

## Original Research Article

# Efficacy of the combination of minocycline and periodontal basic therapy in type 2 diabetes mellitus patients with chronic periodontitis

Chuizhuang Chen<sup>1</sup>, Shaodeng Li<sup>2</sup>, Fuwen Xing<sup>1\*</sup>

<sup>1</sup>Department of Stomatology, Hainan Medical College Second Affiliated Hospital, <sup>2</sup>Department of Periodontitis and Oral Implantation, Affiliated Haikou Hospital, Xiangya Medical School, Central South University, Hainan Provincial Stomatology Centre, Haikou, Hainan, China

\*For correspondence: **Email:** Nkykqk@163.com; **Tel:** +86-013876261045

Sent for review: 19 May 2023

Revised accepted: 3 October 2023

### Abstract

**Purpose:** To investigate the clinical efficacy of the combination of minocycline and periodontal basic treatment on type 2 diabetes mellitus (T2DM) patients with chronic periodontitis (CP) and its impact on inflammatory factors.

**Methods:** A total of 90 T2DM patients with CP admitted to the Department of Stomatology in Hainan Medical College Second Affiliated Hospital, China from January 2020 to October 2022, were enrolled in this study and randomly assigned to two equal groups, viz, study and control group. Study group received minocycline in addition to routine hypoglycemic control and basic periodontal treatment, while control group received only routine hypoglycemic control and basic periodontal treatment. Treatment lasted for 8 weeks. Efficacy of the combination of minocycline and periodontal basic treatment of T2DM patients with CP in the two groups, as well as the periodontal probing depth (PD), clinical attachment loss (CAL), bleeding on probing (sulcus bleeding index, SBI), interleukin-36 (IL-36), interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), glycated hemoglobin (HbA1c) level, chewing function score, Oral Health Impact Profile-14 (OHIP-14) score, and incidence of adverse reactions before and after treatment were determined.

**Results:** The effectiveness/efficacy of treatment in study group (91.11 %) was significantly higher than in control group (75.56 %,  $p < 0.05$ ). After treatment, study group had reduced PD, PLI, and SBI scores than control group ( $p < 0.05$ ). Study group also showed lower levels of IL-1 $\beta$ , IL-36, and TNF- $\alpha$  as well as reduced OHIP-14 score and a higher chewing function score than control group ( $p < 0.05$ ).

**Conclusion:** The combination of minocycline with periodontal basic treatment improves the efficacy in T2DM patients with CP, and reduces the level of inflammatory factors with a good margin of safety.

**Keywords:** Minocycline, Periodontal basic treatment, Type 2 diabetes mellitus, Chronic periodontitis, Inflammatory factors

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, Web of Science, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

## INTRODUCTION

Patients with type 2 diabetes mellitus (T2DM) exhibit abnormal glucose metabolism, which

decreases their resistance to local periodontal inflammatory stimuli, accelerates alveolar bone absorption, and leads to periodontal destruction. This often results in the spread of gingivitis to

deeper periodontal tissues and the formation of chronic periodontitis (CP) [1,2]. The clinical manifestations of T2DM with CP are gingival bleeding and hypertrophy, gingival pocket abscess, and periodontal pocket formation. In severe cases, alveolar bone and tooth mobility occur, requiring timely symptomatic treatment [3]. Currently, the clinical approach for treatment of T2DM with CP involves treating CP based on strict glycemic control. This involves periodontal basic treatment such as scaling, root planning, root surface smoothing, periodontal pocket medication, and oral health maintenance. These measures effectively improve the health of periodontal tissue and decrease the levels of inflammatory factors. However, some patients respond poorly to inflammation and clearance efficacy, hence their clinical condition is not significantly improved [4,5].

Minocycline is a semi-synthetic broad-spectrum antibiotic (tetracycline) that produces bacteriostatic effects by binding with tRNA. It is effective against soft tissue infections, inhibits the activity of collagenase in periodontal tissue, inhibits inflammation reaction in periodontal pockets, and promotes recovery [6]. Previous studies have reported on the effects of combination therapy on efficacy and inflammation in CP patients [7], but their report was limited to the efficacy of combination therapy for T2DM with CP patients. Therefore, this study investigates the clinical effect of the combination of minocycline and periodontal base therapy on T2DM with CP and its effect on inflammatory factors.

## METHODS

### Patients

A total of ninety patients with T2DM and CP admitted to the Department of Stomatology in Hainan Medical College Second Affiliated Hospital, China from January 2020 to October 2022 were enrolled. Patients with T2DM and CP were randomly divided into equal groups, namely, study and control groups. This study was approved by Hainan Medical College Second Affiliated Hospital (approval no. LW2022299) and conducted according to the guidelines provided in the Declaration of Helsinki [8].

### Inclusion criteria

Patients who met the diagnostic criteria for T2DM [9], disease course of more than 1 year, met the diagnostic criteria for CP [10], had 15 or more teeth, and for which informed consent has been obtained were included in the study.

### Exclusion criteria

Patients with concurrent cardiovascular disease, history of antibiotic or nonsteroidal anti-inflammatory drug treatment within the previous six months, history of periodontal treatment within the previous six months, and smoking history were excluded from the study.

### Treatments

Both groups received routine glycemic control treatment and oral hygiene education. Control group received periodontal basic treatment, including supragingival and subgingival cleaning and scraping, adjustment of root surfaces and occlusion, and completed full-mouth subgingival scaling and root planning within 2 weeks. Patients were reviewed after the surgery once weekly to check periodontal hygiene and received physiologic saline rinsing and oral hygiene guidance. Study group received minocycline in combination with periodontal basic treatment. The steps of periodontal basic treatment were the same as those in control group. Minocycline hydrochloride ointment (0.5 g × 5 tubes, registration number H20150106, Sunstar INC, Japan) was injected into the periodontal pocket from the bottom of the pocket to the gingival margin until it overflowed, and was rinsed after 30 min. Both groups received continuous treatment for 8 weeks.

### Evaluation of parameters/indices

Efficacy of treatment administered to the two groups was compared based on improvement in diabetic symptoms and periodontal pockets [11]. Complete disappearance of symptoms and a reduction in pocket depth of more than 2 mm with no purulent discharge was considered markedly effective improvement, while a reduction of more than 1 mm with no purulent discharge and basic disappearance of symptoms was considered an effective treatment. Worsening of symptoms was considered as ineffective. The total effective rate was calculated as the sum of the marked and effective rates in patients with T2DM and CP. Probing depth (PD), clinical attachment loss (CAL), and sulcus bleeding index (SBI) before and after treatment were compared between the two groups of patients with T2DM and CP. A CAL of 0 indicated that the tooth surface was free of plaque, while a CAL of 1 indicated that thin plaque was visible with the naked eye. A CAL of 2 indicated the presence of visible gingival plaque, and a CAL of 3 indicated the presence of a large amount of plaque. Probing depth (PD) was determined by measuring depth of the periodontal pockets using

a periodontal probe with higher scores indicating worse periodontal health status. Sulcus bleeding index (SBI) was assessed by gently probing the periodontal pocket (20 - 25 g) and observing bleeding from the gums within 30 sec after removing the probe. A score of 0 indicated healthy gums, while a score of 5 indicated automatic bleeding of the gums.

Venous blood samples were collected before and after treatment from patients in both groups, and the levels of interleukin-36 (IL-36), interleukin-1 $\beta$  (IL-1 $\beta$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and glycosylated hemoglobin (HbA1c) were measured using enzyme-linked immunosorbent assay (ELISA). A D-10B multi-glucose meter from Olympus Corporation of Japan was used to measure HbA1c levels, and the reagent kit was provided by Shenzhen Jingmei Biotechnology Co., Ltd. (Shenzhen, China). Masticatory function score (food sensitivity and chewing) [12] and the Oral Health Impact Profile 14 (OHIP-14) score [12] before and after treatment were compared between the two groups. Masticatory function has a maximum score of 12 and a higher score indicates better masticatory function in patients. The OHIP-14 questionnaire comprises 14 items related to oral health knowledge, attitudes, and behaviors, with a total score of 56. Higher score indicates better quality of life. Adverse reactions, such as ataxia, reduced appetite, rash, nausea and vomiting, were recorded during the treatment period for both groups.

### Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS) 22.0 software (IBM, Armonk, NY, USA). Measurement

data, such as PD, PLI, and SBI in the two groups are expressed as mean  $\pm$  standard deviation (SD). Differences were analyzed using the t-test. Count data, such as clinical efficacy in the two groups are expressed as percentages and differences were analyzed using the chi-square test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Efficacy

The overall response rate of T2DM patients with CP in study group was 91.11 %, significantly higher than 75.56 % in control group ( $p < 0.05$ ) (Table 1). There were no significant differences in baseline data between the two groups ( $p > 0.05$ ).

### Periodontal health status indicators

Before treatment, there was no significant difference in PD, PLI, and SBI between the two groups of T2DM patients with CP ( $p > 0.05$ ). However, PD, PLI, and SBI in both groups were significantly lower after treatment in study group than control group ( $p < 0.05$ ; Table 2).

### Inflammatory factor levels

There were no significant differences in levels of IL-1 $\beta$ , IL-36, and TNF- $\alpha$  levels between the two groups ( $p > 0.05$ ). Inflammatory factors (IL-1 $\beta$ , IL-36, and TNF- $\alpha$ ) levels were significantly lower after treatment in study group than control group ( $p < 0.05$ ; Table 3).

**Table 1:** Comparison of clinical efficacy (n = 45)

Group	Markedly effective	Effective	Ineffective	Overall response rate
Study group	29 (64.44)	12 (26.67)	4 (8.89)	41 (91.11)
Control group	23 (41.82)	11 (24.44)	11 (24.44)	34 (75.56)
$\chi^2$ value				3.920
<i>P</i> -value				0.048

**Table 2:** Periodontal health status indicators (n = 45)

Group	PD (mm)		PLI (mm)		SBI (%)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
A	2.73 $\pm$ 0.31	2.01 $\pm$ 0.17*	3.86 $\pm$ 0.49	3.18 $\pm$ 0.26*	2.91 $\pm$ 0.28	1.54 $\pm$ 0.16*
B	2.79 $\pm$ 0.35	2.66 $\pm$ 0.24*	3.85 $\pm$ 0.51	3.49 $\pm$ 0.35*	2.93 $\pm$ 0.25	1.87 $\pm$ 0.19*
<i>t</i> value	0.861	14.826	0.095	4.769	0.357	8.912
<i>P</i> -value	0.392	0.000	0.924	0.000	0.721	0.000

\* $P < 0.05$  compared with that before treatment in the same group

**Table 3:** Inflammatory factor levels (n = 45)

Group	IL-1 $\beta$ (ng/L)		IL-36 (ng/L)		TNF- $\alpha$ (ng/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Study group	7.13 $\pm$ 0.95	4.28 $\pm$ 0.41*	29.54 $\pm$ 4.16	19.36 $\pm$ 2.98*	8.86 $\pm$ 0.75	6.15 $\pm$ 0.49*
Control group	7.16 $\pm$ 0.92	5.69 $\pm$ 0.53*	29.12 $\pm$ 4.29	24.77 $\pm$ 3.65*	8.82 $\pm$ 0.71	7.09 $\pm$ 0.52*
<i>t</i> value	0.152	14.115	0.471	7.702	0.259	8.825
<i>P</i> -value	0.879	0.000	0.638	0.000	0.795	0.000

\**P* < 0.05 compared with that before treatment in the same group

**Table 4:** Blood glucose level, masticatory function, and quality of life (n = 45)

Group	Hb1AC (%)		Masticatory function score (points)		OHIP-14 Score (ng/L)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Study	7.13 $\pm$ 0.45	9.04 $\pm$ 0.2*	4.23 $\pm$ 0.72	7.98 $\pm$ 0.35*	14.86 $\pm$ 1.75	7.15 $\pm$ 0.49*
Control	7.16 $\pm$ 0.42	8.95 $\pm$ 0.23*	4.19 $\pm$ 0.75	6.05 $\pm$ 0.31*	14.82 $\pm$ 1.71	8.39 $\pm$ 0.52*
<i>t</i> value	0.327	1.938	0.258	27.691	0.109	11.642
<i>P</i> -value	0.745	0.056	0.796	0.000	0.912	0.000

\**P* < 0.05 compared with that before treatment in the same group

**Table 5:** Incidence of adverse reactions during treatment (n=45)

Group	Appetite decreased	Nausea and vomiting	Rash	Ataxia	Overall incidence of adverse reactions
Study	3 (6.67)	2 (4.44)	1 (2.22)	1 (2.22)	7 (15.56)
Control	2 (4.44)	2 (4.44)	0 (0.00)	0 (0.00)	4 (8.89)
$\chi^2$ value					0.932
<i>P</i> -value					0.334

### Blood glucose level, masticatory function, and quality of life

There was no significant difference in Hb1AC, masticatory function score, and OHIP-14 score levels between the two groups before treatment ( $p > 0.05$ ). The OHIP-14 scores of the two groups were significantly lower after treatment. Glycated haemoglobin (Hb1AC) and masticatory function score were significantly higher after treatment ( $p < 0.05$ ). The OHIP-14 score of study group was significantly lower than control group, but the masticatory function score of study group was significantly higher than that of control group ( $p < 0.05$ ). There was no significant difference in Hb1AC after treatment between the two groups ( $p > 0.05$ ; Table 4).

### Incidence of adverse reactions during treatment

The incidence of treatment-emergent adverse reactions in T2DM patients with CP in study group was 15.56 %, which was not significantly different from 8.89 % in control group ( $p > 0.05$ ; Table 5).

## DISCUSSION

Chronic periodontitis (CP) is a destructive chronic infectious oral disease caused by an aberrant immune response to dental plaque microbiota. The hallmark lesion of this disease is the formation of periodontal pockets that harbor a large amount of dental calculus and bacteria and readily cause bleeding gums [14]. It has been found that metabolic disorders commonly present in patients with type 2 diabetes mellitus (T2DM) are risk factors for the coexistence of CP. Excessive activation of pro-inflammatory factors, such as TNF- $\alpha$  and IL-36, induces an inflammatory response in soft and hard tissues of the periodontal area, leading to severe alveolar bone loss, which requires timely intervention with an effective treatment plan [15]. In the past, the basic treatment of periodontitis was mainly used to treat CP, but its therapeutic effect on patients with T2DM and CP is limited, which has restricted its clinical application [16]. Local targeted administration of minocycline directly to the bottom of the periodontal pocket ensures sufficient concentration and prolongs antibacterial activity in the gingival crevicular fluid, achieving good therapeutic effect [17].

This study investigated the clinical efficacy of minocycline in combination with periodontal basic treatment for T2DM patients with CP and its effect on inflammatory factors. The results of this study showed that the total effective rate of study group was 91.11 %, which was significantly higher than control group (75.56 %). In addition, the levels of periodontal parameters PD, PLI, and SBI of study group were significantly lower than those control group, indicating that minocycline combined with periodontal basic treatment effectively improves the clinical efficacy of patients with T2DM and CP. Probing depth (PD), clinical attachment loss (CAL), and sulcus bleeding index (SBI) are common objective indicators for evaluating the severity of CP in T2DM patients.

Inflammation separates the normal gingival crevice bottom from the pressure surface, and the gingival crevice gradually deepens to form a periodontal pocket, while recession and exposure of the enamel-cementum junction result in loss of attachment. Inflammation stimulates bleeding of the gingival crevice, which reflects the periodontal health status of patients with CP [18]. Periodontal basic treatment removes plaque and eliminates plaque retention factors in the gingiva and periodontal pockets, thereby terminating the deepening of the periodontal pocket, attachment loss, and gum bleeding. However, it cannot eliminate inflammatory stimulation of the periodontal tissues, and it requires combined local drug treatment for antibacterial therapy [19].

Previous studies have shown that the main pathogenic bacteria of CP are anaerobic bacteria such as *Fusobacterium nucleatum* and *Porphyromonas gingivalis*, which are often attached to periodontal tissues and periodontal pockets inducing chronic progressive inflammatory reactions and are sensitive to most antibiotics [20]. Minocycline is a lipophilic tetracycline antibiotic with high efficiency, long duration, and high absorbability. Minocycline inhibits pathogenic bacteria such as *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, and *Actinobacillus actinomycetemcomitans*, reduces periodontal inflammation, and promotes alveolar bone repair [21]. The combination of minocycline and periodontal basic treatment in the clinical treatment of T2DM patients with CP helps to control periodontal inflammation and repair alveolar bone at the root surface.

Levels of IL-1 $\beta$ , IL-36, and TNF- $\alpha$ , as well as OHIP-14 scores of T2DM patients with CP in study group, were significantly lower than those

in control group after treatment. Moreover, chewing function score in study group was significantly higher than control group. However, there was no significant difference in Hb1AC level between the two groups after treatment, indicating that the combination of minocycline and periodontal basic therapy effectively reduces levels of inflammatory factors, improves chewing function and quality of life in T2DM patients with CP, but had no significant impact on hypoglycemic treatment.

The mechanism by which T2DM affects CP is complex and is often related to the accumulation of glycosylated end-products that chemoattract monocytes and macrophages due to the patient's high blood glucose status. Binding to their surface receptors promotes the secretion of inflammatory factors such as IL-1 $\beta$ , IL-36, and TNF- $\alpha$ , resulting in significantly increased levels of serum IL-1 $\beta$ , IL-36, and TNF- $\alpha$ . Long-term inflammatory stimulation and dental plaque corrode the teeth, reduce the resistance of periodontal tissues, and cause tooth loosening in CP patients. Minocycline has a strong inhibitory effect on various pathogens associated with periodontal disease [22]. In this study, local administration of minocycline in combination with periodontal basic therapy for T2DM patients with CP ensures slow release of the drug which effectively controls blood concentration and duration of minocycline. This exerts a strong inhibitory effect on dental plaque aggregation, leading to lower levels of inflammatory factors, improved chewing function, and quality of life. The incidence of adverse reactions was not significantly different between the two groups in this study, indicating that the combination of minocycline and periodontal basic therapy did not increase the treatment risk for T2DM patients with CP and has good safety.

#### Limitations of the study

This study has some limitations. The results were obtained from a small sample size and thus, there could be sampling bias. Further studies with larger sample sizes are needed to verify this result.

#### CONCLUSION

Combination of minocycline and periodontal basic therapy improves clinical efficacy, reduces levels of inflammatory factors, and improves periodontal health, chewing function, and quality of life of T2DM patients with CP, without increasing the incidence of adverse reactions.

## DECLARATIONS

### Acknowledgements

None provided.

### Funding

None provided.

### Ethical approval

This study was approved by Hainan Medical College Second Affiliated Hospital (approval no. LW2022299).

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

### Open Access

This is an Open Access article that uses a funding model which does not charge readers or their institutions for access and distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>) and the Budapest Open Access Initiative (<http://www.budapestopenaccessinitiative.org/read>), which permit unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited.

## REFERENCES

1. Preshaw PM, Bissett SM. Periodontitis and diabetes. *Brit Dent J* 2019; 227(7): 577-584.
2. Luong A, Tawfik AN, Islamoglu H, Gobriel HS, Ali N, Ansari P, Shah R, Hung T, Patel T, Henson B, et al. Periodontitis and diabetes mellitus co-morbidity: A molecular dialogue. *J Oral Biosci* 2021; 63(4): 360-369.
3. Portes J, Bullon B, Quiles JL, Battino M, Bullon P. Diabetes Mellitus and Periodontitis Share Intracellular Disorders as the Main Meeting Point. *Cells-Basel* 2021; 10(9): 2411
4. Simpson TC, Clarkson JE, Worthington HV, MacDonald L, Weldon JC, Needleman I, Iheozor-Ejirofor Z, Wild SH, Qureshi A, Walker A, et al. Treatment of periodontitis for glycaemic control in people with diabetes mellitus. *Cochrane Db Syst Rev* 2022; 4(4): D4714.
5. Bian Y, Liu C, Fu Z. Application value of combination therapy of periodontal curettage and root planing on moderate-to-severe chronic periodontitis in patients with type 2 diabetes. *Head Face Med* 2021; 17(1): 12.
6. Paquette DW. Minocycline microspheres: a complementary medical-mechanical model for the treatment of chronic periodontitis. *Compend Contin Educ Dent* 2002; 23(5 Suppl): 15-21.
7. Chen X, Wang L, He Z, Zhao H, Cai Y, Song S, Yu Q. Effects of xipayi mouth rinse combined with minocycline on localized aggressive periodontitis' therapeutic effect and the levels of CRP, TNF-alpha, IL-6 in serum. *Ann Palliat Med* 2020; 9(5): 3410-3417.
8. World Medical Association. World Medical Association Declaration of Helsinki: Ethical principles for medical research involving human subjects. *JAMA*. 2013; 310: 2191-2194.
9. Cosentino F, Grant PJ, Aboyans V, Bailey CJ, Ceriello A, Delgado V, Federici M, Filippatos G, Grobbee DE, Hansen TB, et al. 2019 ESC Guidelines on diabetes, pre-diabetes, and cardiovascular diseases developed in collaboration with the EASD. *Eur Heart J* 2020; 41(2): 255-323.
10. Smiley CJ, Tracy SL, Abt E, Michalowicz BS, John MT, Gunsolley J, Cobb CM, Rossmann J, Harrel SK, Forrest JL, et al. Evidence-based clinical practice guideline on the nonsurgical treatment of chronic periodontitis by means of scaling and root planing with or without adjuncts. *J Am Dent Assoc* 2015; 146(7): 525-535.
11. Li F, Xu HS. Effects of low-level laser combined with basic periodontal therapy on cytokines and LPS, leptin in gingival crevicular fluid of diabetes mellitus complicated with chronic periodontitis patients. *Shanghai Kou Qiang Yi Xue* 2018; 27(6): 637-640.
12. Ji PH, Jiang ZM, Xu QL. The clinical evaluation of tinidazole-iodoform root canal paste on treatment of chronic periapical periodontitis associated with ectopic sinus. *Shanghai Kou Qiang Yi Xue* 2003; 12(1): 73-74.
13. Su N, van Wijk A, Visscher CM. Psychosocial oral health-related quality of life impact: A systematic review. *J Oral Rehabil* 2021; 48(3): 282-292.
14. Saxena S, Venugopal R, Chandrayan RR, Yuwanati MB, Awasthi H, Jain M. Association of chronic periodontitis and type 2 diabetes mellitus with salivary Del-1 and IL-17 levels. *J Oral Biol Craniofac Res* 2020; 10(4): 529-534.
15. Shi LX, Zhang L, Zhang DL, Zhou JP, Jiang XJ, Jin YL, Chang WW. Association between TNF-alpha G-308A (rs1800629) polymorphism and susceptibility to chronic periodontitis and type 2 diabetes mellitus: A meta-analysis. *J Periodontal Res* 2021; 56(2): 226-235.
16. Rosling B, Hellstrom MK, Ramberg P, Socransky SS, Lindhe J. The use of PVP-iodine as an adjunct to non-

- surgical treatment of chronic periodontitis. *J Clin Periodontol* 2001; 28(11): 1023-1031.
17. Zingale J, Harpenau L, Bruce G, Chambers D, Lundergan W. The effectiveness of scaling and root planing with adjunctive time-release minocycline using an open and closed approach for the treatment of periodontitis. *Gen Dent* 2012; 60(4): 300-305.
  18. Cherepynska Y, Volkova O, Riabokon E, Baglyk T. Analysis of the long-term clinical results of using 940 nm diode laser in patients with generalized moderate chronic periodontitis. *Georgian Med News* 2017; 270: 24-30.
  19. Kocak E, Saglam M, Arslan U, Kayis SA, Kebapcilar L, Loos BG, Hakki SS. 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. *Laser Med Sci* 2020; 35(6): 1403-1410.
  20. Zhang M, Zhu DZ, Li ZS, Zhan XB. Red peony root decoction in treatment of severe acute pancreatitis: a randomized controlled trial. *Zhong Xi Yi Jie He Xue Bao* 2008; 6(6): 569-575.
  21. Rapone B, Ferrara E, Corsalini M, Qorri E, Converti I, Lorusso F, Delvecchio M, Gnoni A, Scacco S, Scarano A. Inflammatory Status and Glycemic Control Level of Patients with Type 2 Diabetes and Periodontitis: A Randomized Clinical Trial. *Int J Env Res Pub He* 2021; 18(6): 3018
  22. Yang C, Wang X, Wang Y. Effect of diode laser combined with minocycline hydrochloride in nonsurgical periodontal therapy: a randomized clinical trial. *Bmc Oral Health* 2022; 22(1): 71.