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DYNAMICS OF MMP8 AND OSTEOPONTIN CONTENT IN ORAL LIQUID OF PATIENTS WITH ORAL DISEASES IN THE PRESENCE OF ONCOPATHOLOGY

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According to WHO, breast cancer is one of the most common forms of cancer in women. In 2020, 2.3 million women were diagnosed with breast cancer and 685,000 deaths from the disease were reported worldwide. This research aims to study the dynamics of the concentration of MMP8, osteopontin, and Ca2+ ions, as well as the pH level in patients with breast cancer during chemotherapy and specific dental treatment. Materials and methods. The study involved 60 patients, both men and women. All respondents were divided into groups by their oncological diseases: group 1 consisted of 20 patients with stage II and III breast cancer after chemotherapy, without prior dental treatment, group 2 comprised 20 patients with breast cancer after chemotherapy, with previous dental treatment, the control group included 20 people without cancer and dental pathology. Results and discussion. Dental diseases in patients of both groups were manifested by significant changes in bone metabolism, which was accompanied with sharp rise of MMP8 concentration in oral fluid during reducing osteopontin concentration. It is worth noting that the above pathobiochemical changes occurred against the background of a fall in the Ca2+ ion content and a pH shift towards the acidic side. The increase in Ca2+ is associated with a decrease in MMP8 level and the elevation in pH and osteoponine. In turn, the pH has medium power feedback with MMP8 and a direct relationship of medium force with osteoponin. MMP8 and osteopontin are associated with strong feedback, that is, an increase in the level of one of them can be displayed about reducing osteopontin. Conclusion. This study has established a statistically significant increase in MMP 8 against the background of the reducing concentration of osteoponitis. Pathobiochemical changes in bone tissue were found against the background of significant loss of Ca2+ ions and pH. Dental treatment for this category of patients has led to the improvement in the concentration of these markers. The data obtained justifies the necessity of their use as diagnostic markers and laboratory screening of the effectiveness of dental treatment in patients of this category.

Key words: breast cancer, dental pathology, laboratory screening, bone tissue metabolism, biological markers

According to WHO, breast cancer is one of the most common forms of cancer in women. In 2020, 2.3 million women were diagnosed with breast cancer and 685,000 deaths from the disease were reported worldwide. At the end of 2020, there were 7.8 million women who had been diagnosed with breast cancer over the past five years that means that this type is the most common cancer in the world. The number of disability-adjusted life years (DALYs) of globally cancer-diagnosed women exceeds that of any other type of women-specific cancer. Breast cancer occurs throughout the world in women of any age after puberty, but the incidence increases with ageing [1, 2].

At present, modern medicine is facing an urgent task that is not only developing effective targeted ways to treat this pathology, but also ensuring a sufficient quality of life for patients with breast cancer, especially during chemotherapy. One of the main criteria for the quality of life today is a healthy dental system [3]. Today, dental health is considered a condition that allows an individual to eat a variety of food without any restrictions, to communicate effectively, to feel confident and satisfied with the facial appearance. At the present stage of social development, the provision of dental care to the population is aimed at achieving and maintaining a functioning, painless, aesthetic and socially acceptable condition of the oral cavity throughout the life. In this respect, the state of the oral cavity in patients with cancerous diseases is of great medical and social relevance [4, 5]. The use of dental criteria for estimating quality of life in patients with cancerous diseases is especially important in dental practice because it enables to assess not only the intensity of dental health loss in presence of the underlying disease but also its psychosocial and financial consequences. A dynamic assessment of quality of life opens the way for estimating the adequacy of the ongoing dental treatment and, along with traditional methods, is a full-fledged indicator of its effectiveness [6, 7].

Concerning the above, there is a need to introduce new informative and minimally invasive methods of assessing a patient's dental status. Recently, methods of laboratory screening of the oral cavity have been extensively introduced into clinical practice. Using various laboratory techniques of oral fluid composition analysis enables to determine the activity of substances involved in the metabolism in dental diseases [7-9]. In this regard, the use of biological markers, which are employed for a more accurate determination of the treatment effectiveness, deserves special attention. The assessment of the content of biomarkers has clinical significance for risk identification, detection of the disease and its progression, as well as for the evaluation of the therapy effectiveness. This is especially promising when is based on a personalized approach to a particular patient [10].

In recent years, numerous studies have made biomarkers more accessible. They complement the clinical and radiological picture of the disease by allowing doctors to make the right decisions. Patients can also use biomarkers to learn about their health and the need for dental care [8, 9].

The last decade reports have stated the high level of microorganisms as one of the main factors contributing to the development of diseases associated with the bone tissue destruction and their pathological procession. It is believed that pathological changes in the periodontium occur with an increase in microbial attack caused by the accumulation of microorganisms (biofilm formation) [11, 12]. The slackening of specific and nonspecific mechanisms of local and general protection is mostly associated with increased activity of microbial assemblies that results in the development of a clinically pronounced inflammatory reaction. This cascade pathogenesis is especially relevant for patients with cancerous diseases, which are directly related to severe immunosuppression and a decrease in the components of the cellular and humoral immune response [12, 13].

A special role in the development and maintenance of chronic inflammation is played by matrix metalloproteinases (MMPs), catabolism enzymes of most extracellular matrix proteins at various stages of the inflammatory process, as well as osteopontin, the state of electrolyte balance, and pH level [14, 15].

This research aims to study the dynamics of the concentration of MMP8, osteopontin, and $Ca2^{+}$ ions, as well as the pH level in patients with breast cancer receiving chemotherapy and specific dental treatment.

Materials and methods

The study involved 60 patients, both men and women. All respondents were divided into groups by their cancerous diseases: group 1 consisted of 20 patients with stage II and III breast cancer after chemotherapy, without prior dental treatment, group 2 comprised 20 patients with breast cancer after chemotherapy, with previous dental treatment, the control group included 20 people without cancer and dental pathology. Permission for enrolment and informed consent were obtained from all the respondents. For statistical data collection, patients were examined at the Department of Therapeutic, Orthopedic and Pediatric Dentistry, Zaporizhzhia State Medical University, and the documentation of patients from the ONCOLIFE Medical Center was used. Clinical and instrumental research methods used to detect and confirm dental diseases included the following: an examination of the oral cavity, probing, palpation and percussion of teeth, and use of caries markers. Hygienic status was assessed with such hygiene indices as OHI-S (Green-Vermillion), GI gingivitis (Silness-Loe) and PMA (papillary marginal index). Detected caries-affected teeth were classified, according to T.V. Vinogradova, by the severity of the process. Teeth affected by pulpitis and periodontitis were classified according to the MMCI classification by the course of the inflammatory process. Detected lesions of the mucous membrane were classified by the WHO classification. Dental treatment included: treatment of acute and chronic caries, endodontic treatment of acute/chronic pulpitis and acute/chronic periodontitis, as well as treatment of oral mucosa inflammation, mucositis. To prevent dental complications, patients with cancer diseases underwent professional hygiene and complete rehabilitation of the oral cavity before chemotherapy that included the removal of damaged mobile teeth and roots.

Enzyme-linked immunosorbent assay was used to determine the content of bone destruction markers in the oral fluid: matrix metalloproteinase 8 (MMP-8, ELISA Kit "Hycult biotechnology b.v.", HK501-01) and osteopontin (N eBioscience (Bender MedSystems) ELISA); potentiometric method using electrolyte analyzer *Elite plus* determined the content of Ca2 + ions and pH level [16].

The research results were processed by modern statistical methods of analysis using PC with the software package Statistica 13, license number JPZ804I382130ARCN10-J. The data were checked for the normality of distribution, as most of them are distributed not entirely normally; the form of median data representation and the quartile interval (Me (Q25; Q75)) were chosen. Comparisons in three independent groups were performed according to the Kruskal-Wallis test, and in two independent groups – according to the Mann-Whitney test, a non-parametric Wilcoxon test was used to determine the effect of preventive treatment. The Chi-square criterion was used to compare qualitative data. Differences were considered statistically significant at a significance level of $p < 0.05$.

Results and Discussion

A clinical examination of patients with breast cancer revealed the presence of acute (10%) and chronic caries (50%), chronic periodontitis (30%), and partial adentia (10%). The control group patients had a similar distribution of dental pathology.

Dental diseases in patients of both groups were accompanied with significant changes in bone metabolism, followed by a sharp increase in MMP8 in the oral fluid against the background of reduced osteopontin concentrations. It should be noted that the above mentioned pathobiochemical changes occurred in presence of the decrease in Ca2 + ions and pH shift to the acidic side (Table 1). In patients of the group receiving dental treatment, the changes in bone metabolism were less pronounced. Thus, the increase in MMP8 concentration was less intense compared with the patients in group 1 and was 91% versus 96% in group 1 compared with the control group. The increase in MMP8 occurred against the background of a decrease in osteopontin concentration, and its decline in patients of group 1 was more intense and amounted to 88% versus 53% in group 2 patients (Table 1).

Table 1

The concentration of MMP8 and osteopontin in oral fluid in patients with breast cancer with chemotherapy

Indicator	Group 1, n=20	Group 2, n=20	Control, n=20	p-value according to the Kruskal-Wallis test
Ca^{2+}, mmol/l	0.80 [0.70; 0.90]	1.11 [0.90; 1.25] *	1.40 [1.28; 1.60]	0.001
pH	6.70 [6.10; 7.10)	7.35 [6.80; 7.70) *	7.60 [7.10; 8.00]	0.016
MMP8, ng/ml	8.10 [7.70; 9.50]	4.15 [3.00; 5.20] *	0.34 [0.27; 0.50]	<0.001
Osteopontin, ng/ml	0.20 [0.20; 0.30]	0.78 [0.40; 0.80] *	1.65 [1.40; 1.90]	<0.001

Note: * *- a statistically significant difference between groups without treatment and with treatment*

Table 2

Values of Spearman correlation coefficients between markers of bone metabolism in patients with breast cancer

Indicators	Ca^{2+}	pH	MMP8, ng/ml	Osteopontin, ng/ml
Ca^{2+}	1.00	0.35	-0.64	0.58
pH	0.35	1.00	-0.52	0.39
MMP8, ng/ml	-0.64	-0.52	1.00	-0.80
Osteopontin, ng/ml	0.58	0.39	-0.80	1.00

The analysis of the obtained data demonstrated the intensification of bone destruction processes and general metabolic disorders in patients with breast cancer that, in our opinion, is mainly due to adverse effects of chemotherapeutic drugs on bone tissue and general immunosuppression in this category of patients.

Moreover, we found a significant correlation between the studied indicators (Table 2).

Table 2 demonstrates that the increase in Ca2 + is associated with a decrease in MMP8 as well as with an increase in pH and osteopontin. In turn, pH has a medium-strength feedback relationship with MMP8 and a direct medium-strength relationship with osteopontin. MMP8 and osteopontin have strong feedback, meaning that an elevation in one of them may indicate a decrease in osteopontin [11, 12, 15].

Determined correlations are due to the general biological functions of matrix metalloproteinase-8 and osteopontin in bone metabolism. Thus, against the background of inflammatory processes and destruction of periodontal tissue, there is an increased production of proteolytic enzymes, including MMP8, in the focus of inflammation, which results from the response of epithelial cells and the immune system to the microbial invasion. This leads to the destruction of the intracellular matrix that consequently results in periodontal pockets, pathological mobility and displacement of teeth, resorption of interalveolar septa, and osteoporosis. Bacterial collagenases act differently: they cleave collagen in different areas, forming many short peptide fragments [9, 13, 14]. Microorganisms interact with gingival epithelial cells through TL-receptors (toll-like receptors). In response, cells secrete interleukin (IL) 1, tumour necrosis factor (TNFα), MMP, and antimicrobial peptides. Cytokines (IL-1, -8, -12, TNFα) stimulate the migration of other types of immune cells to the site of inflammation. Activated macrophages produce iL12 and interferon γ. In the chronic course of the process, the morphological picture in the focus of inflammation resembles that of delayed-type hypersensitivity. The presence of iL12 accelerates chemotaxis and subsequently activates macrophages and type 1 T-helpers [13, 16]. During exacerbation of chronic periodontitis, B-cells, plasma cells, and type 2 T-helpers predominate in the inflammation foci. Stimulation of these cells by bacterial products (e.g., lipopolysaccharides) causes the secretion of the cytokines IL1 and TNFα, which can indirectly induce the differentiation of osteoclast precursors and subsequently their activation. The differentiation of osteoblasts depends on the synthesis of the so-called osteoprotegerin ligand (RANKL) by these cells. In contrast, activated T-cells, through their RANKL synthesis, can directly accelerate osteoblast differentiation. RANKL is predominantly synthesized in Th-1 cells. Thus, Th-1 type T-cells are mainly involved in the action of T-cells on bone resorption in periodontitis [17]. Accordingly, the suppression of humoral and especially cellular immunity in patients with breast cancer leads to an even more pronounced disintegration of these molecular units, which was recorded in patients of group 1.

Thus, we have registered a decrease in osteopontin during the destruction of bone tissue cells and an increase in MMP8 in the oral fluid that points out the degradation of osteosynthesis processes. It is known that osteopontin is an extracellular structural protein and an organic component of bone tissue. It is located on the q arm of chromosome 4, made up of a total of 300 amino acid resi-

dues, and rich in aspartic or glutamic acid residues, which give it a high negative charge. Osteopontin is expressed in various tissues such as bones, teeth, kidneys, endometrium, and epithelium, and it is also found in macrophages, various types of tumours, T-cells and smooth muscle cells. This protein is involved in bone resorption, immune function, angiogenesis, cell survival, wound healing, and cancer development [14-16]. Osteopontin is associated with bone remodelling, chemotaxis, cell activation, and apoptosis. In bone tissue, osteoclasts destroy old bone tissue and this process is a precursor for the formation of new bone tissue. Osteopontin may influence bone homeostasis by enhancing osteoclast differentiation or osteoclast activity. A less pronounced decrease in this indicator in group 2 patients, in our opinion, is directly related to the ongoing therapeutic measures, limiting the inflammatory processes of periodontal tissue destruction [15].

Conclusions

This study has established a statistically significant increase in MMP 8 against the background of a decrease in the osteopontin concentration. Pathobiochemical changes in bone tissue proceeded against the background of a significant decrease in Ca2+ ions and a shift in pH. The ongoing dental treatment in this category of patients improves the concentration of these markers. The data obtained substantiate the appropriateness of their use as markers for diagnostic purposes, as well as for laboratory screening of dental treatment effectiveness in such patients.

The role of both MMP8 and osteopontin in the pathogenesis of dental diseases in patients with cancerous diseases highlighted in this study determines the relevance and prospects of their use as targets of pharmacological intervention in bone tissue and the development of effective ways of targeted therapy.

References

1. Ren, J-X, Gong Y, Ling H, et al. Racial/ethnic differences in the outcomes of patients with metastatic breast cancer: contributions of demographic, socioeconomic, tumor and metastatic characteristics. Breast Cancer Res. Treat. 2019; 173: 225–237.
2. Bray F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J. Clin. 2018; 68: 394–424,
3. Luísa Comerlato Jardim, Priscila Trindade Flores, Maria do Carmo Dos Santos Araújo, et al. Oral health-related quality of life in breast cancer survivors. Support Care Cancer. 2020 Jan;28(1):65-71.
4. Ines Willershausen, Irene Schmidtmann, Adriano Azaripour, et al. Association between breast cancer chemotherapy, oral health and chronic dental infections: a pilot study. Odontology. 2019 Jul;107(3):401-408.
5. Luísa Comerlato Jardim, Priscila Trindade Flores, Felipe de Araújo Sensever, et al. Oral lesions and associated factors in breast cancer survivors. J Investig Clin Dent. 2019 Nov;10(4):e12447.
6. Masahiro Umeda, Yoshihide Ota, Kosuke Kashiwabara, et al. Oral care and oral assessment guide in breast cancer patients receiving everolimus and exemestane: subanalysis of a randomized controlled trial (Oral Care-BC) Ann Transl Med. 2021 Apr;9(7):535.
7. A. de Sire, M. Ferrillo, A Gennari, et al. Bone health, vitamin D status and oral hygiene screening in breast cancer women before starting osteoporosis treatment: a cross-sectional study J Biol Regul Homeost Agents. Jan-Feb 2021;35(1):397-402.
8. Ying-Ying Wu, E Xiao, Dana T. Graves Diabetes mellitus related bone metabolism and periodontal disease Int J Oral Sci. 2015 Jun 26;7(2):63-72.
9. Christopher A Barwacz, Kim A Brogden, Clark M Stanford, et al. Comparison of pro-inflammatory cytokines and bone metabolism mediators around titanium and zirconia dental implant abutments following a minimum of 6 months of clinical function Clin Oral Implants Res. 2015 Apr;26(4):e35-e41.
10. Kazuhiro Maeda, Yasuhiro Kobayashi, Masanori Koide, et al. The Regulation of Bone Metabolism and Disorders by Wnt Signaling Int J Mol Sci. 2019 Nov 6;20(22):5525.
11. Leming Jia, Ye Tu, Xiaoyue Jia, et al. Probiotics ameliorate alveolar bone loss by regulating gut microbiota Cell Prolif. 2021 Jul;54(7):e13075.
12. Matthieu Minty, Thibault Canceil, Matteo Serino, et al. Oral microbiota-induced periodontitis: a new risk factor of metabolic diseases Rev Endocr Metab Disord. 2019 Dec;20(4):449-459.
13. Kaliyatz TV, Solovykh EA, Gershtein ES, Kushlinskii NE. Comparative Analysis of MMP-8 and MMP-9 Concentrations in Crevicular and Peri-Implants Sulcular Fluids Bull Exp Biol Med. 2020 Nov;170(1):19-23.
14. Ulvi K Gursoy, Joonas Liukkonen, Antti Jula, et al. Associations Between Salivary Bone Metabolism Markers and Periodontal Breakdown J Periodontol. 2016 Apr;87(4):367-75.
15. Dileep Sharma CG, Pradeep AR. Gingival crevicular fluid osteopontin levels in periodontal health and disease. J Periodontol. 2006 Oct;77(10):1674-80.
16. Allan VJ. Basic immunofluorescence. Protein localization by fluorescence microscopy: a practical approach. Oxford University Press, United Kingdom, 2006. P. 1–26.
17. Masayuki Tsukasaki RANKL and osteoimmunology in periodontitis J Bone Miner Metab. 2021 Jan;39(1):82-90.

Реферат

ДИНАМІКА ВМІСТУ ММР8 ТА ОСТЕПОНТИНУ В РОТОВІЙ РІДИНІ ПАЦІЄНТІВ ІЗ ЗАХВОРЮВАННЯМИ РОТОВОЇ ПОРОЖНИНИ НА ТЛІ ОНКОПАТОЛОГІЇ

Філон А.М.

Ключові слова: рак молочної залози, стоматологічна патологія, лабораторний скринінг, метаболізм кісткової тканини, біологічні маркери

За даними Всесвітньої організації охорони здоров'я рак молочної залози є однією з найпоширеніших форм онкологічних захворювань у жінок. У 2020 році рак молочної залози діагностований у 2,3 мільйона жінок, тоді як у світі зареєстровано 685 000 випадків смерті від цього захворювання.

Мета роботи. Вивчення динаміки концентрації ММР8, остеопонтину, Са2+іонів, а також рН у пацієнтів з раком молочної залози на тлі хіміотерапії та проведеного лікування зубів.

Матеріали і методи. В дослідженні взяли участь 60 хворих, чоловіків та жінок. Всі респонденти були поділені на групи за онкологічними захворюваннями: першу групу склали 20 хворих жінок на рак молочної залози II та III стадії з хіміотерапією, без попередньо проведеного стоматологічного лікування, другу - 20 хворих жінок на рак молочної залози з хіміотерапією, з попередньо проведеним стоматологічним лікуванням, контрольну групу – 20 осіб, які не страждають на онкологічне захворювання та мають стоматологічну патологію.

Результати. Стоматологічні захворювання пацієнтів обох груп супроводжувались значними змінами метаболізму кісткової тканини, що супроводжувались ризьким приростом у ротовій рідини ММР8 на

тлі зменшення концентрації остеопонтину. Варто зазначити, що вищезазначені патобіохімічні зміни протікали на тлі падіння вмісту іонів Са2+ та зсувом рН у кислу сторону. Приріст ММР8 відбувався на тлі зменшення концентрації остеопонтину. Збільшення значення Ca^{2+} пов'язано зі зменшенням рівня ММР8 та підвищенням рівня рН та остеопонтина. В свою чергу рН має зворотний зв'язок середньої сили з ММР8 та прямий зв'язок середньої сили з остеопонтином. ММР8 з остеопонтином пов'язані сильним зворотним зв'язком, тобто збільшення рівня одного з них може відчити про зменшення остеопонтину.

Висновки. Дослідження встановили статистично значне збільшення ММП 8 на тлі зменшення концентрації остеопонтину. Патобіохімічні зміни кісткової тканини були встановлені на тлі значної втрати іонів Са2+ та зміщення рН. Проведене стоматологічне лікування в цій категорії пацієнтів призвело до нормалізації концентрації цих маркерів. Дані, отримані нами, виправдовує доцільність їх використання як маркерів діагностики, а також лабораторного скринінгу ефективності лікування захворювань зубів у пацієнтів цієї категорії.