

V. V. Khattou, O. S. Ivanytska, V. M. Havrylyev, O. P. Buhanchenko, I. V. Boyko,  
K. P. Lokes, D. S. Avetikov  
Poltava State Medical University, Poltava

## DYNAMICS OF BONE FORMATION AND OSTEORESORPTION MARKERS IN PATIENTS WITH POSTOPERATIVE MANDIBULAR BONE DEFECTS

e-mail: v.khattu@pdmu.edu.ua

The defect that appears after a tooth extraction leads to remodeling of bone tissue. The processes of remodeling and mineralization of the newly formed bone are affected by substances with an antioxidant effect, among which are quercetin and placenta cryoextract. The study involved 90 procedures in which tooth extraction was performed: using placenta cryoextract (group 1), combination of placenta cryoextract with quercetin (group 2) and standard treatment protocol (group 3). The content of osteocalcin and matrix metalloproteinase-8 in blood serum was determined. The formation of a postoperative defect in bone tissue was accompanied by antagonistic changes in the content of osteocalcin (due to its increase) and MMP-8 (due to its decrease) in the blood serum and oral fluid of patients, which characterizes the activation of bone tissue resorption processes at earlier times and bone formation at later times.

**Key words:** tooth extraction operation, bone defect, bone defect healing, bone tissue, mandibular molars.

В.В. Хатту, О.С. Іваницька, В.М. Гаврильєв, О.П. Буханченко, І.В. Бойко,  
К.П. Локес, Д.С. Аветіков

## ДИНАМІКА МАРКЕРІВ КІСТКОФОРМУВАННЯ ТА ОСТЕОРЕЗОРБЦІЇ У ХВОРИХ З ПІСЛЯОПЕРАЦІЙНИМИ ДЕФЕКТАМИ КІСТКИ НИЖНЬОЇ ЩЕЛЕПИ

Після проведення операції видалення зуба виникає дефект, який, у свою чергу, призводить до ремоделювання кісткової тканини. На процеси ремоделювання та мінералізації новоутвореної кістки мають вплив речовини із антиоксидантною дією, одними з яких є кверцетин та кріоекстракт плаценти. В дослідженні брали участь 90 пацієнтів, яким проводилося видалення зуба: з використанням кріоекстракту плаценти (1 група), комбінація кріоекстракту плаценти із кверцетином (2 група) та згідно стандартного протоколу лікування (3 група). Визначали вміст остеокальцину та матричної металопротеїнази-8 у сироватці крові пацієнтів. Утворення післяопераційного дефекту кісткової тканини супроводжувалося антагоністичними змінами вмісту остеокальцину (за рахунок його підвищення) та ММП-8 (внаслідок зниження) у сироватці крові та ротовій рідині пацієнтів, що характеризує активацію процесів резорбції кісткової тканини на більш ранніх термінах та кісткоутворення – на пізніх.

**Ключові слова:** операція видалення зуба, кістковий дефект, загоєння кісткового дефекту, кісткова тканина, моляри нижньої щелепи.

*The study is a fragment of the research project "Diagnosis, surgical and medical treatment of patients with inflammatory diseases of maxillofacial localization", state registration No. 0119U102862.*

The most common surgical intervention in the world is tooth extraction. After carrying out this operation, a bone tissue defect occurs, which leads to bone tissue remodeling, with a reduction in its volume in various directions. During this process the deposition of osteoblasts on alveolar bone forms newly formed bone tissue, and osteoclastic activity leads to its destruction [9, 11].

The first stage of the reparative regeneration of the alveolus is the formation of a stable blood clot, inside which fibrin forms a natural support structure that contributes to the formation of osteoid and its subsequent calcification. Then the hole is filled with epithelium, which contributes to the regeneration of bone tissue [1, 8, 12]. This process is completed in about 120 days; the periosteum is completely stabilized after 180 days [3, 6]. At the same time, the processes of remodeling and mineralization of the newly formed bone tissue take place during periods that differ greatly in different patients and are unpredictable in each case [7, 9].

A significant number of exo- and endogenous factors influences the processes of formation and resorption of bone tissue. One of these factors is quercetin, which is a powerful antioxidant that has attracted considerable attention from researchers due to its strong antioxidant and biological activity against oxidative stress-related diseases through the scavenging of free radicals and the regulation of many anti-inflammatory pathways [4, 14, 15].

Cryoextract of the placenta has pronounced antioxidant properties and anti-inflammatory effect, which, according to literature sources, contributes to the elimination of pro-inflammatory modulation of reactions of the blood system by active forms of oxygen and products of lipid peroxidation as a result of stimulation of the physiological antioxidant system. The anti-inflammatory properties of this drug have been revealed, the mechanism of which is apparently related to the action of the hormones present in it (progesterone, estradiol, prolactin, gonadotropin, etc.) [2, 10].

**The purpose** of the study was to optimise the prevention of inflammatory complications and stimulation of osteogenesis of mandible by local administration of cryopreserved placenta as monotherapy and in combination with quercetin.

**Materials and methods.** The research was carried out at the Poltava Regional Clinical Hospital in the department of maxillofacial surgery. The study included 90 patients (male and female) with indications for tooth extraction against the background of an inflammatory process, the age of the patients ranged from 18 to 58 years. Patients were divided into three clinical groups:

The 1<sup>st</sup> clinical group (n=30) – patients to whom the cryopreserved placenta was used after tooth extraction,

The 2<sup>nd</sup> clinical group (n=30) – patients to whom the combined therapy of cryopreserved placenta in combination with quercetin was used,

The 3<sup>rd</sup> clinical group (control) (n=30) – patients after tooth extraction without any additional types of treatment.

The osteocalcin and matrix metalloproteinase-8 content in the blood serum of patients of all clinical groups was determined on an empty stomach on the 1st, 10th, 20th, 30th, 90th and 180th day after surgery. The content of osteocalcin was determined using immunochemiluminescence analysis, MMP-8 – using the enzyme immunoassay method.

The research results were processed by the method of variational statistics on a personal computer with the determination of the reliability of differences between the values of the studied indicators, as well as by the method of correlation using the Statistica program package and "Excel 2010" spreadsheets.

**Results of the study and their discussion.** Osteocalcin (bone glutamine protein - BGP) is the most common non-collagen protein of bone tissue, which is involved in the process of binding calcium and hydroxylapatite to collagen, which, in turn, contributes to the organization of the extracellular matrix of bone tissue, participates in metabolism and mineralization and is a marker of bone formation. This is due to the fact that osteocalcin is expressed by osteoblasts mainly during the phase of bone formation in the regenerate, participates in the process of osteoid mineralization.

During the surgical intervention, the content of this marker in the patients' blood serum did not undergo significant changes in all three studied groups (Table 1, Fig 1), the absence of a difference in this indicator in comparison between the groups is due to the fact that at this stage of observation, the treatment protocols had not yet been applied.

Table 1

**The content of osteocalcin in the blood serum of patients with postoperative defects of the mandible against the background of inflammatory processes (M±m, ng/ml)**

	1 <sup>st</sup> clinical group	2 <sup>nd</sup> clinical group	3 <sup>rd</sup> clinical group
1 <sup>st</sup> day	28.05±1.037	27.43±1.051	26.17±0.949
10 <sup>th</sup> day	18.87±0.926 *	19.61±1.048 * **	15.59±0.766*
20 <sup>th</sup> day	15.67±0.853	17.26±0.725 **	13.81±0.568
30 <sup>th</sup> day	14.70±0.608	16.64±0.657	13.22±0.401
90 <sup>th</sup> day	19.65±0.859 *	20.73±0.731 * **	17.40±0.746 *
180 <sup>th</sup> day	23.59±0.822 *	25.09±1.247 *	23.16±1.119 *

Notes: \* –  $p \leq$  relative to the previous term; \*\* –  $p \leq$  relative to the control (3rd group).

On the 10th day after the surgical intervention, a statistically significant decrease in the content of osteocalcin in the blood serum of patients in all three studied groups was noted by 32.7 %, 28.5 % and 40.4 %, respectively, which indicates the activation of the processes of resorption of mandibular bone tissue. At the same time, in the second clinical group, this indicator was significantly higher than the control by 25.8 % for the same period of observation.

When analyzing the data of the studied indicator on the 20th and 30th day after tooth extraction, no statistically significant changes were noted in the studied groups. Only in the 2nd clinical group, the content of osteocalcin was significantly higher by 25.0 % compared to the data of the control group for the same period of observation. The obtained results may be due to the fact that osteocalcin is not a specific marker of bone formation, and its content in the blood serum of patients can be characterized by significant variability.

On the 90th day after the surgical intervention, a gradual statistically significant increase in the level of osteocalcin in blood serum was noted by 33.7 %, 24.6 % and 31.6 %, respectively, for the 1st, 2nd and 3rd clinical groups, which may determine the restoration of bone formation processes at this stage of observation. At the same time, the level of this indicator under the conditions of using cryopreserved placenta in combination with quercetin was significantly higher by 19.1 % than the indicators of the control group, in which the surgical intervention was carried out according to the classical method.

On the 180th day after surgery, serum osteocalcin continued to experience a gradual but statistically significant increase of 20.1 %, 21.0 % and 33.1 %, respectively, in all three studied groups, which may characterize the gradual mineralization of bone tissue in the area of the postoperative defect of the mandible.

In order to assess reparative osteogenesis after the formation of a postoperative defect or alveola of an extracted tooth of the mandible against the background of an inflammatory process, the content of matrix metalloproteinase 8 was determined in blood serum, which is a key enzyme that begins the destruction of the extracellular matrix during inflammatory processes and has an effect on the destruction of bone tissue.

During analyzis of the data of this marker, no statistically significant difference was noted between the various studied groups on the 1st and 10th days of observation (Table 2, Fig 1).

The unreliability of the obtained results may be due to the significant variability of MMP-8 content in the blood serum of patients and correlated with serum osteocalcin levels.

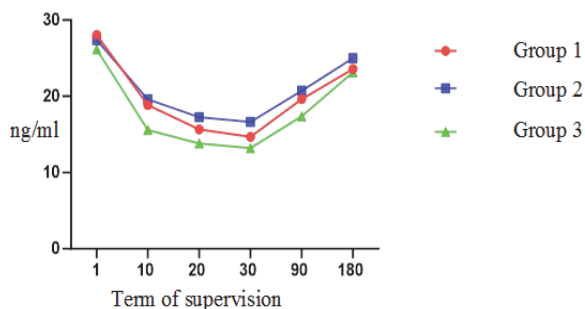


Fig. 1. Dynamics of osteocalcin in blood serum of patients with postoperative defects of the mandible against the background of inflammatory processes

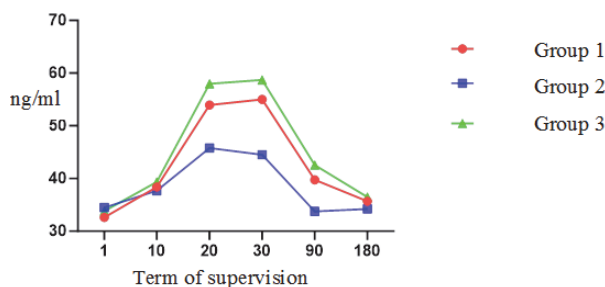


Fig. 2. Dynamics of MMP-8 in blood serum of patients with postoperative defects of mandible against the background of inflammatory processes

Table 2

**The content of MMP-8 in the blood serum of patients with postoperative defects of the mandible against the background of inflammatory processes (M±m, ng/ml)**

	1 <sup>st</sup> clinical group	2 <sup>nd</sup> clinical group	3 <sup>rd</sup> clinical group
1 <sup>st</sup> day	32.60±1.629	34.49±1.656	33.82±1.773
10 <sup>th</sup> day	38.38±1.947	37.69±1.896	39.35±1.758
20 <sup>th</sup> day	53.95±1.917 *	45.80±1.943 * ** ***	57.98±1.755 *
30 <sup>th</sup> day	55.03±1.757	44.52±1.507 ** ***	58.72±1.543
90 <sup>th</sup> day	39.74±1.412 *	33.76±1.755 * **	42.56±1.735 *
180 <sup>th</sup> day	35.68±1.632	34.22±1.519	36.47±1.489

Notes: \* – p ≤ relative to the previous term; \*\* – p ≤ relative to the control (3rd group); \*\*\* – p ≤ relative to the 1st group.

A statistically significant increase in the level of MMP-8 in the blood serum of patients who underwent surgical intervention against the background of an inflammatory process, which led to the formation of a partial bone defect, was noted on the 20th day of observation by 40.6 %, 21.5 % and 47.3 %, respectively, for the 1st, 2nd and 3rd clinical groups. It should be noted that under the conditions of the combined use of placenta cryoextract with quercetin, a significant decrease of the studied index was noted by 21.0 % in comparison with the group of patients who underwent surgical treatment according to the classical method and by 15.1 % – under the conditions of the use of cryopreserved placenta as monotherapy. The obtained data may determine the less pronounced processes of bone tissue destruction of the jaw under the conditions of using this treatment protocol, compared to other treatment methods.

On the 30th day of observation, changes in the level of MMP-8 in the blood serum of patients were not statistically significant, but the indicator of the 2nd clinical group was significantly lower than the data of the control group and the first research group by 24.2 % and 19.1 %, respectively. Similar results can be obtained due to a decrease in the activity of bone resorption processes in the area of the postoperative defect of mandible under the conditions of the combined use of cryoextract of the placenta with quercetin in comparison with the results under the conditions of the use of other treatment methods.

Three months after surgical treatment, a statistically significant decrease in the content of MMP-8 in blood serum was noted in all studied groups by 27.8 %, 24.2 % and 27.5 %, respectively. In the second clinical group, the level of MMP-8 was 20.7% lower than the results of the control group for the same period of observation.

On the 180th day of observation, changes in the level of MMP-8 were not statistically significant in all studied groups in any comparison. Such data may be due to the gradual inhibition of mandible bone tissue resorption processes at the later stages of reparative osteoregeneration when a postoperative bone tissue defect of the mandible is formed, regardless of the applied treatment method of these patients.

Thus, it was established that the dynamics of the content of the bone formation marker – osteocalcin and the osteoresorption marker – MMP-8 in blood serum after surgical treatment against the background of the inflammatory process, which led to the formation of a bone tissue defect, was antagonistic, with the best results in the second clinical group, in which tooth extraction against the background of the inflammatory process was carried out according to the author's method with the combined use of quercetin and placenta cryoextract. The obtained results confirm the data of other researchers regarding the inverse correlation of the indicated markers [5, 13].

It should be noted that the changes in the studied indicators in all studied groups had a similar nature, but different amplitude, which was minimally expressed in the second research group, where cryopreserved placenta in combination with quercetin was used in the treatment process, and the maximum dynamics of cyst formation and osteoresorption markers was noted in the control group, in which the surgical intervention was carried out according to the classical method. This statement indicates a positive effect on osteoregeneration processes of the proposed method of replacing bone defects of the jaws that occur after tooth extraction [2, 6, 10].

### Conclusion

The formation of a postoperative bone tissue defect was accompanied by antagonistic changes in the content of osteocalcin (due to its increase) and MMP-8 (due to its decrease) in the patient's blood serum, which characterizes the activation of the processes of bone tissue resorption at earlier times and bone formation at later times. Moreover, changes in these indices on the 180th day of observation were mostly not statistically significant. It should also be noted that the lowest dynamics of the studied parameters occurred in patients whose standard treatment protocol was accompanied by the combined use of placenta chiroextract and quercetin.

### References

1. Abellán D, Barallat L, Vilarrasa J, Cabezas M, Pascual La Rocca A, Valles C. Ridge preservation in molar sites comparing xenograft versus mineralized freeze-dried bone allograft: A randomized clinical trial. *Clin Oral Implants Res.* 2022 May;33(5):511–523. doi: 10.1111/clr.13911.
2. Avetikov DS, Cuong VV, Stavickij SO, Yacenko IV, Lokes KP. The role of antihypoxants and antioxidant in treatment of odontogenic of maxillofacial area. *Likars'ka sprava.* 2014; 12:82–6.
3. Avetikov DS, Lokes KP, Ishchenko VV. Changes of the mineral component of mandibular bone in the dynamics of reparative osteogenesis under conditions of chronic nitrate intoxication. *Visnyk problem biolohiyi i medytsyny.* 2014;2(1):37–9.
4. Chekalina NI, Kazakov YM, Mamontova TV, Vesnina LE, Kaidashev IP. Resveratrol more effectively than quercetin reduces endothelium degeneration and level of necrosis factor  $\alpha$  in patients with coronary artery disease. *Wiadomosci lekarskie.* 2016;69(3):475–9.
5. Chen ZY, Yang J, Tian CY, Jia W. The Relationship Between Bone Metabolism and Peripheral Artery Disease in Patients on Hemodialysis: The Potential Role of Osteocalcin. *Diabetes Metab Syndr Obes.* 2023; 16:3331–3337. doi: 10.2147/DMSO.S432345
6. de Baranda BS, Javier Silvestre F, Márquez-Arrico CF, Silvestre-Rangil J. Surgical difficulty and postoperative course of the third molar extraction under general anesthesia: an intervention trial. *J Stomatol Oral Maxillofac Surg.* 2023;101663. doi: 10.1016/j.jormas.2023.101663.
7. Dubyna V.A., Skrypnikov P.N., Khavalkina, L.M., Korobeinikova Yu.L. Substantiation of use of nanopreparation in complex treatment of patients with chronic generalized periodontitis of I-II degree. *World of Medicine and Biology.* 2019;3(69):63–66. doi: 10.26724/2079-8334-2019-3-69-63-66.
8. Faustova MO, Ananieva MM, Basarab YO, Dobrobolska OV, Vovk IM, Loban' GA. Bacterial factors of cariogenicity (literature review). *Wiadomosci lekarskie (Warsaw, Poland: 1960).* 2018;71(2):378–82.
9. Guo R, Zhang L, Hu M, Huang Y, Li W. Alveolar bone changes in maxillary and mandibular anterior teeth during orthodontic treatment: A systematic review and meta-analysis. *Orthod Craniofac Res.* 2021 May;24(2):165–179. doi: 10.1111/ocr.12421
10. Hladkykh FV. The effect of meloxicam and cryopreserved placenta extract on initial inflammatory response - an experimental study. *Ceska Slov Farm.* 2021;70(5):179–85. doi: 10.5817/CSF2021-5-179.
11. Huang J, Gan Y, Li D, Xu H, Han S, Zhu H et al. Clinical applications and outcomes of the surgical tooth extrusion technique: A bibliometric analysis from 1982 to 2023. *J Prosthet Dent.* 2023;S0022-3913(23)00630–3. doi: 10.1016/j.prosdent.2023.09.016
12. Loban' GA, Faustova MO, Chereda VV, Ananieva MM. Epidemiological and Etiological Aspects of Dental Caries Development. *Acta facultatis medicae Naissensis.* 2021;38(1):27–34. doi: 10.5937/afmna38-27564
13. Saskianti T, Nugraha AP, Prahasanti C, Ernawati DS, Tanimoto K, Riawan W et al. Study of Alveolar Bone Remodeling Using Deciduous Tooth Stem Cells and Hydroxyapatite by Vascular Endothelial Growth Factor Enhancement and Inhibition of Matrix Metalloproteinase-8 Expression in vivo. *Clin Cosmet Investig Dent.* 2022 Mar 24; 14:71–78. doi: 10.2147/CCIDE.S354153
14. Tsuber V, Kadamov Y, Tarasenko L. Activation of antioxidant defenses in whole saliva by psychosocial stress is more manifested in young women than in young men. *PLoS ONE.* 2014;9(1219): e115048.
15. Wang N, Zhang K, Chen Y, Hu J, Jiang Y, Wang X, Ban Q. Tuning whey protein isolate/hyaluronic acid emulsion gel structure to enhance quercetin bioaccessibility and in vitro digestive characteristics. *Food Chem.* 2023 Dec 15;429:136910. doi: 10.1016/j.foodchem.2023.136910.

Стаття надійшла 13.12.2022 р.