12:e0179592. Available from: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0179592. Accessed on: 19th November 2018. [DOI: 10.1371/journal.pone.0179592].