Original Article

Impact of Nonsurgical and Antibiotics Treatment on Periodontitis

Azhar Abdullah Imran, Zeyad Nazar M. Al-Talib[1], Fatima Al-Rubea Malik

Departments of Microbiology, College of Dentistry, [1]Periodontology, Faculty of Dentistry, University of Babylon, Babylon, Iraq

Abstract

Background: Periodontitis is one of the most prevalent chronic inflammatory noncommunicable and multifactorial diseases. **Objectives:** The present study was aimed to detect the effect of systemic antibiotics as disjunctive to nonsurgical treatment of generalized periodontitis (grade I and grade II) on the bacterial count and clinical periodontal parameters. **Materials and Methods:** Subgingival plaque samples were collected from 40 patients with generalized periodontitis, 20 patients were treated with scaling and root planning only, and 20 patients were treated with a combination of ciprofloxacin 500 mg and metronidazole 500 mg with scaling and root planning. Moreover, 20 samples were collected from healthy patients as a control group. Specific periodontal pathogen genes for *Tannerella forsythia* (BspA, CIpB genes) were identified using real time polymerase reaction (RT-PCR). **Results:** In this study, 120 samples were detected for the presence of *T. forsythia* by RT-PCR technique, and the specific ClpB, and BspA primers' genes were used for the amplification of a fragment of these genes for the identification of *T. forsythia*. The results showed that the presence of ClpB and BspA in all (100%). BspA and ClpB showed a decrease but non-significant differences before and after treatment, whereas the periodontal parameter, probing pocket depth, plaque score, and bleeding on probing showed a significant decrease after treatment except clinical attachment lose showing a non-significant difference. **Conclusion:** Clinical treatment (scaling and root planning) is often helpful in the treatment of generalized periodontal disease (grade I and grade II) without the need for antimicrobial therapy as soon as possible.

Keywords: Antibiotic, nonsurgical treatment, periodontitis, RT-PCR, *Tannerella forsythia*

Introduction

Periodontal disease is an infection of the soft plus solid tissues that support the teeth, and it is regarded as being one of the leading causes of tooth damage in the world.[1] It develops as a result of untreated gingivitis, a condition caused by the accumulation of dental plaque and characterized clinically by inflammation of the margina1 gums by bleeding on probing. If this procedure is not stopped, periodontitis will develop, resulting in irreparable periodontic attachment loss, pockets, recessions, and finally tooth quality and loss.[2] Periodontal disease may be a chronic disease triggered by microorganism infections that activate the host' immune responses, thereby inflicting damage of the assistant tissues of the teeth, forming periodontic pockets, and finally resulting in tooth loss.[3] These challenges could be caused by the advanced periodontal bacteria, which have a lot of variations across people. Also, it has been well established that there are differences in the distribution of periodontal bacteria among populations.[4]

The goal of periodontal cure is to remove bacterial deposits and thereby reverse the inflammatory process. Scaling and root planning (SRP) is a nonsurgical gold-operative treatment that removes dental plaque and calculus while also polishing root surfaces.[5] The most relevant drawback of SRP is the lack of long-term results as a result of ultimate microorganism recolonization following medical treatment.[6] As a result, related treatments such as antibiotics, photodynamic therapy, antioxidants, natural substances, supplements (e.g., melatonin), and, more recently, probiotic therapy, have been introduced to SRP.[7] This last strategy, in particular, is gaining traction in the

Access this article online

Quick Response Code:

Website: https://journals.lww.com/mjby

DOI: 10.4103/MJBL.MJBL_99_23

Address for correspondence: Ms. Azhar Abdullah Imran, Department of Microbiology, College of Dentistry, University of Babylon, Hillah, Babylon, Iraq.
E-mail: azhar.imran.hd124@student.uobabylon.edu.iq

Submission: 30-Jan-2023 **Accepted:** 27-Feb-2023 **Published:** 27-Sep-2023

How to cite this article: Imran AA, Al-Talib ZNM, Malik FA-R. Impact of nonsurgical and antibiotics treatment on periodontitis. Med J Babylon 2023;20:486-90.

scientific community due to the lack of antibiotic-related side effects.[8]

Probiotics, according to the Food and Agriculture Organization and therefore the World Health Organization, are "live microorganism that impart a health benefit on the host when provided in suitable proportions."[9] Probiotics effects will be clarified through a variety of processes, including competition with periodontopathogens, the assembly of antimicrobial compounds, the enhancement of the membrane barrier, and immunomodulatory effect.[10] The large number of microorganism species seen in standard periodontic microbiota can make determining microorganism profiles in sub-animal tissue plaques even more difficult.[11] It is possible that low-level dental medicine pathogens can be found in periodontally healthy people. These choices for periodontal microbiota, which can induce inflammation, highlight the necessity for a second proper microbe designation to aid in clinical diagnosis and lead to a more comprehensive treatment strategy. People with and without periodontal inflammation have the same five bacteria species in their sub-animal tissue biofilms.[12] The red complex, which includes *Porphyromonase gingivalis*, *Tannerella forsythia*, and *Spirochete denticola*, is an example of this.[13]

Tannerella forsythia, a gram-negative anaerobic organism, inhabits the mature biofilm gift at sites expressing progressive periodontitis. It is a neighborhood of "red complex" cluster that contributes to the pathologic process of periodontitis. The BspA macromolecule and ClpB sequence encoded amino acid enzyme play an important role within the virulence of *T. forsythia*.[14] ClpB could be a crucial part of protein internal control, which maintains a protein physiological condition (proteostasis) in microorganism cells and supports their existence below environmental stresses by mediating the reactivation of protein collections.[15] BspA proteins are leucine-rich repeat (LRR)-containing proteins characterized by a 23 organics compound-long repetitive motif (called TpLRR) that is generally found in the N-terminal region.[16] These surface proteins mediate host pathogen interactions and promote cell aggregations. Moreover, BspA-deficient mutants of *T. forsythia* are less probable to induce an alveolar bone loss in mice. BspAs are found in different eukaryotic pathogens appreciate, wherever they were found to be concerned in taxis toward a tumor sphacelus issue.[17] It is thought that genes encryption BspA was introduced into the genomes of *T. forsythia* and contemplated virulence factors.[18]

The aim of the present study was to detect the effect of systemic antibiotics as adjunctive to nonsurgical treatment of generalized periodontitis (grade I and grade II) on the bacterial count and clinical periodontal parameters.

Materials and Methods

In this study, 40 patients with generalized periodontitis (grade I and grade II) from both sex (35 male patients and 5 female patients) with age range from 25 to 60 years were divided into two groups, in which 20 patients were treated with nonsurgical periodontal treatment only, and 20 patients were treated with combination of amoxicillin 500 mg and metronidazole 250 mg. A total of 10 patients apparently with healthy periodontium were added as control study group. A total of 120 samples were collected (two samples from each subject).

The clinical periodontal parameters were measured from six sites for each subject. Two sub-gingival plaque samples were collected from each subject from the sites that were measured to have CAL between 1 and 4 mm. The test locations were air-dried and maintained dry with cotton rolls after gentle removal of supragingival plaque. To obtain the sub-gingival plaque, sterile paper points (size #30) were placed for 30 s into the desired location (periodontal pockets or gingival sulcus). Any paper that had been contaminated with blood was discarded. Each paper point was immediately placed into a microfuge tube containing 1ml phosphate buffer saline from each sampling site. After that, the samples for real-time PCR analysis were kept at –20°C. *Tannerella forsythia* was quantified with primer design, and bacterial genomic DNA was quantified with primer design according to the manufacturer's methodology. The reaction mix was designed to include enough reactions for the negative control and standard curve wells were prepared. The clpB and bspA genes amplification plots using qPCR samples contained all samples, according to the real-time PCR program parameters and reaction volume provided in Table 1. The photo was taken straight from the qPCR machine.

Ethical approval

Before the collection of samples, all the participants included in this study were informed, and their consent was obtained using consent form with document number 566 to conduct the tests and publish the results (containing the number and date in 30/01/2022).

Statistical analysis

All statistical analyses are conducted by using SPSS version 26.0 software (SPSS, IBM Company, Chicago, IL).

Table 1: The primers, sequences, and PCR conditions of specific primer genes (*ClpB*, *BspA*) for the identification of bacteria

Gene name	Primer sequence (5´- 3´)
ClpB	F: i5' GAAGCCATCCGACGTAAACC '
	R: 5' GTCTTCGTCTCGGCAATCAC '3
BspA	F: 15' TCACTATTGTGTCTCGCTG '3
	R: 15' TCTCTCCGATTGTGGTTA '3i

DNA extraction

Method was developed using the manufacturing company's genomic DNA kit (EASY PURE® Micro Genomic DNA Kit). Table 1 lists the primers utilized for the amplification of fragment-specific primer genes (ClpB, BspA) for *T. forsythia* identification [Table 1].

Results

In this study, 60 samples were detected for presence of *T. forsythia* by using RT-PCR technique. The specific ClpB and BspA primers' genes were used for the amplification of a fragment these genes for identification of *T. forsythia*. The results showed the presence of ClpB and BspA in all samples (100%) as shown in Figures 1, 2.

A total of 40 patients were asked to participate in the study after meeting the inclusion criteria. All the patients agreed to participate and received the allocated intervention. Twenty control patients without periodontitis (periodontal health) with mean age (21.5 ± 1.3) years (2 male patients and 8 female patients) and 40 patients with generalized periodontitis were divided into two groups: Group A included 20 patients who were treated by SRP with mean age (51.5 ± 8.7), and group B included 20 patients who were treated with amoxicillin and metronidazole with mean (49.9 ± 7.2). The results are shown in Table 2.

In Table 3, descriptive data included primer 2/ bacterial load, primer 1/bacterial load, and clinical periodontal parameters among the study groups. The result showed that there were no significant difference in bacterial counts between the study groups and control.

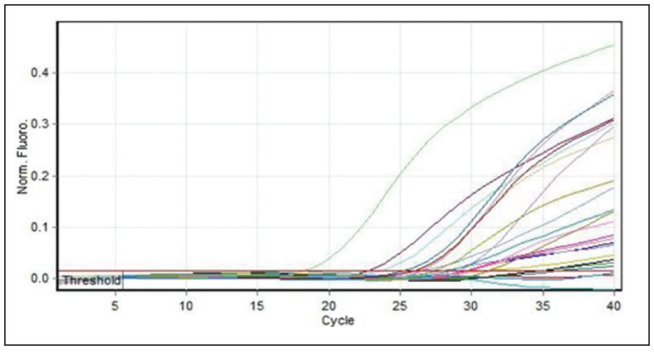

Figure 1: ClpB gene amplification by real time PCR

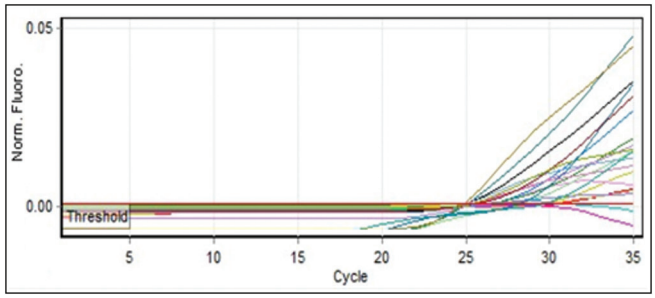

Figure 2: BspA gene amplification by real time PCR

In Table 4, the effect of scaling and root planning on the bacterial counts and clinical periodontal parameters was investigated. The results showed that there were non-significant differences between the bacterial counts before and after nonsurgical treatment. A decrease was noted in the counts of *T. forsythia* after the nonsurgical treatment than before it, whereas, there was a significant difference, in which all the periodontal parameters, as results showed decreased after SRP.

In Table 5, the effect of amoxicillin and metronidazole on the bacterial counts and clinical periodontal parameters was studied. The results showed that the counts of *T. forsythia* decreased after the administration of systemic antibiotics but also this decrease was statistically not significant, whereas the clinical periodontal parameters showed marked decrease after the antibiotic usage, in which the difference before and after the treatment was statistically significant.

Discussion

In the current study, 120 sub-gingival plaque samples collected from 60 subjects to detect the presence of *T. forsythia* by RT-PCR technique, and the precise *ClpB* and *BspA* primers genes were used for the amplification of these genes for identification of *T. forsythia*. The results showed the presence of *ClpB* and *BspA* in all periodontitis subjects (100%). The results were compatible with several authors.[19-21] RTPCR is a DNA amplification technology that allows for the accurate assessment of macromolecule levels by observing fluorescent signals at a cycle-to-cycle pace. It plays an acute role in the detection of infective microorganisms in mixed oral infections.[22] According to Saito *et al*.[23], PCR will provide up to 41-fold better sensitivity compared to colony enumeration, offering 36 % to 51 %, which will increase within the specified cases of *T. forsythia*. The study of ribosomal sequences is used to detect and quantify oral microorganisms at a molecular level.[24] Variations in ribosomal DNA copy counts among species and strains, however, have been proven to impede accurate cell-level assessment.[25]

The bspA and ClpB protein-encoding genes have been used as a replacement marking for microorganism ecology studies, offering biological process resolutions similar to the116S rRNA sequence at many compartmentalization levels. Similarly, the single-copy supermolecule encryption genes clpB and bspA are used as distinct markers for the quantification of *T. forsythiai* in dental samples, with satisfactory detection sensitivity.[26] This study showed that there was a decrease in the count of *T. forsythia* after SRP and ciprofloxacin + metronidazol (CI + MT). These results were consistence with the study of Feres *et al.*,[27] who found that there was no statistical significance in the count of *T. forsythia* between two groups (SRP, CI + MT at baseline and after treatment). While the results of Octavia *et al*.,[28] were incompatible with results of this study. They found

Table 2: The demographic data of the subjects who were involved in the study

Parameters		Rooting planning and scaling group	Ciprofloxacin and metronidazole treatment group	Control group
Age/years	Mean ± SD	51.5 ± 8.745	49.9 ± 7.232	21.5 ± 1.318
Sex No. (%)	Male	14 (70%)	20 (100%)	4 (20%)
	Female	6 (30%)	0	16 (80%)

Table 3: Descriptive data comparing average of two treatment groups with control group

Parameters	Nonsurgical treatment group	Ciprofloxacin and metronidazole treatment group	Control group	*P value
	Mean ± SD	Mean ± SD	Mean ± SD	
Primer 2/bacterial load	1054 ± 197	1089 ± 746	755 ± 779	**ns
Primer 1/bacterial load	1216 ± 549	829 ± 679	785 ± 812	**ns
Probing pocket depth/mm	2.98 ± 0.57	3.20 ± 0.64	1.69 ± 0.32	*s
Clinical attachment loos/ mm	3.67 ± 1.25	3.53 ± 0.48	0	**ns
Plaque score	34.18 ± 7.68	44.78 ± 15.64	10.61 ± 3.07	*s
Bleeding on probing/ %	32.91 ± 9.40	35.19 ± 9.48	5.13 ± 1.32	*s

*One-way ANOVA test

Table 4: The effect of scaling and root planning on the bacterial counts and clinical periodontal parameters

Parameters	Before treatment	After treatment	*P* value
	Mean ± SD	Mean ± SD	
Primer 2/bacterial copy number	1054 ± 197	623 ± 793	**ns
Primer 1/bacterial copy number	785 ± 812	67350 ± 832	**ns
Probing pocket depth/mm	2.98 ± 0.57	2.75 ± 0.55	*s
Clinical attachment loos/ mm	4.38 ± 0.8	4.66 ± 1.2	**ns
Plaque index/%	34.18 ± 7.68	27.6 ± 5.37	*s
Bleeding on probing/%	32.91 ± 9.40	27.81 ± 8.27	*s

*Paired sample *t* test

**Wilcoxon signed ranks test

Table 5: The effect of amoxicillin and metronidazole on the bacterial counts and clinical periodontal parameters

Parameters	Ciprofloxacin and metronidazole treatment group		P value
	Before treatment	After treatment	
	Mean ± SD	Mean ± SD	
Primer 2 /bacterial copy number	1089 ± 746	103 ± 892	**ns
Primer 1/bacterial copy number	829 ± 679	995 ± 724	**ns
Probing pocket depth/mm	3.20 ± 0.642	2.70 ± 0.53	*s
Clinical attachment loos/ mm	3.53 ± 0.48	4.69 ± 0.91	**ns
Plaque index/ %	44.78 ± 15.64	31.3 ± 8.19	*s
Bleeding on probing/ %	35.19 ± 9.48	26.4 ± 7.71	**s

*Paired sample T test

**Wilcoxon signed ranks test

that a total count of *T. forsythia* was decreased significantly compared with the count before scaling and root planning (SRP). Teughels *et al.*[29] also found that CI+MT treatment provided same microbiological outcomes with control subjects and who had received SRP after treatment.

In another study utilizing real-time PCR, Abdelbary *et al*.[30] reported that *T. forsythia* level was significantly decreased in antibiotic therapy group. Kocak *et al*.,[31] revealed that the application of diode laser, as an adjunct to nonsurgical periodontal therapy, has an effect on the reduction of red complex microorganisms in patients with chronic periodontitis. Moreover, Abdelbary *et al*.,[30] also documented that better clinical outcomes are related with ciprofloxacin + metronidazol (CI+MT) supplementation. The results of some other study shown by Ardila *et al*.,[32] have also supported the clinical parameter of AM+MT adjunction to SRP. However, Yaprak *et al*.,[33] reported that CI+MT supplementation to SRP did not exhibit any significant difference in the clinical parameters compared with controls. Systemic CI+MT treatment did not exhibit any additional effect in the decrease of *T. forsythia* count in periodontitis patients.[34] This may be explained by the

high degree of antibiotic resistance pattern of *T. forsythia*. Besides, *T. forsythia* is thought as a reservoir for antibiotic resistance genes and potential source of other species.[35,36]

Conclusion

This study concluded the effect of nonsurgical treatment of periodontitis on *T. forsythia* seems to be of great effect in the reduction of their counts, without subjecting the patients to the side effects of systemic antimicrobial therapy.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Baelum V, López R. Periodontal disease epidemiology—Learned and unlearned? Periodontology 2000 2013;62:37-58.
2. Preshaw PM, Alba AL, Herrera D, Jepsen S, Konstantinidis A, Makrilakis K, *et al*. Periodontitis and diabetes: A two-way relationship. Diabetologia 2012;55:21-31.
3. Könönen E, Gursoy M, Gursoy UK. Periodontitis: A multifaceted disease of tooth-supporting tissues. J Clin Med 2019;8:1135.
4. Genco RJ, Sanz M. Clinical and public health implications of periodontal and systemic diseases: An overview. Periodontology 2000 2020;83:7-13.
5. Berezow AB, Darveau RP. Microbial shift and periodontitis. Periodontology 2000 2011;55:36-47.
6. Mombelli A. Microbial colonization of the periodontal pocket and its significance for periodontal therapy. Periodontology 2000 2018;76:85-96.
7. Invernici MM, Salvador SL, Silva PH, Soares MS, Casarin R, Palioto DB, *et al*. Effects of Bifidobacterium probiotic on the treatment of chronic periodontitis: A randomized clinical trial. J Clin Periodontol 2018;45:1198-210.
8. Francino MP. Antibiotics and the human gut microbiome: Dysbioses and accumulation of resistances. Front Microbiol 2016:15-43.
9. İnce G, Gürsoy H, İpçi ŞD, Cakar G, Emekli-Alturfan E, Yılmaz S. Clinical and biochemical evaluation of lozenges containing *Lactobacillus reuteri* as an adjunct to non-surgical periodontal therapy in chronic periodontitis. J Periodontol 2015;86:746-54.
10. Joint FAO. WHO working group report on drafting guidelines for the evaluation of probiotics in food. London, Ontario, Canada 2002;30.
11. Curtis MA, Diaz PI, Van Dyke TE. The role of the microbiota in periodontal disease. Periodontology 2000 2020;83:14-25.
12. Majeed ZN, Alabsi AM, Swaminathan D, Philip K. Reliability of periodontal pathogens and human cathelicidine Ll-37 in the prediction of the different stages of periodontal disease. Modern Res Dent 2019;4:MRD. 000588.
13. Singh M, Sahota JK, Singh P, Kour H. Association of red complex bacteria with periodontal disease: A clinico-microbiological study. Apollo Med 2021;18:224.
14. Mahalakshmi K, Krishnan P, Chandrasekaran SC. Detection of *Tannerella forsythia* bspA and prtH genotypes among periodontitis patients and healthy subjects—A case-control study. Arch Oral Biol 2018;96:178-81.
15. Kędzierska-Mieszkowska S, Zolkiewski M. Hsp100 molecular chaperone ClpB and its role in virulence of bacterial pathogens. Int J Molecular Sci 2021;22:5319.
16. Sharma A, Sojar HT, Glurich I, Honma K, Kuramitsu HK, Genco RJ. Cloning, expression, and sequencing of a cell surface antigen containing a leucine-rich repeat motif from *Bacteroides forsythus* ATCC 43037. Infect Immun 1998;66:5703-10.
17. Handrich MR, Garg SG, Sommerville EW, Hirt RP, Gould SB. Characterization of the BspA and Pmp protein family of trichomonads. Parasites Vectors 2019;12:1-15.
18. Sharma, A. Virulence mechanisms of *Tannerella forsythia*. Periodontology 2000 2010;54:106-16.
19. Ali RAH, Radeef SM, Mohammed NB, Diab BS. Oral health-related quality of life among dental implant patients in relation to temporomandibular joint function. Med J Babylon 2022;19:609.
20. Hassan NU, Hassan Y, Ahmad PA, Shah OJ, Bhat MY. Hepatic artery anomalies and its impact in patients undergoing pancreaticoduodenectmy. A comparative study from Kashmir Valley. Med J Babylon 2022;19:350.
21. Santhosh Kumar K. Evaluation of the microbiome in Periodontal Health and Plaque Covering Supragingival calculus using Next Generation Sequencing Technology. Doctoral dissertation, Ragas Dental College and Hospital, Chennai; 2019.
22. Komarova N, Barkova D, Kuznetsov A. Implementation of high-throughput sequencing (HTS) in aptamer selection technology. Int J Mol Sci 2020;21:8774.
23. Saito D, Coutinho LL, Saito CPB, Tsai SM, Höfling JF, Gonçalves RB. Real-time polymerase chain reaction quantification of *Porphyromonas gingivalis* and *Tannerella forsythia* in primary endodontic infections. J Endodont 2009;35:1518-24.
24. Jin J, Yamamoto R, Takeuchi T, Cui G, Miyauchi E, Hojo N, *et al*. High-throughput identification and quantification of single bacterial cells in the microbiota. Nat Commun 2022;13:1-13.
25. Highfield J. Diagnosis and classification of periodontal disease. Aust Dent J 2009;54:S11-26.
26. Byrne SJ, Dashper SG, Darby IB, Adams GG, Hoffmann B, Reynolds EC. Progression of chronic periodontitis can be predicted by the levels of *Porphyromonas gingivalis* and *Treponema denticola* in subgingival plaque. Oral Microbiol Immunol 2009;24:469-77.
27. Feres M, Retamal-Valdes B, Fermiano D, Faveri M, Figueiredo LC, Mayer MP, *et al*. Microbiome changes in young periodontitis patients treated with adjunctive metronidazole and amoxicillin. J Periodontol 2021;92:467-8.
28. Octavia M, Soeroso Y, Kemal Y, Sunarto H, Bachtiar BM. Microbial effects (*Porphyromonas gingivalis*, *Tannerella forsythia*) after scaling and root planing. J Phys Conf Ser 2018;1073:062011.
29. Teughels W, Feres M, Oud V, Martín C, Matesanz P, Herrera D. Adjunctive effect of systemic antimicrobials in periodontitis therapy: A systematic review and meta-analysis. J Clin Periodontol 2020;47:257-81.
30. Abdelbary MM, Schittenhelm F, Yekta-Michael SS, Reichert S, Schulz S, *et al*. Impact of three nonsurgical, full-mouth periodontal treatments on total bacterial load and selected pathobionts. Antibiotics 2022;11:686.
31. Kocak E, Sağlam M, Arslan U, Kayis SA, Kebapcilar L, Loos BG, *et al*. Effect of diode laser application as an adjunct to nonsurgical periodontal therapy on the reduction of red complex microorganisms in type 2 diabetics with chronic periodontitis. Lasers Med Sci 2020;35:1403-10.
32. Ardila CM, Hernández-Casas C, Bedoya-García JA. Effects on clinical outcomes of adjunctive moxifloxacin versus amoxicillin plus metronidazole in periodontitis patients harboring *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Tannerella forsythia*: Exploratory analyses from a clinical trial. Quintessence Int 2021;52:20.
33. Yaprak E, Arslan U, Ataoğlu T. Effect of adjunctive amoxicillin/metronidazole treatment on subgingival *Tannerella forsythia*, *Prevotella intermedia* and *Fusobacterium nucleatum* recolonization in periodontitis patients. Selcuk Dental J 2020;6:169-76.
34. Ridha HSH, Kadri ZHM. Assessment of some immunological biomarkers in saliva and serum of Iraqi patients with chronic periodontitis disease. J Pharmaceut Sci Res 2018;10:2998-3000.
35. Kadhim EM, Amin BK, Amin BK. The antibacterial effect of green tea on *Enterococcus faecalis*, Iraq. Med J Babylon 2022;19:676-9.
36. Al-Shukri MS, Hmood AM, Al-Charrakh AH. Sequencing of *Clostridium perfringens* toxin genes (cpa, etx, iap) from Iraqi hospitals and detection by PCR of the genes encoding resistance to metronidazole, tetracycline, and clindamycin. Indian J Med Microbiol 2021;39:289-94.