ORIGINAL ARTICLE

CONTENTS

Correction of periodontal status and evaluation of disorders in biomarkers of local oxidative metabolism in patients with orthodontic anomalies on the background of generalized periodontitis

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ABSTRACT

Aim: To determine clinical efficacy of developed therapeutic and prophylactic complex (TPC) in orthodontic patients with periodontal pathology and studying its correction after installation of fixed orthodontic appliances. Evaluation of this efficacy will be based on assessment of periodontal indices and changes in activity of enzymes of antioxidant defense, as well as the activity of enzymatic markers of local stress response in oral fluid.

Materials and Methods: 118 patients with orthodontic anomalies and generalized periodontitis were divided into two groups: 60 (main), and 58 (comparison). Clinical and paraclinical parameters for assessing periodontal health, including biochemical parameters of enzymes of oral fluid, were studied. In main group, TPC was applied, and in comparison group, traditional treatment regimen was used. The indicators were evaluated before and after periodontal treatment.

Results: The developed TPC made it possible reducing by 3-3.5 times the preparation for active orthodontic period and achieve stabilization of pathological process in the periodontal tissues at different time after braces installation. In patients of main group, indicators of studied enzymes in oral fluid were improved by 1.5-2.4 times, compared to results obtained in comparison group, where, after using traditional treatment regimen, level of oxidative stress and local oxidative metabolic disorders remained high.

Conclusions: Changes in in periodontal status and activity of SOD and LDH activity relative to CA and AA measured in orthodontic patients with periodontal disease were shown to be important biomarkers of treatment effectiveness and controlling the state of reduced periodontium comparing to background of oxidative stress caused by fixed orthodontic appliances.

KEYWORDS: treatment, periodontitis, enzymes, orthodontic appliance, orthodontic anomalies, periodontal indices

Wiad Lek. 2026;79(9):2129-2141. doi: 10.36740/WLek/225978 DOI

INTRODUCTION

The development of oxidative stress (OS) is one of the biological reactions of oral tissues to the installation of fixed orthodontic appliances [1]. During the active period of orthodontic treatment, two different conditions coexist that can cause OS: on the one hand, it is the fixed appliance, and on the other, it is the biomechanics of tooth movement. At the same time, the use of braces often creates a complicated situation that provokes an inflammatory reaction around the teeth being moved, especially in patients with compromised periodontium. [2]. Since there is compelling evidence that periodontal tissue inflammation is one of the main sources of reactive oxygen forms (ROS) in the oral cavity, it can be assumed that aseptic inflammation is associated with damage caused by OS [3]. Most orthodontic appliances made of metal, ceramic, or plastic (organic polymer) materials can have significant oxidative potential and release metals or other elements through a phenomenon known as corrosion. This causes an increase in the level of ROS through various reactions with those free radicals that are catalyzed by metals [4].

Medication is extremely important in preparing periodontal patients with orthodontic anomalies (OA) for the active period of orthodontic treatment and throughout its course. Particular attention should be paid to drugs with antioxidant, antihypoxic, cytoprotective, and metabolic properties, which are pathogenetically justified and perform well in OS conditions. The

world literature shows that flavonoids, as free radical scavengers, have pronounced antioxidant, anti-inflammatory, anti-allergic, antiviral, antibacterial, and antiaggregant effects [5]. The phenolic structure allows the molecules of these substances to interact with free radicals. As a result, the intensity of lipid peroxidation decreases and the formation of the main negative factor, malondialdehyde, is inhibited [6]. Along with this, physiotherapeutic methods of treating periodontal diseases are of considerable interest, as they potentiate the penetration of the drug into the periodontal tissues, have a reflex effect, favorably affect local hemodynamics, eliminate hypoxia, and stimulate nonspecific tissue reactivity and the body's defenses [7]. As a result, blood and lymph circulation, trophism, and metabolism improve in periodontal tissues, inflammation and congestion decrease, and the anti-inflammatory, antimicrobial, and analgesic effects of medications are prolonged, accelerating reparative processes [8].

Currently, it is relevant to analyze the activity of oral fluid enzymes using biochemical methods, which allow for a more accurate assessment of the degree of periodontal tissue damage before and after periodontal treatment, as well as to fully monitor the condition of periodontal tissues during the active period of orthodontic treatment [9].

AIM

To determine clinical efficacy of the developed therapeutic and prophylactic complex (TPC) in the orthodontic patients with periodontal pathology and study its correction after the installation of fixed orthodontic appliances. The evaluation of this efficacy will be based on the assessment of periodontal indices and changes in the activity of enzymes of the antioxidant defense, as well as the activity of the enzymatic markers of local stress response in oral fluid.

MATERIALS AND METHODS

The design of this study consisted of the following steps: 1) use of main criteria for inclusion of patients with orthodontic anomalies complicated by generalized periodontitis; 2) clinical and diagnostic characterization of these patients; 3) biochemical analysis of oral fluid of selected patients; 4) development of treatment plan for patients of the main and comparison groups; 5) statistical processing of the obtained results; 6) research hypothesis based on the results of using the developed therapeutic and prophylactic complex.

A randomized controlled clinical trial aimed at evaluating the effectiveness of a developed therapeutic and prevention complex (TPC) in patients with orthodontic anomalies (OA) complicated by generalized periodontitis (GP) was carried out. A total of 118 patients aged 20-35 years of both sexes diagnosed with stage I–II (grade A) generalized periodontitis who sought orthodontic treatment were included in the study. The patients were randomly divided into two groups: the main group (n = 60), in which periodontal treatment was performed as part of preparation for active orthodontic treatment and during orthodontic therapy using the developed TPC; the comparison group (n = 58), which received standard treatment according to the generally accepted methodology in accordance with 'Guideline 00163. Periodontal diseases (gingivitis and periodontitis) [10].

The criteria for inclusion of patients in the study were as follows:

- patients with OA complicated by periodontal disease;
- patients of both sexes aged 20 to 35 years;
- the patient's individual consent to participate in the study;
- the ability to visit the dentist at the appointed time.

The exclusion criteria for the study were as follows:

- patients with severe systemic diseases;
- patients with cancer, with a history of chemotherapy and radiation therapy;
- presence of genetic diseases, immunodeficiency states, polyvalent allergy;
- acute and chronic infectious and viral diseases;
- patients who took various medications (corticosteroids, immunosuppressants, antibiotics).
- patients with secondary adentia (partial or complete);
- pregnancy and lactation;
- harmful habits (smoking);
- patient refusal to participate in the research program.

CLINICAL AND DIAGNOSTIC METHODS

The following methods and materials were used to achieve the set goal: in addition to 118 medical record of a dental patient (Form No. 043/o), 118 periodontal cards and 118 orthopantomograms (was performed to clarify periodontal diagnosis) were created before treatment and the same number were created 6 months after treatment. According to the course of the study based on the collection of medical history and clinical and radiological assessment of the condition of periodontal tissues to diagnose GP, the classification of diseases and conditions of periodontal and peri-implant tissues was used (World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions, EFP & AAP World Workshop, Chicago, 2017) [11]. To

evaluate the effectiveness of periodontal treatment, the following indices were calculated: BOP (Bleeding on Probing, Ainamo & Bay, 1975), the Approximal Plaque Index (API) (Lange D.E., Plagmann H., 1977) for dental plaque in the interdental spaces, and the Gingival Index (GI) (J. Sillness, H. Loe, 1967), which allows determining the location and severity of gum inflammation (mild, moderate, and severe) [12]. X-ray examination of the jaw bones was performed using intraoral and panoramic radiography on a «VillaRotograPH-D» (Italy) device. The structure, shape, and height of the interalveolar septa, the distinctness of the cortical plate, and the condition of the cancellous bone of the alveolar processes of the jaws were evaluated.

The diagnosis of the OA was performed according to the classifications of D.A. Calvelis and E H. Angle [13, 14], in which the following occlusion pathologies were diagnosed and examined in patients of both groups: prognathia (distal occlusion), deep bite, crossbite, open bite and progenia (mesial occlusion). Also, the patients had dentition abnormalities combined with occlusion pathology, such as disrupted dentition formation, abnormalities of individual teeth, crowded teeth, vestibular and oral overjet, mesial and distal overjet, tortoanomalies, supra- and infraposition of teeth, diastemas and tremas.

BIOCHEMICAL ANALYSIS OF ORAL FLUID

Enzymatic activity of catalase, amylase, superoxide dismutase, and lactate dehydrogenase were measured in oral fluid of patients of both groups before and after treatment. According to the course of studies in patients of both groups catalase activity (CA) of oral fluid was measured using method based on the ability of H_2O_2 to form a colored complex with the molybdenum salts [15]. The reference values of normal CA are 180-200 U/ml. Amylase activity (AA) in the oral fluid was measured using a modification of the Wolgemuth method that is based on the ability of amylase to hydrolyze starch [16, 17]. The reference values of AA are 10,000 – 11,000 U/L [15, 18]. Measurement of the activity of superoxide dismutase (SOD) in oral fluid was performed using conjugated nitroblue tetrazolium conversion reaction that is based on the ability of SOD to compete with nitroblue tetrazolium for superoxide anions formed as a result of aerobic interaction of the reduced form of nicotinamide nucleotide (NADH) in the presence of phenazine methasulfate (PMS) [19-21]. The reference values for the norm of SOD are approximately 30%. The colored products of the reaction were determined spectrophotometrically at the corresponding absorption wavelengths on a multichannel microphotometer «Plate Reader BioTek» 76883 (BioTek, USA) [15].

The automated biochemical analysis of lactate dehydrogenase (LDH) was performed on «Alinity c» analyzer (Abbott Laboratories, USA) using the «Lactate Dehydrogenase2 Reagent Kit» in the UNILAB LLC laboratory (License of the Ministry of Health of Ukraine Series AG No. 597497 dated by 09.02.2012). The reference values of LDH levels in the oral fluid were 135.0-214.0 U/L [21, 22].

The Treatment Plan for 60 patients in the main group prior to the active period of orthodontic treatment and during its course was as follows: a treatment plan was developed an extemporaneous periodontal gel composition under the patented name «Benzydaflaziverdyne» (GCB), containing two active components – benzidamine hydrochloride (BH) in tablet form «T-Sept®» (ICN Polfa Rzeszow S.A, Poland), which has anti-exudative, anti-edematous, antioxidant and analgesic properties, and «Proteflazid®» drops (LLC SPC Ecopharm, Ukraine) containing antioxidants of plant origin (flavonoids) in a gel base consisting of sodium alginate, nipagine and water for injection [23]. The above mentioned GCB was included in the treatment and prevention complex (TPC) (Table 1). The initial periodontal therapy in the main group was a part of oral cavity sanitation which began with teaching patients rational hygiene and controlled toothbrushing (Table. 1). Removal of dental plaque was combined with the SRP (Scaling and Root Planing) protocol which was performed using an instrumental method (Gracie curettes (Hu-Friedy, USA) and Kohler (Germany)) in combination with an ultrasonic scaler «Woodpecker UDS L» (China). Before the procedure, the oral cavity was irrigated with «Tantum Verde®» solution in which BH is the active ingredient. Additionally, for rinsing the mouth, patients of the main group were recommended 15 ml of this drug 3-4 times a day for 7-8 days.

In order to prolong the effect of GCB in patients of the main group, two approaches were applied: 1. Individually made mouthguards; 2. Modified electrophoresis procedure (Table 1). For this purpose, individually made mouthguards for GCB and electrodes made of thin sheet metal 0.5 mm thick were used. Metal plates of active electrodes on the vestibular side and plates of passive electrodes on the oral/palatal side were placed in the mouthguards (Fig. 1 - A), filled with GCB, which has a distinctive emerald color, and fitted in the oral cavity (Fig. 1 - B). With the patient's mouth closed, the «Potok-1» effectrophoresis device (SMEP) was turned on. The duration of one GCB electrophoresis procedure was 45-50 seconds for one jaw. This time interval was established on an experimental model of in vitro

Table 1. Algorithm of application of treatment and prophylactic complex developed for orthodontic patients with GP stage I-II (gradeA) of the main group

Before the active period of the orthodontic treatment:		
General therapy:	Individual oral hygiene:	Local therapy:
1. «Biotus Ca, Mg, Z with Vitamin D3 BIO-530982» (Biotus, Ukraine) - 1 capsule 3 times a day with meals (recommended course of treatment 3-4 weeks x twice a year)) 2. «Resveratrol» (GREEN PHARMA, Ukraine) - 1 capsule 2-3 times a day after meals; (recommended course of treatment is 4 weeks x twice a year)	1. «Vitis Aloe Vera Apple» toothpaste (DENTAID, Spain); 2. Interdental brushes - Interprox 4G and 2G (DENTAID); 3. Dental floss (VITIS), (DENTAID); 4. Bass cleaning method 5. Use of the Mira-2-Ton plaque revelerator (Miradent, (HAGER Werken, Germany))	1. «Tantum Verde» mouthwash (Angelini Francesco A.C.R.A.F. S.p.A., Italy); 2. Professional hygiene; 3. SRP protocol; 4. Oral cavity sanitation; 5. Use of individual mouthguards with GCB (for 2 hours once a day, a course of 4-5 days); 6. Conducting gingival electrophoresis with GCB (4-5 procedures)
In the active period of the orthodontic treatment:		
General therapy: 1. «Biotus Ca, Mg, Z with Vitamin D3 BIO-530982» Repeat the course in 6 months; 2. «Resveratrol» – repeat the course in 6 months	Individual oral hygiene: 1. Toothpaste «VITIS orthodontic» (DENTAID); 2. Interdental brushes – Interprox 4G and 2G (DENTAID)	Local therapy: Follow-up therapy every month: 1. Professional hygiene and rinsing of the mouth with «Tantum Verde» solution (7-8 days course once every 2 months); 2. Individual mouthguards with GCB (2-3 procedures); 3. Electrophoresis with GCB (2-3 procedures)

Source: compiled by the authors of this study

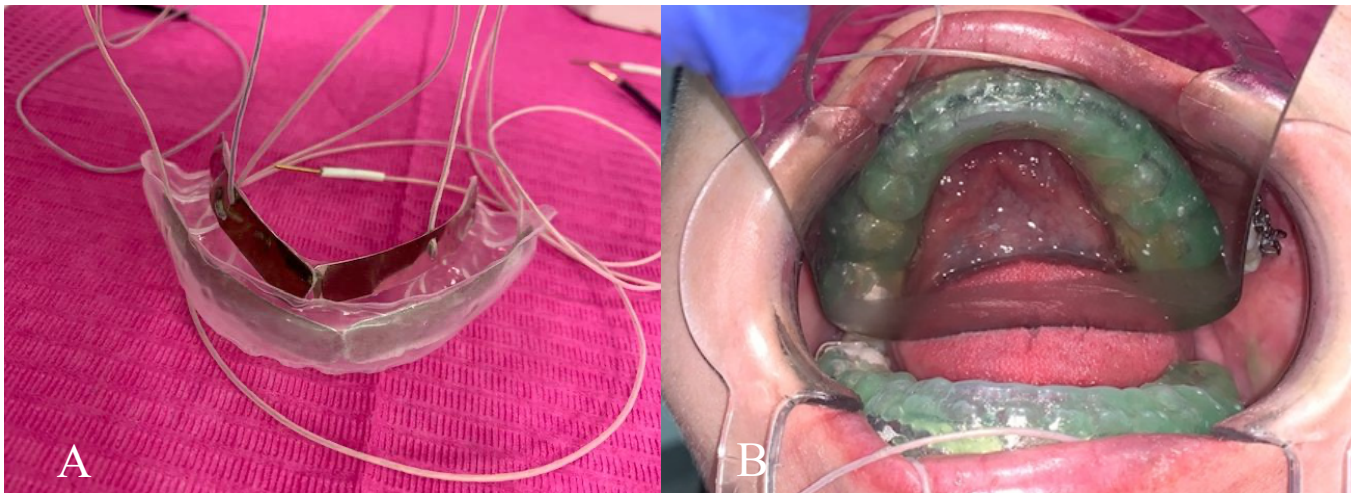


Fig. 1. A – Fitting of electrodes in a custom-made mouthguard on the vestibular and oral sides; B – Fitting of a mouthguard with a GCB in the oral cavity
Picture taken by the authors

electrophoresis with three types of test cells (mouse fibroblasts of the BALB-3T3 line, mouse macrophages of the J774.2 line, and pseudonormal human keratinocytes of the HaCaTi line) cultured in agar-supplemented medium [24].

General drug therapy in the main group included the multimineral complex «Biotus Calcium Magnesium Zinc with Vitamin D3 BIO-530982» and natural antioxidant «Resveratrol» (Table 1).

After achieving stabilization of the periodontal tissues during the active period of orthodontic treatment, patients of the main group were prescribed a low-abrasiveness toothpaste «VITIS orthodontic» containing cetylpyridine chloride, sodium fluoride, Aloe vera and allantoin for individual toothbrushing. Interdental brushes were prescribed – Interprox 4G, Interprox 2G. Control of the level of oral hygiene and maintenance therapy in the main group was carried out once a month using the following therapeutic and prophylactic measures: «Tantum Verde®» mouthwash; use of individual mouthguards with GCB; electrophoresis procedures with GCB. Six months after braces were placed, patients in the main group were prescribed a repeated course of general medications «Biotus Calcium Magnesium Zinc with Vitamin D3 60 Caps BIO-530982» and «Resveratrol» (Table 1).

Patients of the comparison group (58 patients with a same diagnosis) received a traditional treatment regimen according to the the generally accepted methodology in accordance with 'Guideline 00163. Periodontal diseases (gingivitis and periodontitis) [10]. It included training in individual hygiene with a prescription of «Parodontax» toothpaste. For topical treatment before the professional hygiene procedure and SRP protocol, a traditional antimicrobial agent for oral irrigation, chlorhexidine bigluconate 0.05% was used. It was prescribed in accordance with protocols of the conventional treatment. Patients were supplied with a spatula to the gingival mucosa with «Cholisal gel» (Jelfa S.A. Poland) (treatment course – 7 days). In the comparison group, traditional hygiene control and maintenance therapy were performed once every three months.

The observation periods in both groups (main and comparative) were as follows: all indicators were obtained before and after periodontal treatment of stage I-II (grade A) immediately before the installation of fixed orthodontic appliances (G&H® miniPrevail® TWIN Bracket System) and then at 3 and 6 months of the active period of orthodontic treatment.

The research hypothesis is that the use of the developed therapeutic and prophylactic complex (TPC), containing flavonoid-based preparations and benzidamine hydrochloride in combination with electrophoresis and applied to patients in the main group, provides better clinical results in the treatment of stage I-II generalized periodontitis (grade A) in the orthodontic patients. This demonstrates an improvement in periodontal status and enzyme activity in oral fluid and reduces the time required to prepare for the active period of orthodontic treatment, ensuring better stabilization of the pathological process when installing fixed orthodontic appliances compared to the traditional treatment regimen used in patients in the comparison group.

BIOETHICS

The study was performed taking into account the main provisions of the GCP ICH and the Declaration of Helsinki on biomedical research where a person is the object, and the subsequent revision, Seoul, 2008; the Council of European Convention on Human Rights and Biomedicine (2007) and the recommendations of the Bioethics Committee at the Presidium of the National Academy of Medical Sciences of Ukraine (2002), as well as a positive opinion of the commission on ethics of State Non-profit Enterprise «Danylo Halytskyi Lviv National Medical University» (extract from protocol No. 1 of January 15, 2024). No violations of moral and ethical norms were identified during the research.

STATISTICAL PROCESSING OF RESULTS

The obtained numerical results were analyzed using statistical processing according to generally accepted methods. The creation and editing of the primary database was performed in Microsoft Excel. Statistical processing of digital material was performed using OriginPro 7.0 software (OriginLab, Northampton, Massachusetts, USA). The significance level was set at $p \leq 0.05$ (95% confidence interval) for all statistical procedures, which is generally accepted in medical and biological research. The results were considered reliable at $p \leq 0.05$ and highly reliable at $p \leq 0.001$. Quantitative indicators were tested for normal distribution using the Shapiro-Wilk test. A two-tailed Student's (heteroscedastic) t-test, which analyzes the overall difference, was used to analyze the differences between the two groups. A paired t-test was used to analyze the differences before/after treatment. To visualize the data obtained, each indicator presented in the figures (the ordinate of the columns in the diagrams) corresponds to the average value «M» calculated from the results of three measurements for one sample in each of the determination methods. The standard deviation «σ» of the result obtained was calculated based on the mean error «m». This is represented in the illustration next to each indicator by a vertical line, the length of which corresponds to the value «m» [25].

AI STATEMENT

The author states that no AI tools were used in writing this article.

FRAMEWORK

The work is a fragment of scientific research work of Department of Ortodontic State Non-profit Enterprise «Danylo Halytsky Lviv National Medical University»: «The state of dental health and its correction on the basis of systematic analysis of clinical and laboratory, radiological, morphological, functional, aesthetic parameters in people of different ages», state registration number 0120U002143.

This study (authors RS & OK) was funded by "Presidential Discretionary-Ukraine Support Grants" SFI-PD-Ukraine-00014576 from Simons Foundation. In addition, we are grateful to Manko Nazar, PhD, Junior Researcher of the Department of Regulation of Cell Proliferation and Apoptosis, Institute of Cell Biology, National Academy of Sciences of Ukraine, Lviv, Ukraine, for his technical assistance.

RESULTS

In all 118 patients who sought an orthodontic treatment, the examination before braces were installed, there were

no complaints of bleeding and swelling of the gums, bad breath, and discomfort typical of stage I-II GP (grade A). Filling out and analyzing periodontal charts revealed that the average depth of the periodontal pockets was 1.5-3.5 mm. In 40% of patients, the mobility of individual teeth of the first degree was noted. The X-ray images in the areas of the anterior teeth and molars showed a mixed situation, since it was particularly difficult to trace the complete loss of the cortical layer or only the loosening of the tops of the interalveolar septa of the anterior teeth in 67% of patients in both groups (main and comparison) due to teeth crowding.

Before the start of periodontal treatment, the BOP, API, and GI indices in patients in both groups (the main group – 60 people and the comparison group – 58 people) did not differ significantly (Table 2). After treatment, statistically significant differences in the indices of both groups were observed for all three indices. While the average BOP index before treatment in both groups was 77.13%, after the use of TPC in the main group, this index decreased to 1.83 ± 0.71% ($p<0.001$), in contrast to 10.24 ± 9.60% in the comparison group, whose patients received traditional treatment. Accordingly, in the comparison group, these indicators delayed the installation of braces and led to the continuation of the treatment course (Table 2).

The mean values of API and GI indices before periodontal treatment in both groups were 77.32% (according to the evaluation scale, this is an unacceptable level of hygiene) and 1.08 points (according to the interpretation of the values, this is an inflammatory process in the gum tissues of mild to moderate severity). Treatment of patients in the main group and the comparison group with different treatment regimens also led to different statistically significant results. In the main group, the API and GI indices were 3.02 ± 2.3% and 0.05 ± 0.04 points, as opposed to the comparison group – 27.13 ± 9.09% and 0.39 ± 0.21 points, which also led to a delay in the start of the active period of orthodontic treatment in this group.

After the beginning of active period of the orthodontic treatment and installation of braces, despite the high level of motivation, patients always show a tendency to deterioration of the level of individual hygiene due to painful reactions and difficult adaptation to new conditions. However, in the main group (3 months after the start of the active period of the orthodontic treatment), there was a minimal increase in the BOP (7.85±3.18%), ARI (11.13±10.12%) and GI (0.07±0.05 points) indices, which indicated a slight destabilisation of the situation, which required only minor correction. At the same time, patients in the comparison group showed an active increase in index indicators – BOP (43.48±23.12%), API (70.64±17.98%) and GI (0.59±0.33 points), which confirmed the loss of remission and required a repeated full course of treatment with a traditional regimen.

After 6 months of active period of the orthodontic treatment, the repeated indices allowed to suggest in patients of the main group the practical absence of signs of inflammation in the form of gingival edema and bleeding, as evidenced by very low residual values of the BOP bleeding index – 0.28±0.2% in contrast to the comparison group – 17.45±9.94% ($p<0.001$). The absence of inflammation in the periodontal tissues of patients in the main group resulted in better API and GI indices – 3.04±1.85% and 0.06±0.06 points, respectively. While in patients of the comparison group, against the background of exacerbation of the pathological process in periodontal tissues, the API index was statistically significantly higher than the result obtained in patients of the main group – 73.96±29.89% and practically did not differ from the previous indicator «before treatment». GI scores also indicated the presence of inflammatory process in the gingival tissues – 0.34±0.31 points ($p<0.01$), (Table 2).

The analysis of the obtained indicators of the activity of SOD and CA in the oral fluid allowed us to assume that the presence of stage I-II GP (grade A) in patients and the installation of fixed orthodontic appliances caused a shift in the local oxidative metabolism. Before the periodontal treatment in both groups of patients with oral hyperplasia on the background of GP, a low level of CA in the oral fluid was noted (40.04 ± 25.1 U/ml in patients of the main group and 39.56 ± 24.6 U/ml in the comparison group, comparing to 180-200 U/ml – normal values). At the same time, SOD indicators showed almost three times deviation from the norm (89 ± 6.7% and 91 ± 6.8% in patients of the main and comparison groups comparing to 30% that is a normal value of that indicator), ($p<0.001$), (Fig. 2, Fig. 3).

At the stage of the periodontal treatment, the preparation of the periodontal tissues for active period of the orthodontic treatment using the developed TPC in patients of the main group helped to improve significantly the indicators of SOD enzyme to be close to normal level – 29 ± 2.2 %, and an increase in the level of CA enzyme to 101.2 ± 18.9 U/ml ($p<0.001$) (Fig. 2, Fig. 3).

In contrast to the main group, in patients of the comparison group, after applying the traditional treatment regimen, the indicators of SOD activity were still twice as high as normal – 65 ± 4.8 % ($p<0.01$), and the indicator of CA activity was only 58.7 ± 14.1 U/ml ($p<0.001$), that was almost three times lower than the normal one which significantly delayed the start of active orthodontic treatment (Fig. 2, Fig. 3).

Table 2. Values of BOP, API and GI indices in orthodontic patients with GP stage I-II (grade A)

Indicators	Before treatment of GP		Before treatment of GP		3 months after braces are fitted		6 months after braces have been fitted	
	Main group	Comparison group	Main group	Comparison group	Main group	Comparison group	Main group	Comparison group
BOP (%)	79.03 ± 7.67	75.24 ± 18.97	1.83 ± 0.71 *** #	10.24 ± 9.60 ***	7.85 ± 3.18 *** ###	43.48 ± 23.12	0.28 ± 0.2 *** ##	17.45 ± 9.94 ***
API (%)	78.43 ± 22.14	76.22 ± 22.74	3.02 ± 2.3 *** ##	27.13 ± 9.09 **	11.13 ± 10.12 *** ###	70.64 ± 17.98	3.04 ± 1.85 *** ###	73.96 ± 29.89
GI (points)	0.99 ± 0.32	1.18 ± 0.28	0.05 ± 0.04 *** #	0.39 ± 0.21 **	0.07 ± 0.05 *** #	0.59 ± 0.33	0.06 ± 0.06 ***	0.34 ± 0.31 **

Notes: 1. *- significance of differences between the groups «before treatment of CGP» and the groups at the stages of observation, where *- p<0.05, **- p<0.01, ***- p<0.001

2. #- significance of differences between the indicators of the main group and the comparison group at the stages of observation, where #- p<0.05 ##- p<0.01, ###- p<0.001

Source: compiled by the authors of this study

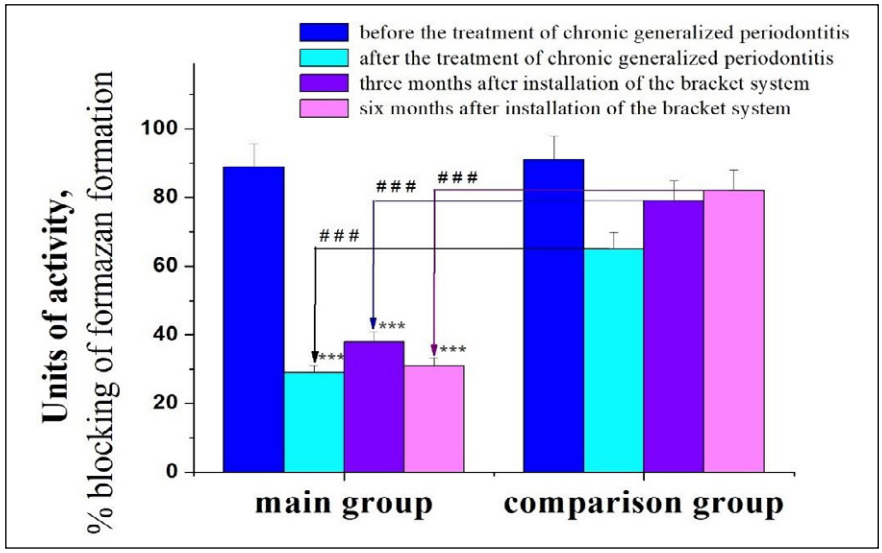


Fig. 2. Indicators of superoxide dismutase enzyme activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment

Picture taken by the authors

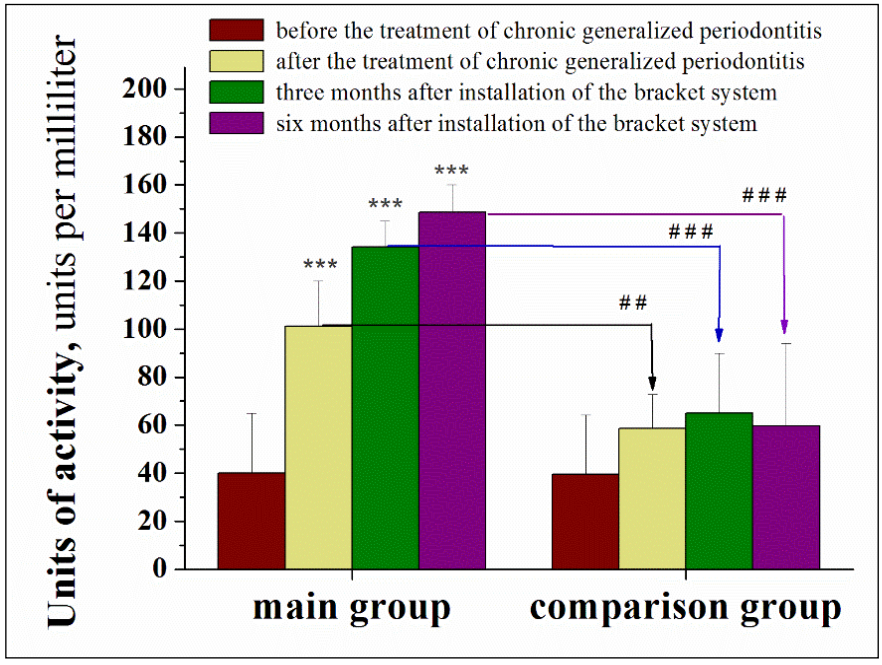


Fig. 3. Indicators of catalase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods

*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment

Picture taken by the authors

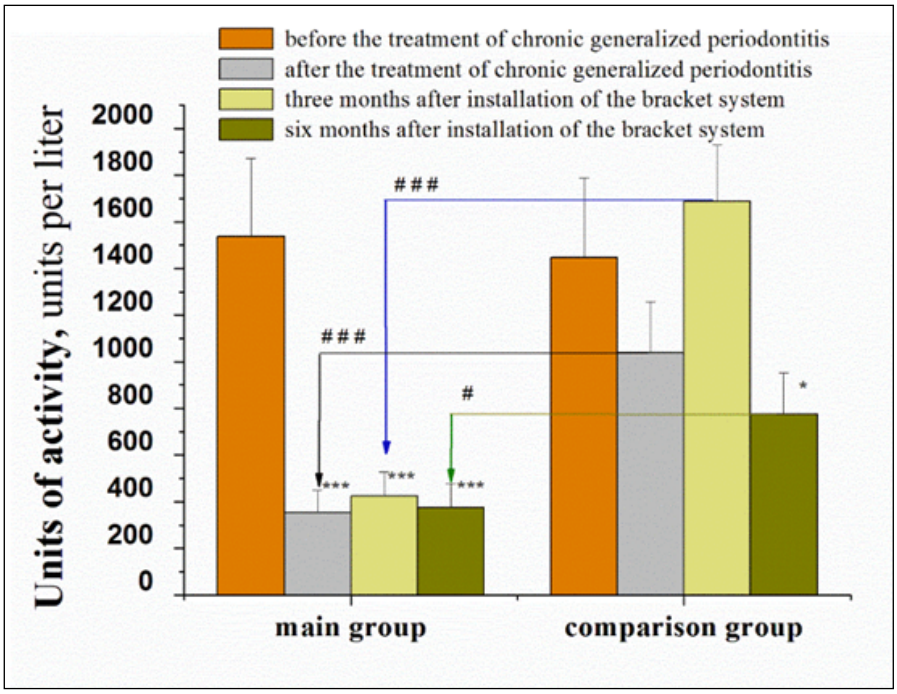


Fig. 4. Indicators of lactate dehydrogenase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

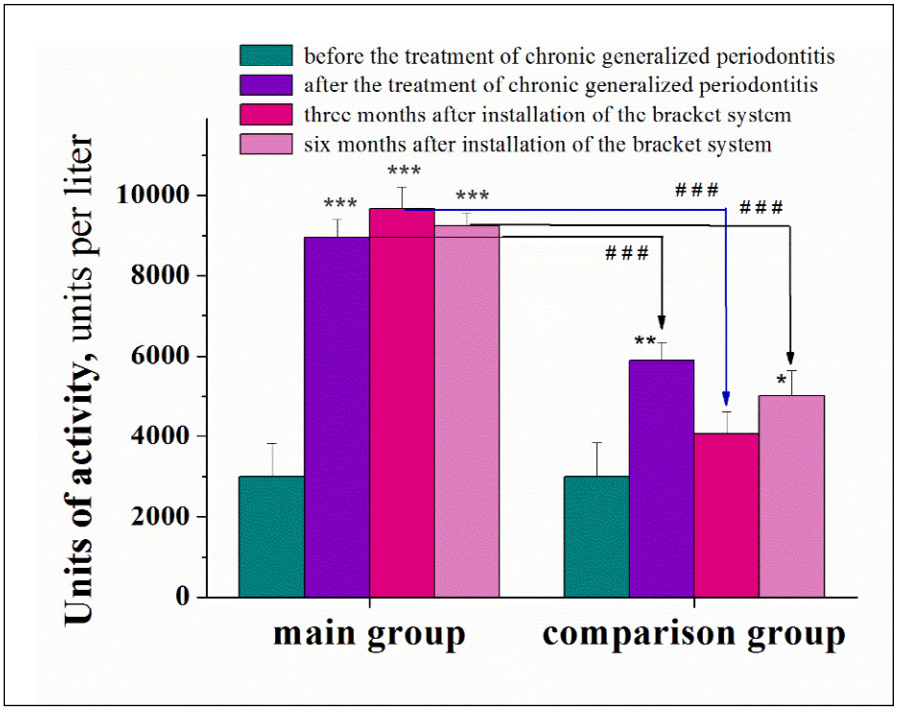


Fig. 5. Indicators of the amylase activity in oral fluid of the orthodontic patients with stage I-II GP (grade A) at different follow-up periods
*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 – statistically significant differences compared to the baseline indicators of the group (before treatment); #P ≤ 0.05, ##P ≤ 0.01, ##P ≤ 0.01, ###P ≤ 0.001 – statistically significant differences compared to the comparison group during treatment
Picture taken by the authors

After three months of wearing fixed orthodontic appliances by patients of both groups, a routine sampling of oral fluid was performed to determine the levels of SOD and CA activities under the influence of OS. The obtained results suggest that SOD activity in the main group did not have a significant shift compared to this indicator after treatment of GP before the installation of braces. It amounted 38 ± 2.8 % that is statistically significantly higher than this indicator in the comparison group (79 ± 5.9 %, p<0.001). The indicator of CA activity after three months of active orthodontic treatment was 134.2 ± 10.6 U/ml that is significantly higher than in the comparison group – 65 ± 25.8 U/ml (p<0.001), (Fig. 2, Fig. 3).

Similar changes were observed after 6 months of active orthodontic treatment comparing the background of regular treatment and preventive measures included in the TPC developed for the main group. The indicators of SOD and CA activities in the main group were 31 ± 2.3 % and 148.6 ± 11.4 U/ml, which suggested stabilization of the clinical situation and the absence of local metabolic shifts. In contrast to the main group, in patients of the comparison group, the values of SOD and CA activities were 82 ± 6.1% and

59.5 ± 34.6 U/ml, that is 2.5-1.5 times higher than the normal values. These data indicated a loss of remission in the comparison group comparing the background of an impaired oxidative metabolism (Fig. 2, Fig. 3).

Thus, after treatment of GP before braces were applied in patients of the main group, the levels of SOD and CA activities were 2.2 and 1.7 times higher than in the comparison group. After 3 months, this difference was 2 times, and after 6 months, 2.5 times.

The analysis of the defined indicators of LDH and AA activities in the oral fluid suggested that the presence of stage I-II GP (grade A) in patients and the installation of fixed braces caused a shift in local energy metabolism. Before the start of periodontal treatment, an increase in LDH activity in the oral fluid was noted in both groups of patients with DA comparing the background of GP (1536.4 ± 436.1 U/l – in the main group and 1446.1 ± 441.1 U/l – in the comparison group comparing 135.0-214.0 U/l that is reference value in the norm) and a decrease in AA activity (2997 ± 831.7 U/l and 3002 ± 831.7 U/l, respectively, comparing 10,000-11,000 U/l that are normal values), (Fig. 4, Fig. 5). At the stage of periodontal treatment, application of TPC in the main group led to a significant reduction in the LDH levels bringing them close to the norm – 353.1 ± 94.7 U/l ($p<0.001$) comparing 1040 ± 217.1 U/l in the comparison group, and a significant increase in AA activity was observed – 8958 ± 436.2 U/l ($p<0.001$) comparing to 5897 ± 433.9 U/l in the comparison group, (Fig. 4, Fig. 5).

After the installation of fixed orthodontic appliances in patients of the main group after 3 and 6 months, the values of LDH activity were statistically significantly different from those detected «before treatment». There was a clear confirmation of a quantitative decrease in the OS in periodontal tissues (424.52 ± 103.5 U/l, $p<0.001$ and 375.8 ± 102.4 U/l, $p<0.001$), compared to the LDH values in the comparison group – 1687.6 ± 240.8 U/l. Only after 6 months, the level of LDH activity in oral fluid in the comparison group was shown to be slightly decreased (774.5 ± 177.8 U/l) that was probably due to a greater extent of general adaptive mechanisms (Fig. 4). Thus, the LDH activity after treatment of GP in patients of the main group was three times higher than in the comparison group, in 3 months, this difference was 4.2 times, and in 6 months – 2.2 times.

Three months after the beginning of active period of the orthodontic treatment in the main group, the indicator of AA activity was normalized even to a higher extent – from 9673 ± 536.7 U/l to 4072 ± 532.5 U/l in the comparison group ($p<0.001$). After 6 months of active orthodontic treatment, the difference in the main and comparison groups remained almost at the same level (Fig. 5). Thus, the AA activity after treatment of GP before the formation of fixed orthodontic appliances was 1.5 times higher than in the comparison group, and after 3 months of active orthodontic treatment, this difference was 2.4 times, and after 6 months – 1.9 times.

Thus, we have shown that the applied TPC with GCB best contributed to the normalization of the activity of four enzymes, namely decreased levels of LDH and SOD activities, and increased levels of AA and CA activities. That also led to stabilization of clinical picture under the initial periodontal therapy. After 3 and 6 months of active periodontal treatment, the absence of signs of inflammation in the form of gingival edema and bleeding in the patients of the main group was evidenced. This made it possible to reduce by 3-3.5 times the period of their preparation for the active period of the orthodontic treatment. At the same time, in patients of the comparison group, the level of the OS and local oxidative metabolic disorders remained high due to the traditional local treatment regimen. The comparison group which used a traditional treatment regimen required a longer period (about 4-5 months) to stop all signs of inflammation before starting teeth removal, in contrast to the main group, which took only 1 month to prepare for the installation of fixed orthodontic appliances and for achieving remission.

DISCUSSION

The clinical and laboratory effectiveness of the therapeutic and prophylactic complex (TPC) in the treatment of stage I-II periodontitis (grade A) in orthodontic patients, which included a modified electrophoresis procedure in periodontal tissues using individually manufactured caps with a patented gel composition «Benzydaflaziverdyne» (GCB) based on a flavonoid complex and benzidamine hydrochloride. The essence of the method of subgingival electrophoresis with GCB before active orthodontic treatment was to remove all signs of chronic inflammation in periodontal tissues and ensure an adaptive transitional preparatory period before installing fixed orthodontic appliances. After three months of active orthodontic treatment, application of the developed TPC served to maintain remission.

The inclusion of the electrophoresis procedure in combination with GCB in the complex of therapeutic and preventive measures during the active period of orthodontic treatment in patients with OA against the background of treated GP led to a more intensive normalisation of BOP, API, GI and LDH levels. There was a faster improvement in the condition of periodontal tissues, which ensured a favourable course of the active period of the orthodontic treatment [2, 26].

In general, electrophoresis is recommended by researchers in the field of periodontology, as it has a reflective effect, favorably affecting the nervous system, its vegetative division, and hemodynamics. As a result, blood and lymph circulation, trophism, and metabolism improve in the periodontal tissues, granulation growth in periodontal pockets is suppressed, and inflammatory and congestive phenomena are reduced. An increase in the activity of connective tissue elements, phagocytic activity of leukocytes and elements of the reticuloendothelial system, acceleration of the regeneration process were demonstrated [26]. This is because physical procedures affect the body through nervous-reflex and nervous-humoral pathways. As a result of the body's adaptation to the action of the therapeutic stimulus, its defenses are enhanced. Therefore, electrophoresis procedures are pathogenetically justified by the need to prepare the patient for the stress-modulating effect of fixed orthodontic appliances, and the stage with electrophoresis and local delivery of GCB deep into the tissues is best suited for this, ensuring a kind of adaptation of the periodontal complex [24].

At present, tracing the main aspects of the pathogenesis of the periodontal tissue diseases in the orthodontic patients is impossible without a deep understanding of the role of metabolic mechanisms, in particular in the lipid peroxidation-antioxidant defense systems, which are the basis of many pathological reactions. It was reported that disturbances in local and/or general indicators of the OS are responsible for the mechanisms of the etiopathogenesis of periodontitis [27]. The human body which uses oxygen for its metabolic processes, is equipped with defense systems against ROS. They are usually called the «antioxidant barrier». Among the main enzymatic antioxidants that have been widely studied in gingival fluid, oral fluid, and blood serum of patients with periodontitis are SOD and CA [19, 21]. The reports on the activity of these enzymes in different periodontal diseases (chronic or acute) and at different stages of periodontitis are non-uniform. In the gingival tissue, the activity of CA was usually increased, and in the oral fluid it was decreased [20, 22]. These variations could be the result of using different methods of biochemical assessment of the enzymatic activity and do not reflect the stage and/or risk of the periodontitis progression [28].

Taking in consideration that the aseptic inflammation in the orthodontic patients may be associated with damage caused by the OS, the inclusion of «Proteflazid®» in the composition of the GCB was crucial in our study. This agent is a drug with pronounced antioxidant properties, it does not cause immunotoxic effects or refractoriness (hyporesponsiveness) of the immune system. The medicine inhibits the course of free radical processes, prevents the accumulation of ROS products, enhances the antioxidant status of cells, and thus, reduces intoxication of the organism [29]. In the present study, this was confirmed through measuring the activity of the antioxidant defense enzymes – SOD and CA, which is consistent with the results published in literature [30].

There are reports that flavonoids can counteract the pro-inflammatory effects of proteins associated with pathogenic molecular structures through Toll-like receptor (TLR) responses. The effect of flavonoids on the epithelial cells and fibroblasts of the gums and periodontal ligament, as well as the osteoblasts that support the alveolar bone, is potentially beneficial [5, 29]. The antibacterial mechanism of action of flavonoids was described. Specifically, it inhibits energy metabolism, cell proliferation, nucleic acid synthesis, reduces biofilm formation and cell adhesion, reduces pathogenicity and damage to membranes via producing hydrogen peroxide [31].

In scientific literature, salivary α-amylase was proposed as a marker of stress tension, the secretion of which into oral fluid is regulated by β-adrenergic stimulation [32]. In our study, this biomarker was monitored in oral fluid before treatment of stage I-II GP in patients of the main and comparison groups. It was significantly decreased before treatment which could be due to a damage of the secretory cells of the salivary gland caused by the metabolic processes in the OS and their negative effect on the tissues of the periodontal complex [33]. In turn, these disorders might cause a shift in the pH to the acidic side and induce a decrease in the AA, since this activity is manifested at neutral or slightly alkaline pH [33].

There are several reports concerning the level of the LDH in the oral and crevicular fluid. This enzyme is released from the cytoplasm into the extracellular space during a programmed cell death in the gingivitis or periodontitis, as well as at the orthodontic treatment [34, 35]. The time of increase in LDH levels varied depending on the level of the applied force.

The local analgesic effect of «Tantum Verde®» solution that is another effective component of the drug product, caused stabilization of sensory receptors lasting for 90 min after topical application. Its effectiveness observed after topical application is due to its ability to penetrate into the epithelial layer and reach effective concentrations in inflamed tissues. The antibacterial ability of the BH is manifested by rapid penetration through the outer membranes of microorganisms with a subsequent damage to cellular structures, disruption of metabolic processes and cell lysis. It is important that the antimicrobial effect of the

BH is enhanced in a neutral and alkaline environment (in the periodontal pocket, the pH is on average 8.0) [1, 5].

Multimineral complex «Biotus Calcium Magnesium Zinc with Vitamin D3 BIO-530982» containing calcium, magnesium, zinc and vitamin D3, is an additional source of these minerals and vitamin in order to maintain their optimal levels in the body. The drug supports normal functioning of the musculoskeletal system, maintaining the condition of teeth, bones, basic functions of the immune system and plays an important role in structure of cell membranes. Vitamin D3, in turn, is essential for better calcium absorption and is involved in mineralization, skeletal growth and restructuring.

«Resveratrol» is another drug with a systemic effect and it is known to be natural antioxidant. It contains resveratrol (150 mg), grape seed extract (300 mg) and food gelatin, and is used as an additional source of polyphenolic compounds that are needed to normalize metabolic processes and restore the body›s internal strength [6].

Summerising, the developed TPC is shown to be clinically effective in reducing local stress-induced metabolic disorders in the periodontal tissues, since achieving a stable remission is an important condition at tooth movement in the periodontal pathology that may lead to a rapid and irreversible deterioration in tooth support apparatus.

CONCLUSIONS

1. Based on the results of changes in the periodontal status indicators in the orthodontic patients with GP stage I-II (grade A), the developed periodontal gel composition based on a flavonoid complex and a nonsteroidal anti-inflammatory medicine (benzidamine hydrochloride) in combination with electrophoresis into the tissues of the periodontal complex can be recommended before and during active orthodontic treatment. The clinical efficacy of the developed TPC was confirmed by the BOP API and GI indices. Its application can reduce the preparation time for the active orthodontic period by 3-3.5 times.
2. The measurement of the activity LDH, SOD, CA and AA enzymes in oral fluid, reflecting changes in gum tissue under the stress-modulating effect of fixed orthodontic appliances, is important at the use of the TPC developed for patients in the main group. 3. Adaptation of the periodontal tissues to the installation of braces and reducing the level of local oxidative sterss after their installation are more efficient than in the comparison group, where a persistent loss of remission was observed as soon as 2-3 months after the start of the active period of orthodontic treatment.

REFERENCES

1. Primožič J, Poljšak B, Jamnik P et al. Risk Assessment of Oxidative Stress Induced by Metal Ions Released from Fixed Orthodontic Appliances during Treatment and Indications for Supportive Antioxidant Therapy: A Narrative Review. Antioxidants (Basel). 2021;10(9):1359. doi: 10.3390/antiox10091359. DOI
2. Kovac V, Poljsak B, Perinetti G, Primozic J. Systemic Level of Oxidative Stress during Orthodontic Treatment with Fixed Appliances. Biomed Res Int. 2019;2019:5063565. doi: 10.1155/2019/5063565. DOI
3. Gharbi A, Hamila A, Bouguezzi A et al. Biochemical parameters and oxidative stress markers in Tunisian patients with periodontal disease. BMC Oral Health. 2019;19(1):225. doi: 10.1186/s12903-019-0912-4. DOI
4. Velasco-Ibáñez R, Lara-Carrillo E, Morales-Luckie RA. et al. Evaluation of the release of nickel and titanium under orthodontic treatment. Sci Rep. 2020;10:22280. doi:10.1038/s41598-020-79221-1. DOI
5. Abou Baker DH. An ethnopharmacological review on the therapeutical properties of flavonoids and their mechanisms of actions: A comprehensive review based on up to date knowledge. Toxicol Rep. 2022;9:445-469. doi: 10.1016/j.toxrep.2022.03.011. DOI
6. Fernández-Rojas B, Gutiérrez-Venegas G. Flavonoids exertmultiple periodontic benefits including anti-inflammatory, periodontal ligament-supporting, and alveolar bone-preserving effects. Life Sci. 2018;15(209):435-454. doi: 10.1016/j.lfs.2018.08.029. DOI
7. Rochette L, Mazini L, Malka G et al. The Crosstalk of Adipose-Derived Stem Cells (ADSC), Oxidative Stress, and Inflammation in Protective and Adaptive Responses. Int J Mol Sci. 2020;21(23):9262. doi: 10.3390/ijms21239262. DOI
8. Danylevskyi MF, Borysenko AV, Antonenko MU. Terapevtychna stomatolohiya: pidruchnyk: u 4 tomakh. T. 3. Zakhvoryuvannya parodontu. 2-he vyd., pereroblene. ta dod. [Therapeutic dentistry: textbook: in 4 volumes. T. 3. Periodontal diseases. 2nd ed., reworked. and additional]. Kyiv: Medicine. 2018, p.624. (Ukrainian)
9. Viglianisi G, Tartaglia GM, Santonocito S et al. The Emerging Role of Salivary Oxidative Stress Biomarkers as Prognostic Markers of Periodontitis: New Insights for a Personalized Approach in Dentistry. J Pers Med. 2023;13(2):166. doi: 10.3390/jpm13020166. DOI
10. Guideline 00163. Periodontal diseases (gingivitis and periodontitis) https://guidelines.moz.gov.ua/documents/3060 [Accessed 17 December 2025]
11. Caton J, Armitage G, Berglundh T et al. A new classification scheme for periodontal and peri-implant diseases and conditions – Introduction and key changes from the 1999 classification. J Periodontol. 2018;89(1): S1–S8. doi: 10.1002/JPER.18-0157. DOI

12. Oshman S, Chaar EE, Lee YN, Engebretson S. Effect of patient age awareness on diagnostic agreement of chronic or aggressive periodontitis between clinicians; a pilot study. BMC Oral Health. 2017;17(27). doi: 10.1186/s12903-016-0258-0. DOI
13. Flys PS, Leonenko GP, Filonenko VV, Doroshenko NM. Orthodontics. Dental and jaw anomalies and deformities. Kyiv: Medicine. 2015, p.176.
14. Ghodasra R, Brizuela M. Orthodontics, Malocclusion. StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2025. https://www.ncbi.nlm.nih.gov/books/NBK592395/. [Accessed 17 December 2025]
15. Alexandrova KV, Skoda OS, Vasiliev DA. Vyznachennya aktyvnosti fermentiv u biolohichnykh seredovyshchakh. Odynytsi aktyvnosti fermentiv. Enzymopatiyi. Medychna enzymolohiya: Metodychnyy posibnyk z dystsypliny «Biolohichna khimiya» dlya vykladachiv. [Determination of enzyme activity in biological media. Units of enzyme activity. Enzymopathies. Medical Enzymology: A Methodological Guide to the Discipline «Biological Chemistry» for Teachers]. Zaporizhzhia: ZMU. 2015, p.45. (Ukrainian)
16. Ryabushko NO, Dvornik VM, Baranova AF, Balya GM. Zminy aktyvnosti a-amilazy rotovoyi ridyny v protsesi kompleksnoho likuvannya patsiyentiv z ishemichnoyu khvoroboyu sertsya z defektamy zubnykh ryadiv. [Changes in the activity of a-amylase of oral fluid in the process of complex treatment of patients with coronary heart disease with defects in the dentition]. Ukrayins'kyy stomatolohichnyy al'manakh. 2016; (2):48-50. (Ukrainian)
17. Singh S, Sharma M, Rohilla N et al. Assessment of Salivary Catalase, a-Amylase, and Cotinine Levels in Chronic Smokers: A Comparative Study. J Contemp Dent Pract. 2018;19(3):253-256.
18. Ostapchenko LI, Kompanets IV, Skopenko OV. Bioorhanichna khimiya. Praktykum. [Bioorganic chemistry. Practicum]. Kyiv: University of Kyiv. 2019, p.400. (Ukrainian)
19. Miricescu D, Totan A, Calenic B et al. Salivary biomarkers: relationship between oxidative stress and alveolar bone loss in chronic periodontitis. Acta Odontol. Scand. 2014;72(1):42-47. doi: 10.3109/00016357.2013.795659. DOI
20. Peluso I, Raguzzini A. Salivary and urinary total antioxidant capacity as biomarkers of oxidative stress in humans. Pathol Res Int. 2016;2016:14. doi: 10.1155/2016/5480267.5480267. DOI
21. Abdul Wahab RM, Abu Kasim N, Senafi S et al. Enzyme activity profiles and ELISA analysis of biomarkers from human saliva and gingival crevicular fluid during orthodontic tooth movement using self-ligating brackets. Oral Health Dent. Manag. 2014;13(2):194-199.
22. Viglianisi G, Tartaglia GM, Santonocito S et al. The Emerging Role of Salivary Oxidative Stress Biomarkers as Prognostic Markers of Periodontitis: New Insights for a Personalized Approach in Dentistry. J Pers Med. 2023;13(2):166. doi: 10.3390/jpm13020166. DOI
23. Hodovanyi OV, Hodovana OI, Shandra MS et al. Zasib u formi helevoi kompozytsii «Benzydaflaziverdyne» z prolonhovanoiu diieiu dlia likuvannia zakhvoriuvan tkanyn parodonta. Patent na vynakhid №125272, Ukraina, MPK, A61K 9/00, № a 2020 08196. - Zaiavl. 21.12.2020; Opubl. 09.02.2022. - Biul. №6. [Drug in the form of a gel composition "Benzydaflaziverdyne" with prolonged action for the treatment of periodontal tissue diseases. Patent for the invention No. 125272, Ukraine, MPK, A61K 9/00, No. a 2020 08196. - Application. 21.12.2020; Publication. 09.02.2022. - Biul. No. 6.]. (Ukrainian)
24. Hodovanyi O, Chukhray N, Martovlos O et al. Eksperymental'na model' doslidzhennya vidpovidi klityn na vplyv parodontal'nykh helevykh kompozytsiy ta breketiv, potentsiyovanykh elektroforezom. [An experimental model for studying the response of cells to the effects of periodontal gel compositions and braces potentiated by electrophoresis]. Pratsi Naukovoho tovarystva im. Shevchenka. Medychni nauky. 2024;2(76). (Ukrainian)
25. Welsby PD, Weatherall M. Statistics: an introduction to basic principles. Postgraduate Med J. 2022;98:793-798. doi: 10.1136/postgradmedj-2020-139446. DOI
26. Chen C, Bai X, Ding Y, Lee IS. Electrical stimulation as a novel tool for regulating cell behavior in tissue engineering. Biomater Res. 2019;23:25. doi: 10.1186/s40824-019-0176-8. DOI
27. Buczko P, Knaś M, Grycz M et al. Orthodontic treatment modifies the oxidant-antioxidant balance in saliva of clinically healthy subjects. Adv Med Sci. 2017;62(1):129-135. doi: 10.1016/j.advms.2016.11.004. DOI
28. Toczewska J, Konopka T. Activity of enzymatic antioxidants in periodontitis: A systematic overview of the literature. Dent Med Probl. 2019;56(4):419-426. doi: 10.17219/dmp/112151. DOI
29. Tóthová L, Celec P. Oxidative Stress and Antioxidants in the Diagnosis and Therapy of Periodontitis. Front Physiol. 2017;8:1055. doi: 10.3389/fphys.2017.01055. DOI
30. Kubesy AA, Salem NY, Jaheen AH. Altered blood oxidative stress biomarkers in association with canine pyoderma and allergic contact dermatitis. Comparative Clin Pathol. 2017;26(3):643-646. doi: 10.1007/s00580-017-2418-2. DOI
31. Murugan S, Solanki H, Purusothaman D et al. Safety Evaluation of Standardized Extract of Curcuma longa (NR-INF-02): A 90-Day Subchronic Oral Toxicity Study in Rats. Biomed Res Int. 2021;2021:6671853. doi: 10.1155/2021/6671853. DOI
32. Ali N, Nater UM. Salivary Alpha-Amylase as a Biomarker of Stress in Behavioral Medicine. Int J Behav Med. 2020;27:337-342. doi:10.1007/s12529-019-09843-x. DOI
33. Antonio-Zancajo L, Montero J, Albaladejo A et al. Pain and Oral-Health-Related Quality of Life in Orthodontic Patients During Initial Therapy with Conventional, Low-Friction, and Lingual Brackets and Aligners (Invisalign): A Prospective Clinical Study. J Clin Med. 2020;9(7):2088. doi: 10.3390/jcm9072088. DOI

34. Kumar BD, Singh N, Verma SK et al. A Study to Analyze the Alkaline Phosphatase and Lactate Dehydrogenase Enzyme Activity in Gingival Crevicular Fluid During Orthodontic Tooth Movements. J Pharm Bioallied Sci. 2022;14(1):S490-S493. doi: 10.4103/jpbs.jpbs_51_22. DOI
35. Rasha R, Maha M. Salivary lactate dehydrogenase and salivary total protein as potential biomarkers for screening periodontal disease. Saudi Dent J. 2023;35(7):827-833. doi: 10.1016/j.sdentj.2023.07.011. DOI

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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RECEIVED: 17.02.2025
ACCEPTED: 15.07.2026

CREATIVE COMMONS 4.0