KARBALA JOURNAL OF MEDICINE

Review

The Impact of Orthodontic Therapy on the Periodontal Health and Oral Microbiome

Zainab M. Kadhom[1], Sara M. Al-Mashhadany[1], Hiba M. Hussein Al-Chalabi[1]

[1]*Department of Orthodontics, College of Dentistry, University of Baghdad, Baghdad, Iraq.*

Article information:
Received: 2024-08-16
Accepted: 2024-09-23
Correspondence: Sara M. Al-Mashhadany
Email:drsara.mshhdany@codental.uobaghdad.edu.iq
https://doi.org/10.70863/karbalajm.v17i2.2152

Abstract

The demand for dental treatment with multiple orthodontic appliances has recently increased for aesthetic purposes. Still, these appliances are not free from some negative influences that may affect the teeth and gingiva, especially periodontium, due to plaque accumulation during treatment. Therefore, special recommendations must be given to orthodontic patients, and they must be encouraged to take care of oral and dental hygiene during the orthodontic treatment period. Thus, this review aimed to identify and summarize the different effects of the various orthodontic appliances on the periodontium and the composition of the microbial community.
Key words: Orthodontic, periodontal health, oral microbiome

Introduction

Maintaining periodontal health is significant and requires particular consideration while implementing an orthodontic treatment regimen, including adult and pediatric populations [1]. Ensuring the preservation of periodontal tissues is a primary focus for orthodontic professionals, prompting the establishment of distinct hygiene procedures tailored to orthodontic patients [2]. Orthodontic treatment has many advantages; in most instances, the benefits outweigh the potential disadvantages. Orthodontic therapy has the potential to significantly improve patients' aesthetics, functionality, and self-esteem [3]. The adverse effects of orthodontic treatment might be apparent in local and systemic forms. Those conditions include dental discolorations, decalcification, root resorption, periodontal problems, psychological disturbances, gastrointestinal problems, allergic responses, infective endocarditis, and chronic fatigue syndrome [4]. Studies have shown that orthodontic pressures can induce an inflammatory response inside the periodontium [5].

Periodontitis is a chronic multifactorial inflammatory disease characterized by the destruction of mineralized and nonmineralized connective tissues of the oral cavity which, represents the late colonization of plaque [6]. In contrast, the initial colonization includes members of the yellow, green, and purple complexes and the *Actinomyces* species [7]. Regarding the increasing need for orthodontic intervention among adults with a greater susceptibility to periodontal disease, the assessment of the effects of orthodontic therapy on the oral microbiota and the duration of any resulting microbiological and clinical changes is of utmost importance [8]. The orthodontist's care for the patient's well-being, satisfaction, and relationship with the orthodontist is shown by the post-procedure communication between the orthodontist and the patients [9].

The Periodontium and oral cavity environment

Gingival epithelium: In periodontal disease, the physical barrier separating the biofilm from the gingival tissue is the gingival epithelium. It functions as the first defense against bacterial invasion. The inflammatory response is initiated by disrupting the gingival epithelial barrier, leading to the infiltration of external pathogens into the host tissues, resulting in a chronic infection [10].

The periodontal ligament: It is a fibrous connective tissue band or sheet that serves an important role in force-induced orthodontic tooth movement and is mechanically connected between the alveolar bone and tooth [11]. The oral cavity serves as a habitat for microbial communities. The complexity of its anatomical composition, interconnections with the external environment, and humid conditions contribute to the ecological distinction and complexity of the

microbiome that inhabits that particular place. The occurrence and growth of the oral microbiota are influenced by a combination of complex endogenous and exogenous factors, which contribute to its maintenance in a dynamic equilibrium [12].

Oral cavity environment: In the digestive system, the mouth cavity harbors the most varied microbiome consisting of about 700 species of bacteria, fungi, viruses, archaea, and protozoans [13]. These microorganisms are naturally present in the mouth cavity. However, they may also cause infections [14-15]. Maintaining a healthy and symbiotic interaction between the host and oral microbiota is of utmost importance during orthodontic treatment to reduce adverse effects, including enamel demineralization, dental caries, and periodontal disease. Several factors, including higher biofilm development, alterations in plaque composition, difficulties with maintaining dental hygiene, and modifications in dietary habits, might contribute to a discrepancy in the original state of the oral microbiome, often known as dysbiosis [16].

Pathophysiology

The beneficial microbiota refers to the living, non-pathogenic microorganisms that possess preventative and therapeutic properties against oral infectious diseases [17]. *Candida albicans*, a harmless commensal fungus, is present in the oral cavity of around 53% of the population [18]. The inhibitory effects of *Streptococcus oralis* and *Streptococcus uberis* on pathogen development have been shown in laboratory and animal models. The identification of *S. oralis* and *S. uberis* indicates the overall health status of periodontium [19]. The direct and indirect interactions of probiotics contribute to enhancing dental health [20]. Another example of pathogenic bacteria, *Streptococcus mutans* and *Lactobacillus* spp., is found in individuals receiving orthodontic treatment, these bacteria are potentially pathogenic gram-positive bacteria [21].

The periodontal ligament mostly comprises type I collagen, with type III collagen fibers. periodontal ligament (PDL) primarily serves the purpose of transmitting proprioceptive signals to the brain and enduring compressive stresses during mastication [22]. The blood flow may undergo alterations due to the tension induced by orthodontic therapy [23]. Moreover, a range of immunoregulatory substances, including interleukin-1 a, interleukin-6, and tumor necrosis factor-a, all are secreted in the context of inflammation and play a role in the periodontal remodeling process [24].

Orthodontic appliance's effects on oral biofilm

The microbiome of the oral cavity is often seen as a multicellular accumulation enclosed inside an extracellular matrix composed of polysaccharides and proteins. The bacterial clusters are attached to a surface and/or to each other and embedded in a self-produced matrix called bacterial biofilms. The biofilm matrix is composed of polysaccharides (e.g., alginate), substances like proteins (e.g., fibrin), as well as eDNA [25].

Dental Plaque is a complex biofilm composed of many bacterial complexes that engage in coaggregation, adhesion, and metabolic interactions, resulting in reciprocal benefits for those involved. The structure of dental plaque changes over time and is influenced by its location. Subgingival and supragingival plaques and early and mature plaques exhibit significant differences and are associated with different pathologies, with caries and periodontal diseases being the most common [26].

The components and types of orthodontic appliances that effects on oral biofilm

1. Brackets and ligation technique

Research has shown that metal brackets with elastomeric ligatures have a higher tendency to enhance bleeding upon probing and retain plaque and a more pronounced plaque index than steel ligatures [27]. Tuckerman *et al*. (2005) studied the effects of two archwire ligation procedures (elastomeric rings and steel ligatures). The study's findings revealed that teeth ligated with elastomeric rings exhibited a higher incidence of bleeding than those ligated with steel ligatures [28]. In contrast, Papaioannou *et al*. (2007) observed no variations in the adhesion of *S. mutans* to brackets made of plastic, stainless steel, or ceramic [29]. Although polymer materials such as composites, glass ionomers, and acrylics provide greater incubation environments for microbes in their pores and surface imperfections, they are not as effective as smoother surfaces like metals, ceramics, and enamel [30]. Kaklamanos *et al*. (2017) observed no difference in gingival index (GI) scores and periodontitis disease (PD) between subjects who used standard brackets and those who used Damon3TM self-ligating brackets [31]. According to research done by Bergamo *et al*. (2016) who found that there was a significant difference in the amount of gingival crevicular fluid in subjects who had treatment with self-ligating brackets. Additionally, there was an increase in plaque index (PI), suggesting that the brackets' design may impact the formation of plaque [32]. The self-ligating bracket is a type of

ligation commonly employed for archwire ligation. Several studies have reported different associations between this system and worse plaque index, higher gram-positive and gram-negative bacteria, particularly *Streptococci* spp. and *Lactobacilli* spp., and increased bleeding. However, statistically, these studies showed non-significant differences between conventional brackets ligated with stainless steel ligatures [33]. In addition, introducing Damon brackets during orthodontic movement decreased adverse effects on the periodontium. Yet, insufficient scientific evidence supports such beneficial effects [34].

2. Molar band vs. bonded molar tubes

A study by Mártha *et al*. (2016) who used the DNA strip to evaluate the existence of eleven periodontopathogenic bacteria in the subgingival plaque of bonded and banded molar teeth based on performed pre-fixed orthodontic treatment showed that the major bacteria during the first two months of treatment were *F. nucleatum* (92%), *E. corrodens* (76%), and *Capnocytophaga* spp. (*C. ochracea, C. sputigena, C. gingivalis*), whereas the remaining species (*P. gingivalis, P. intermedia, A. actinomycetemcomitans, T. denticola, C. rectus, T. forsythia, Eubacterium nodatum and Parvimonas micra*), were detected less commonly. Within the group of participants with bonded molars throughout treatment, *E. nodatum* had the lowest presence [35].

Gingival inflammation around orthodontic bands results in the formation of pseudo pockets, which often resolve with the removal of the appliance. Typically, this problem disappears after a few weeks after the debanding process. Nevertheless, some studies have shown a decreased likelihood of developing gingivitis when plaque, orthodontic force, and tooth movements are absent [36].

3. Functional orthodontic appliances

Interceptive orthodontics employs a range of functional and orthodontic appliances, particularly for children requiring rapid maxillary expansion. Ortu *et al.* (2017) investigated microbial alterations linked to these particular appliances. The researchers assessed the microbial level of *Lactobacillus* spp. and *S. mutans* in a sample of 30 children aged 6-9 years. The participants were divided into three groups: 10 subjects who received rapid palatal expander (RPE) treatment, 10 who received Mc Namara expander treatment, and 10 who served as the control group. They observed a significant increase in the level of *Lactobacilli* spp. and *Streptococci* spp. within each group. However, non-significant statistical differences were seen among the groups, except the Mc Namara expander treatment group which significantly exhibited high levels of Lactobacilli following a 6-month treatment, exceeding both the RPE and control groups [37].

4. Lingual orthodontic appliances

Compared to labial brackets, lingual orthodontic appliances were associated with higher counts of *S. mutans* and *A. actinomycetemcomitans*, increased plaque retention, and gingival irritation. However, the Lactobacillus counts, salivary flow rate, and saliva buffer capacity did not differ [38]. In a 30-day randomized clinical experiment, Sfondrini *et al.* (2012) found no significant difference in bleeding on probing, periodontal pocket depth, and the number of CFUs of Streptococci spp. or anaerobic bacteria between the labial and lingual brackets [39]. Demling *et al.* (2010) assessed the impact of the lingual bracket system on clinical and microbiological factors. The frequency of *P. gingivalis* and *A. actinomycetemcomitans* in the crevicular fluid was determined using a 16SrRNA-based PCR technique. There was a minor microbial shift after four weeks of lingual bonding, *A. actinomycetemcomitans* also showed an increase, while *P. gingivalis* did not. In another research done by the same researcher, after 3 months of therapy, the prevalence of *A. actinomycetemcomitans* and *P. gingivalis* showed non-significant changes. This might be attributed to the high baseline level of *A. actinomycetemcomitans*, which may have masked a bacterial shift [40]. Lombardo *et al.* (2013) observed a higher level of *S. mutans*, and increased gingival inflammation, eight weeks after lingual bonding, a statistically significant increase in plaque index was observed compared to the labial appliances [38].

5. Clear aligners

Different studies showed that clear aligner therapy improves patients' periodontal health more than fixed appliances [41]. There were no statistically significant changes in probing depth and bleeding on probing in patients using clear aligners in both the long and short term [42]. The accumulation of plaque is the primary cause of gingivitis. Patients using aligners may clean their teeth and aligners at any time, enabling them to remove plaque layers readily. Han *et al.* (2015) evaluated the effects of two orthodontic appliances on periodontal tissue in individuals with periodontitis. Relatively, patients with clear aligners had good orthodontic treatment outcomes and good periodontal health. Patients' gingival index, bone level, plaque index, and probing depth all improved following orthodontic therapy [43].

Effect of bonding materials on oral microbiota

Composite resins are used for bracket bonding in fixed orthodontics. However, glass ionomer cements are also utilized, their close contact with the hard tooth tissues and the chance for bacterial adhesion they provide lead to the development of white spot lesions and even caries [44]. Sukontapatipark *et al*. (2001) demonstrated that excess composite material around brackets enhances the formation of a mature plaque biofilm. Still, the adjacent enamel surface only possessed plaque in its first stages of formation. The maturation of dental plaque facilitates and is essential for the emergence and proliferation of particular infections [45].

Orthodontic treatment's clinical implications for periodontal health:

1. Changes in soft tissue

Placing orthodontic attachments, including bands and brackets, creates new retentive areas, which in turn increases plaque formation and triggers an inflammatory response [46]. Gingivitis risk may be raised by orthodontic brackets and elastics that restrict the elimination of dental plaque. Because metal brackets, bands, and auxiliaries are cytotoxic. They may produce localized inflammation. Gingivitis is often seen in conjunction with metal bands because of their subgingival position [47].

In the orthodontic population, gingival hypertrophy is a common problem that may result in forming a false pocket with or without attachment loss. It was thought to be an inflammatory response to the accumulation of bacterial plaque or other factors, like food impaction between teeth and orthodontic devices, food persistence between gingival tissue and orthodontic devices, or chemical irritation from bonding brackets or other orthodontic devices [48]. This reaction is characterized clinically by gingival swelling, pseudo-pocket formation, especially at the proximal areas, and increased signs of inflammation, which usually disappear with the brackets debonding. However, after debonding, this normally resolves in a few weeks [49].

2. Changes that occur in the bone

Loss of alveolar bone and deepening of the gingival crevice resulting lead to pathological periodontal pocket formation also gingival recession which causes root exposure and increased tooth mobility. Severe forms of the disease compromised esthetics, impaired masticatory function, tooth migration, and tooth loss ultimately [50]. Furthermore, it has been reported in the histological study by Melsen *et al.* (2005) that, in the presence of good dental hygiene, orthodontic intrusion can result in new cement formation and new collagen attachment [51]. The presence of protozoa, fungi, and viruses is highly correlated with the severity of chronic periodontitis [52].

Conclusion

Orthodontic appliances of various types significantly impact the oral microbiota, independent of appliance type. Therefore, periodontal health should be checked regularly before orthodontic treatment. Removable appliances had less effect on oral bacteria than fixed ones. Also, in periodontally compromised patients, aligners should be used instead of fixed appliances because they are removable and maintain good oral hygiene. So, the patient must be informed of this beforehand. The patients should be motivated to maintain oral hygiene during orthodontic treatment so the microbial imbalance caused by orthodontic treatment will be prevented.

Funding: There is no funding for this review

Conflict of interest: Authors declare that they have no conflicts of interest that could influence their judgment or review. (Financial, professional, or otherwise).

Author contributions: Conceptualization: Z.M.K. and S.M.A., Methodology: Z.M.K. and S.M.A. Formal analysis and investigation: Z.M.K. and H.M.H., Writing: Z.M.K, Resource: S.M.A. and H.M.H., Supervision: Z.M.K, and S.M.A.

Reference

1. Alfuriji S, Alhazmi N, Alhamlan N, et al. The effect of orthodontic therapy on periodontal health: a review of the literature. International Journal of Dentistry. 2014;585048.
2. Migliorati M, Isaia L, Cassaro A, et al. Efficacy of professional hygiene and prophylaxis on preventing plaque increase in orthodontic patients with multi bracket appliances: a systematic review. Eurepean Journal of Orthodontics.2015;37(3):297–307.
3. Meeran NA. Iatrogenic possibilities of orthodontic treatment and modalities of prevention. Journal of Orthodontic Science. 2013; 73:2-3.
4. Sonwane S, Ganesh P, Kumar PS. Is orthodontic treatment causes bacterial endocarditis? a review based random study. International Journal of Molecular Medical Science. 2013;3(2).
5. Tripuwabhrut P, Brudvik P, Fristad I, Rethnam S, Experimental orthodontic tooth movement and extensive root resorption: periodontal and pulpal changes. European Journal of Oral Sciences. 2010 ;118 (6): 596–603.
6. Rahman MU, Ali Sh, Arafah A. Immunogenetic: molecular and clinical over view. Clinical Application of Immunogenetics. Elsevier/Academic Press. 2022; (22). DOI.10.1016/C2020-0-01767-6

7. Socransky SS, Haffajee AD. Periodontal microbial ecology. Periodontol 2000. 2005;38:135–187.
8. Nazir MA. Prevalence of periodontal disease, its association with systemic diseases and prevention. International Journal of Health Science (Qassim). 2017;11(2):72–80.
9. Eppright, M, Shroff, B, Best, AM, Barcoma, E, Lindauer, SJ. Influence of active reminders on oral hygiene compliance in orthodontic patients. The Angle Orthodontist, 2014;84(2): 208-213.
10. Takahashi N, Sulijaya B, Yamada-Hara M, Tsuzuno T, Tabeta K, Yamazaki K. Gingival epithelial barrier: regulation by beneficial and harmful microbes. Tissue Barriers. 2019; 7(3):1651158.
11. Li Z, Yu M, Ji, S, Wang Y, Luo R, Huo B, Liu D, He D, Zhou Y, Liu Y. Stress distribution and collagen remodeling of periodontal ligament during orthodontic tooth movement. Frontiers in Pharmacology. 2019; 10: 1263.
12. Li X, Liu Y, Yang X, Li C, Song Z. The oral microbiota: community composition, influencing factors, pathogenesis, and interventions. Frontiers in Microbiology. 2022; 13.
13. Maukonen, J., Matto, J., Suihko, M.L. and Saarela, M, Intra-individual diversity and similarity of salivary and faecal microbiota. Journal of Medical Microbiology. 2008;57(12): 1560-1568.
14. Sheiham A. Dietary effects on dental diseases. Public Health Nutrition. 2001; 4(2b):569-591.
15. Peterson SN, Snesrud E, Liu J, Ong AC, Kilian M, Schork NJ, Bretz W. The dental plaque microbiome in health and disease. Public Library of Science One. 2013;8(3):58487.
16. Kilian M, Chapple ILC, Hannig M, Marsh PD, Meuric V, Pedersen AML, Tonetti MS, Wade WG, Zaura E. The oral microbiome–an update for oral healthcare professionals. British Dental Journal. 2016 ;221(10):657-666.
17. Zaura E, Twetman S. Critical appraisal of oral pre-and probiotics for caries prevention and care. Caries Research. 2019;53(5):514-526.
18. Zunt SL.Oral candidiasis: diagnosis and treatment. The Journal of Practical Hygiene. 2000; 9: 31–36.
19. Elavarasu S, Jayapalan P, Murugan T. Bugs that debugs: Probiotics. Journal of Pharmacy & Bioallied Sciences. 2012;4(Suppl 2):319.
20. ReddyRS, Swapna LA, Ramesh T, Singh TR, Vijayalaxmi N, Lavanya R. Bacteria in oral health-probiotics and prebiotics a review. International Journal of Biological and Medical Research. 2011;2(4):1226-1233.
21. Contaldo M, Lucchese A, Lajolo C, Rupe C, Di Stasio D, Romano A, Petruzzi M, Serpico R. The oral microbiota changes in orthodontic patients and effects on oral health: An overview. Journal of Clinical Medicine. 2021; 10(4):780.
22. Vandevska-Radunovic V, Kvinnsland IH, Kingsland S, Jonsson R,. Immunocompetent cells in rat periodontal ligament and their recruitment incident to experimental orthodontic tooth movement. European Journal of Oral Sciences. 1997;(105)1: 36–44.
23. Santamaria M, Milagres D, Sasso Stuani A, Sasso Stuani MB, de Oliveira Ruellas AC. Initial changes in pulpal microvasculature during orthodontic tooth movement: a stereological study. European Journal of Orthodontics. 2006 ;(28) 3:217–220.
24. Bletsa A, Berggreen E, Brudvik P. Interleukin-1α and tumor necrosis factor-α expression during the early phases of orthodontic tooth movement in rats. European Journal of Oral Sciences. 2006;(114) 5: 423–429.
25. Stoodley P, Hall-Stoodley L. Evolving concepts in biofilm infections. Cell Microbial. 2009; 11:1034–1043.
26. Kwon T, Lamster IB, Levin L. Current concepts in the management of periodontitis. International Dental Journal.2021; 71(6):462-476.
27. Contaldo M, Lucchese A, Lajolo C, Rupe C, Di Stasio D , Romano A, Petruzzi M., Serpico R. The oral microbiota changes in orthodontic patients and effects on oral health: an overview. Journal of Clinical Medicine. 2021; 10:780.
28. Türkkahraman H, Sayin MO, Bozkurt FY, Yetkin Z, Kaya S, Onal S. Archwire ligation techniques, microbial colonization periodontal status in orthodontically treated patients. Angle Orthodontics. 2005;75: 231–236.
29. Papaioannou W, Gizani S, Nassika M, Kontou E, Nakou M. Adhesion of *Streptococcus mutans* to different types of brackets. Angle Orthodonics. 2007; 77:1090–5. doi: 10.2319/091706-375.1.
30. ØiloM, Bakken V. Biofilm and Dental Biomaterials. Materials. 2015; 8:2887–900. doi: 10.3390/ma8062887
31. Kaklamanos EG, Mavreas D, TsalikisL, Karagiannis V, Athanasiou AE. Treatment duration and gingival inflammation in Angle's ClassI malocclusion patients treated with the conventional straight-wire method and the Damon technique: a single center, randomized clinical trial. Journal of Orthodontics. 2017;44(2):75–81.
32. Bergamo AZ, Nelson-Filho P, Romano FL, et al. Gingival crevicular fluid volume and periodontal parameters alterations after use of conventional and self-ligating brackets. Journal of Orthodontics. 2016;43(4):260–267.
33. Uzuner FD, Kaygisiz E, Çankaya ZT. Effect of the bracket types on microbial colonization and periodontal status. Angle Orthodontics. 2014; 84: 1062–1067.
34. Willeit FJ, Cremonini F, Willeit P, et al. Stability of transverse dental arch dimension with passive self-ligating brackets: a6-year follow-up study. Progress in Orthodontucs. 2022;23(1):19.
35. Mártha K, L″orinczi L, Bica C, Gyergyay R, Petcu B, Lazar L. Assessment of periodontopathogens in subgingival biofilm of banded and bonded molars in early phase of fixed orthodontic treatment. Acta Microbiologica et Immunologica Hungarica. 2016; 63:103–113.
36. Dannan A. An update on periodontic-orthodontic interrelationships. Journal of Indian Society of Periodontology. 2010; (14) 1:66–71, 2010.
37. Ortu E, Sgolastra F, Barone A, Gatto R, Marzo G, Monaco A. Salivary *Streptococcus mutans* and *Lactobacillus* spp. Levels in patients during rapid palatal expansion. Eurpean Journal of Paediatric Dentistry. 2014; 15: 271–274.
38. Lombardo L, Ortan YÖ, Gorgun Ö, Panza C, Scuzzo G, Siciliani G. Changes in the oral environment after placement of lingual and labial orthodontic appliances. Progress in Orthodontics. 2013; 14:1–8. doi: 10.1186/2196-1042-14-28.
39. Sfondrini MF, Debiaggi M, Zara F, Brerra R, Comelli M, Bianchi M, et al. Influence of lingual bracket position on microbial and periodontal parameters *in vivo*. Journal of Applied Oral Science. 2012; 20:357–61. doi: 10.1590/S1678-77572012000300011.

40. Demling A, Demling C, Schwestka-Polly R, Stiesch M, Heuer W. Short-term influence of lingual orthodontic therapy on microbial parameters and periodontal status. Apre-liminarystudy AngleOrthodontists. 2010;80(3): 480–484.
41. Jiang Q,LiJ ,MeiL,et al. Periodontal health during orthodontic treatment with clear aligners and fixed appliances: a meta-analysis. Jam Dent Assoc 2018; 149(8): 712–20.
42. Azaripour A, Weusmann J, Mahmoodi B, et al. Braces versus Invisalign: gingival parameters and patients satisfaction during treatment: a cross-sectional study. BMC Oral Health. 2015;15:69.
43. Han JY. A comparative study of combined periodontal and orthodontic treatment with fixed appliances and clear aligners in patients with periodontitis. Journal of Periodontal and Implant Science. 2015;45(6):193–204.
44. Øgaard B, Rølla G, Arends J, Ten Cate JM. Orthodontic appliances and enamel demineralisation Part 2. Prevention and treatment of lesions. American Journal of Orthodontics and Dentofacial Orthopedics. 1988; 94(2), :123-128.
45. Sukontapatipark W, El-Agroudi MA, Selliseth NJ, Thunold K, Selvig KA. Bacterial colonisation associated with fixed orthodontic appliances. A scanning electron microscopy study. The European Journal of Orthodontics. 2001;23(5):475-484.
46. Alexander SA. Effects of orthodontic attachments on the gingival health of permanent second molars. American Journal of Orthodontics and Dentofacial Orthopedics. 1991;100(4):337-340.
47. Sallum EJ, Nouer DF, Klein MI., Gonçalves RB, Machion L, Sallum AW, Sallum EA. Clinical and microbiologic changes after removal of orthodontic appliances. American Journal of Orthodontics and Dentofacial Orthopedics. 2004; 126(3):363-366
48. Eid HA, Assiri HAM, Kandyala R, Togoo RA, Turakhia VS. Gingival enlargement in different age groups during fixed Orthodontic treatment. Journal of International Oral Health. 2004; 6(1):1.
49. Kouraki E, Bissada, NF, Palomo JM, Ficara AJ. Gingival enlargement and resolution during and after orthodontic treatment. New York State Dental Journal. 2005: 71(4):34.
50. Newman MG. Classification and epidemiology of periodontal diseases. In: Newman MG, Takei H, Carranza FA, editors. Carraza's Clinical Periodontology. 10th ed. Philadelphia: WB Saunders Company; 2007: 100–29.
51. Melsen B, Allais D. Factors of importance for the development of dehiscences during labial movement of mandibular incisors: a retrospective study of adult orthodontic patients. American Journal of Orthodontics and Dentofacial Orthopedics. 2005;127(5):552–561.
52. Horz HP, Robertz N, Vianna ME, Henne K, Conrads G. Relationship between methanogenic archaea and subgingival microbial complexes in human periodontitis. Anaerobe. 2015; 35:10-12.