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O.Ya. Barabash, N.M. Voronych-Semchenko, G.P. Huchenko, T.P. Kryvenkyi

Changes in the nitric oxide system of oral fluid during adaptation to different types of dental prostheses for dental rows defects

Ivano-Frankivsk National Medical University; e-mail: obarabash@ifnmu.edu.ua

The dynamics of nitric oxide metabolism in oral fluid, specifically nitrite ions, the combined level of nitrites and nitrates, peroxynitrite, and the L-arginine/arginase system, were studied in patients during adaptation to various types of dental prostheses. The study included 30 patients aged 45 to 89 years with partial tooth loss treated using bridge prostheses (BP, n = 9) or partial removable lamellar prostheses (n = 11; 6 first-time users, 5 undergoing repeated prosthetics), and with complete tooth loss treated using complete removable lamellar prostheses (n = 10; 6 first-time users, 4 repeated). Oral fluid samples were collected in the morning, fasting, without salivary stimulation prior to orthopedic treatment and on the 7th, 14th, and 30th days after prosthesis placement. No significant changes in the studied parameters were observed in the BP group. In cases of primary prosthetics with removable structures, a 27.34–61.25% increase in nitric oxide metabolites and a 47.62–54.55% increase in arginase activity were observed in oral fluid by day 7. In repeated prosthetics, the levels of nitric oxide metabolites exceeded baseline by 10.93–21.89%. Similar trends persisted on day 14, while by day 30, the nitric oxide system indices reliably decreased. These changes reflect the stages of patient adaptation to dental prostheses. The studied parameters are informative for predicting the course of process, identifying potential complications, and can be used to personalize rehabilitation strategies in patients with partial or complete tooth loss.
Key words: nitric oxide; oral fluid; orthopedic treatment; adaptation to dental prosthesis.

INTRODUCTION

Adaptation of organism to dental prostheses is a complex psychophysiological process that includes both mechanical and neurobiochemical components. An important role in this mechanism is played by the nitric oxide (NO) system, which ensures the regulation of vascular tone, immune response, the course of metabolic and reparative processes in the oral cavity. NO has the ability to easily exit the cells that synthesised it into the intercellular space, as well as penetrate target cells (without receptor interaction), which characterises its neurotransmitter properties [1]. It affects inflammatory reactions, microcirculation and general homeostasis of the oral mucosa. The NO system protects the mucous membrane from the effects of harmful factors (toxic substances, ischaemia, ischaemia/reperfusion trauma, and others), inhibits the adhesion of neutrophils and platelets. At the same time, excessive NO production can injure the mucous membrane. Therefore, studies of local NO metabolism in the oral fluid during the adaptation to different types of orthopaedic structures deserve special attention, which can be informative for examination of biochemical changes in response to external stimuli [1–4]. Considering that under conditions of hypoxia NO synthesis is possible due to the reaction between L-arginine and hydrogen peroxide, it is of interest to study the L-arginine/arginase system [5].

Modern orthopedic dentistry offers a wide range of prosthetic solutions, from bridge-like structures to complete removable prostheses. However, prostheses that are different in structure, area of contact with the mucous membrane and stability of fixation have different effects on the course of metabolic processes in the oral cavity, including the NO system. However, the data about the changes of NO synthesis and metabolism in the process of adaptation to different types of prostheses, especially considering primary and repeated prosthesis, are rather limited, what determines the relevance in-depth study of this problem. Identification of biochemical changes in the oral fluid will allow not only to better understand the mechanisms of adaptation but also form the basis for the development of personalised approaches to the rehabilitation of patients with partial or complete tooth loss [6, 7].

The aim of the study: to investigate the peculiarities of NO metabolism, the activity of L-arginine/arginase system in the oral fluid during the adaptation to different types of orthopedic structures.

METHODS

30 practically healthy individuals aged 45 to 89 years were under observation. During the formation of groups, considering dental status and type of prosthesis, the preservation of the integrity of dental rows and the type of prosthesis used to replace the dentition defect were taken into account. In particular, patients with partial tooth loss and under conditions of defect replacement: bridge prostheses (BP) (1st group, n = 9), partial removable lamellar prostheses (PRLP) – primary prosthesis (2nd group, n = 6), partial removable lamellar prostheses (PRLP) – repeated prosthesis (3rd group, n = 5); patients with complete tooth loss and under conditions of defects replacement with complete removable lamellar prostheses (CRLP) – primary prosthesis (4th group, n = 6), complete removable lamellar prostheses (CRLP) – repeated prosthesis (5th group, n = 4).

The oral fluid was collected by spitting into sterile tubes in the morning, fasting, without stimulation of salivation, preliminary cleaning and rinsing of the oral cavity. Indexes of NO metabolism (nitrite ion content, sum of nitrite and nitrate, peroxynitrite concentration) and the L-arginine/arginase system (L-arginine content and arginase activity) were examined in the oral fluid [8, 9]. The studied parameters were determined before orthopedic treatment and at different stages of adaptation to dental prostheses: on the 7th, 14th and 30th day after prostheses placement (primary or repeated).

The oral cavity was examined using a set of standard instruments in the dental office. The examinations were conducted with the informed consent of the patients for clinical examinations. The conclusion of the Bioethics Committee of Ivano-Frankivsk National Medical University confirmed compliance with the "Rules for Aesthetic Principles of Scientific Research Involving Human Subjects" approved by the Declaration of Helsinki (1964-2013) and in accordance with the ethical and moral requirements of Order of the Ministry of Health of Ukraine No. 281 from 01.11.2000 (protocol No. 125/22 from 25.03.2022).

The statistical processing of the results was carried out based on the Exel package of Microsoft Office 365 ProPlus using the methods of variation statistics with the help of Student's t test. The difference between the studied indexes was considered reliable at a value of $P < 0.05$.

RESULTS AND DISCUSSION

As a result of the study, in patients on the 7th day after prosthesis by BP an increase the amount of nitrite and nitrate sum in the oral fluid by 12.16% ($P < 0.05$) and a reliable decrease the index to the baseline level on the 30th day after prosthesis was found (Fig. 1). Such changes were observed against the background of relative inertness of the L-arginine/arginase system.

After the primary placement of PRLP on the 7-th day of observation, in the oral fluid increased the content of nitrite ion by 41.45% ($P < 0.05$), the sum of nitrite and nitrate – by 27.34% ($P < 0.05$), the content of peroxynitrite – by

61.25% (P < 0.01) regarding to the baseline values. Such changes occurred against the background of a reliable increase of arginase activity by 54.55% and a decrease the content of L-arginine in the oral fluid by 25.29% compared with the values of similar indexes before orthopedic treatment (Fig. 2). On the 14th day of observation, the content of NO metabolites exceeded the control value by 28.00-33.22% (P < 0.05). On the 30th day, the most of studied parameters did not reliably differ from the baseline.

In the case of repeated prosthesis by PRLP, the content of nitrite ion in the oral fluid increased by 18.90% (P < 0.05) on the 7th day, but decreased on the 14th; the sum of nitrite and nitrate increased by 10.93% (P < 0.05) on the 7th day of observation and by 7.22%

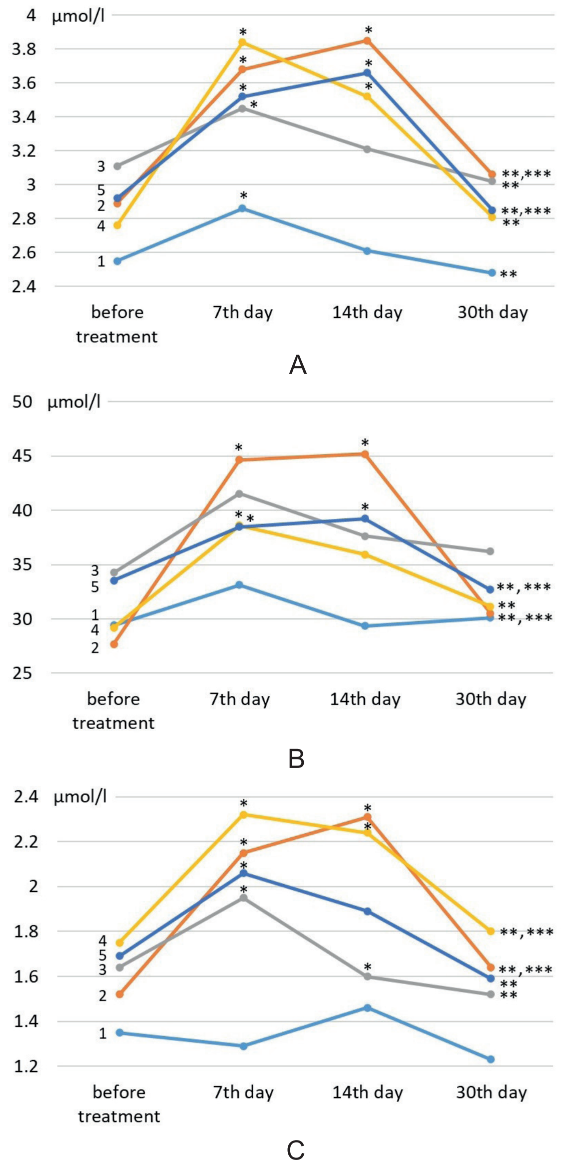

Fig. 1. Changes of the nitrite ion content (A), the sum of nitrite and nitrate (B), peroxynitrite (C) in the oral fluid of patients before orthopedic treatment, on the 7th, 14th and 30th day after the placement of prostheses (M ± m): 1 - placement of bridge prostheses (BP), 2 - primary placement of partial removable lamellar prostheses (PRLP), 3 - repeated placement of PRLP, 4 - primary placement of complete removable lamellar prostheses (CRLP), 5 - repeated placement of CRLP. *P < 0.05 compared to the data before prosthesis; **P < 0.05 compared to the data on the 7th day after prosthesis; ***P < 0.05 compared to the data on the 14th day after prosthesis

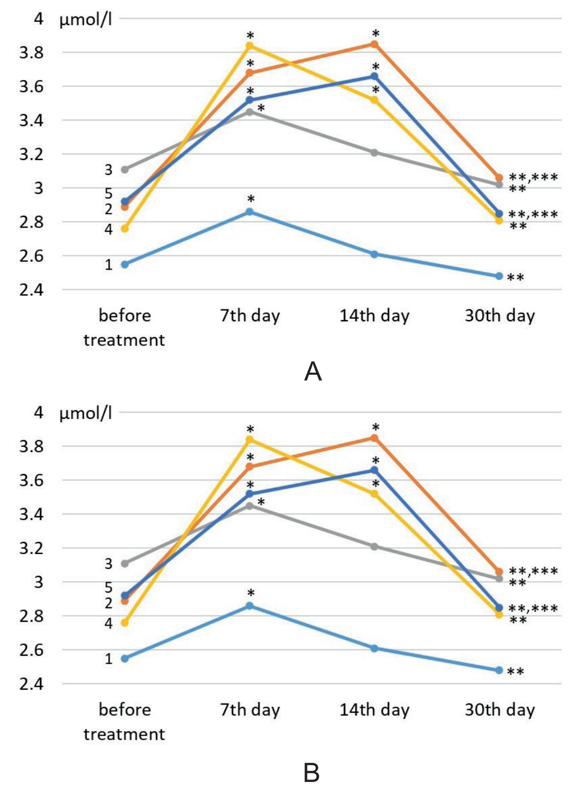

Fig. 2. Changes of the L-arginine content (A), arginase activity (B) in the oral fluid of patients before orthopedic treatment, on the 7th, 14th and 30th day after the placement of prostheses (M ± m): 1 - placement of bridge prostheses (BP), 2 - primary placement of partial removable lamellar prostheses (PRLP), 3 - repeated placement of PRLP, 4 - primary placement of complete removable lamellar prostheses (CRLP), 5 - repeated placement of CRLP. *P < 0.05 compared to the data before prosthesis; **P < 0.05 compared to the data on the 7th day after prosthesis (B); ***P < 0.05 compared to the data on the 14th day after prosthesis

($P < 0.05$) on the 14th, decreasing only on the 30th day after orthopedic treatment. The increase of peroxynitrite content in the oral fluid was not significant at any stage. The reliable decrease of L-arginine content in the oral fluid on the 7th day of observation (by 25.29%) against the background of arginase activation (by 26.09%) compared with the data before prosthesis is noteworthy. On the 14th and 30th day of observation, the changes of studied parameters in the oral fluid of patients of the 3rd group were not so significant.

Replacement of dentition defects by CRLP led to the pronounced changes of indexes of NO system. On the 7th day after primary prosthesis, a reliable maximal increase the content of nitrite ion (by 32.57%), the sum of nitrite and nitrate (by 10.93%) and peroxynitrite (by 32.31%) in the oral fluid was recorded relative to the values of these indexes before the prosthesis (see Fig. 1). In the case of repeated orthopedic treatment, the content of NO and its metabolites reached maximum values on the 14th day (increased by 16.99-30.74%, $P < 0.05$), while on the 7th day only an increase the content of nitrite ion (by 21.85%, $P < 0.05$) was found relative to the baseline values. On the 30th day of observation, the studied parameters did not significantly differ from the values before treatment, and in the case of repeated prosthesis they were even lower.

Under such conditions, in the case of primary prosthesis on the 7th day of observation, the content of L-arginine in the oral fluid was lower by 25.11% ($P < 0.05$), and arginase activity was higher by 47.62% ($P < 0.05$) compared with the values before treatment (see Fig. 2). The L-arginine content returned to the baseline level on the 30th day, and arginase activity – on the 14th day of adaptation. In the case of repeated prosthesis, the content of L-arginine decreased significantly on 14th day (by 17.42%), however already on the 30th day the reliable changes of the L-arginine/arginase system compared with the baseline values were not found.

The revealed during the examination changes in the concentration of NO metabolites in the oral fluid of patients after orthopedic treatment are the result of a complex adaptive response of the oral mucosa to the new prosthetic loading. The placement of dental prostheses, especially partial or complete removable lamellar prostheses, causes the mechanical irritation, changes of the microbial composition of the biofilm and local violation of homeostasis, which can stimulate immune and inflammatory reactions [10, 11].

An increase the content of nitrites and nitrates sum, toxic peroxynitrite on the 7th day after the primary placement of orthopedic structures, may be a consequence of not only the inflammatory process development, but also activation of oxidative reactions. Peroxynitrite and other prooxidants are formed as a result of absorption of NO by superoxide. Its toxicity is caused by damage of proteins and lipids of cell membranes, vascular endothelium, increased platelet aggregation [12–14]. At the same time, a decrease of substrate concentration for NO L-arginine synthesis, was found against the background of arginase activation. L-arginine deficiency can occur in case of its increased metabolism. Arginase hydrolyses L-arginine, competing for it and reducing the activity of endothelial NO-synthase (eNOS). Such competition between enzymatic systems may reflect an attempt to limit the excessive formation of NO by physiological way and, accordingly, a cytotoxic product peroxynitrite formed when NO interacts with reactive oxygen species [15, 16].

In patients with BP and in the case of repeated prosthesis by PRLP and CRLP, the reaction of NO system was less pronounced, which is probably due to lower traumatisation, better adaptation of the mucous membrane or pre-existing tolerance to prosthetic loading.

It should be noted, that the processes of adaptation to removable laminar prostheses usually take longer than to the fixed BP, since they are larger in size and contact surface area with the tissues of oral cavity, and therefore have a greater impact on them. The less pronounced changes of the studied parameters under conditions of repeated prostheses manufacturing are also noteworthy. Under such conditions, the adaptation processes are usually faster, since the tissues of the prosthetic bed are already more adapted to them. The mechanisms of adaptation to dental prostheses involve components of higher nervous activity, because the speed of inhibition and the formation of conditioned reflex connections are significantly affected by the dynamism and balance of excitation and inhibition processes [17, 18]. According to the literature data, adaptation to a newly manufactured prosthesis lasts from 20 to 40 days. During this time, the sensation of a foreign body in the mouth disappears, the vomiting reflex is less pronounced or absent, chewing function and diction are restored. At this time, patients perceive the prosthesis as their own teeth, and therefore a feeling of discomfort appears without it [11, 14]. It is physiologically justified that the development of inhibition to the same conditioned stimuli is a much faster process. Therefore, adaptation in the case of repeated prosthesis can last only 7-10 days.

The detected changes of NO system generally responded the course of adaptation to the dental prostheses and depended on their features. The most pronounced changes of the examined indexes were observed on the 14th day after orthopedic treatment, which characterises the highest intensity of nitrative stress caused by pathophysiological disorders. On the 30th day, the intensity of the processes decreases. Less pronounced changes of the studied processes in the case of repeated prosthesis may be a consequence of the effect of the organism "adaptive memory". The obtained data allow to suggest the feasibility and prospects for the creation of diagnostic algorithms based on the markers of nitro-oxidative stress in order to optimise orthopedic treatment and increasing of its effectiveness.

CONCLUSIONS

The process of adaptation to different types of orthopedic structures for the replacement of dental rows defects is accompanied by the development of nitro-oxidative stress, reflecting the changes of NO system in the oral fluid. Its intensity is the lowest in patients with BP, which indicates the high biocompatibility of such prostheses. In contrast, removable partial and complete lamellar prostheses cause significant changes in the content of NO metabolites (increasing of nitrite ion, the sum of nitrite and nitrate, peroxynitrite), as well as imbalance in the L-arginine/arginase system, which may characterise an excessive loading on the adaptive, immune and reparative mechanisms of the oral mucosa. The primary prosthesis causes more pronounced changes of the studied processes, which may be a consequence of severe inflammatory process and metabolic reactions, while the repeated placement of similar structures is accompanied by less pronounced disorders. Therefore, the dynamic monitoring of biochemical parameters of oral fluid, in particular markers of NO metabolism, can be an important implement for predicting the course of adaptation, identifying the complications and personalising the rehabilitation measures in patients with partial or complete tooth loss.

The authors of this study confirm that the research and publication of the results were not associated with any conflicts regarding commercial or financial relations, relations with organizations and/or individuals who may have been related to the study, and interrelations of co-authors of the article.

О.Я. Барабаш, Н.М. Воронич-Семченко, Г.П. Гученко, Т.П. Кривенький

ЗМІНИ СИСТЕМИ ОКСИДУ АЗОТУ РОТОВОЇ РІДИНИ В ПРОЦЕСІ АДАПТАЦІЇ ДО РІЗНОГО ВИДУ ПРОТЕЗІВ ПРИ ЗАМІЩЕННІ ДЕФЕКТІВ ЗУБНИХ РЯДІВ

Івано-Франківський національний медичний університет; e-mail:obarabash@ifnmu.edu.ua

Вивчали динаміку показників метаболізму оксиду азоту нітрит-іону, суми нітритів і нітратів, пероксинітриту, активності системи L-аргінін/аргіназа у ротовій рідині пацієнтів під час адаптації до різних видів зубних про-

тезів. Обстежено 30 пацієнтів віком від 45 до 89 років із частковою втратою зубів і заміщенням дефектів мостоподібними протезами (МП, n = 9) і частковими знімними пластинковими протезами (ЧЗПП, n = 11, з них 6 протезовані вперше, 5 – повторно) та повною втратою зубів і заміщенням дефектів повними знімними пластинковими протезами (ПЗПП, n = 10, з них у 6 – перше протезування, у 4 – повторне). Зразки ротової рідини збирали вранці, натще, без стимуляції слиновиділення до ортопедичного лікування і на 7-му, 14-ту та 30-ту добу після встановлення протезів. З'ясовано, що МП не зумовлювали суттєвих змін досліджуваних показників. При первинному протезуванні ЧЗПП та ПЗПП на 7-му добу спостерігали значне збільшення вмісту метаболітів оксиду азоту на 27,34 – 61,25%, зростання активності аргінази на 47,62 – 54,55 % у ротовій рідині щодо вихідних значень. При повторному протезуванні вміст метаболітів оксиду азоту перевищив дані до лікування на 10,93 – 21,89 %. Така тенденція зберігалась на 14-ту добу спостереження, тоді як на 30-ту активність системи оксиду азоту вірогідно знижувалась. Такі зміни відображають етапи адаптації до зубних конструкцій, а досліджувані показники є інформативними для прогнозування перебігу процесів, виявлення ускладнень і можуть бути використані для персоналізації реабілітаційних заходів у пацієнтів із частковою або повною втратою зубів.
Ключові слова: оксид азоту, ротова рідина, адаптація до зубних протезів.

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