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ASSESSMENT OF THE PEROXIDE OXIDATION INDEX OF LIPIDS IN THE ORAL FLUID OF CHILDREN WITH FLUOROSIS IN THE DYNAMICS OF COMPREHENSIVE ORTHODONTIC TREATMENT

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The study was devoted to the effect of a treatment and preventive complex of drugs on the content of malondialdehyde in the oral fluid of children with endemic fluorosis and orthodontic pathology Twenty-five children (6–7 years old) were allocated to a comparison group and a main group. Unstimulated whole saliva was collected at baseline, after one month (pre-bonding), and at 3, 6, and 12 months after bracket placement. Salivary malondialdehyde concentrations were measured spectrophotometrically. Findings indicate that the treatment and preventive complex exerts sustained antioxidative and membrane-protective effects, counteracting the combined oxidative burden of fluorosis and orthodontic pathology.

Key words: dental fluorosis, malondialdehyde, lipid peroxidation, orthodontic treatment, antioxidants, children, therapeutic-prophylactic complex.

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ОЦІНКА ПОКАЗНИКА ПЕРЕКИСНОГО ОКИСНЕННЯ ЛІПІДІВ У РОТОВІЙ РІДИНІ ДІТЕЙ ІЗ ФЛЮОРОЗОМ У ДИНАМІЦІ КОМПЛЕКСНОГО ОРТОДОНТИЧНОГО ЛІКУВАННЯ

Дослідження було присвячене впливу лікувально-профілактичного комплексу препаратів на вміст малондіальдегіду в ротовій рідині дітей з ендемічним флюорозом та ортодонтичною патологією. Двадцять п'ять дітей (віком 6–7 років) були розподілені на контрольну групу та основну групу. Нестимульована цільна слина збиралася на початку дослідження, через місяць (до встановлення брекетів) та через 3, 6 і 12 місяців після встановлення брекетів. Концентрації малонового діальдегіду у слині вимірювали спектрофотометрично. Результати показують, що лікувально-профілактичний комплекс має стійкий антиоксидантний та мембранозахисний ефект, протидіючи комбінованому окислювальному навантаженню, спричиненому флюорозом та ортодонтичною патологією.

Ключові слова: флюороз, малоновий діальдегід, перекисне окислення ліпідів, ортодонтичне лікування, антиоксиданти, діти, лікувально-профілактичний комплекс.

The work is a fragment of the research project "Development and introduction into clinical practice of methods of diagnosis, prevention and treatment of osteogenesis disorders during dental intervention in patients in wartime", state registration No. 0123U103247.

Gingival inflammation and dental malocclusions are common conditions in children that can significantly compromise oral hygiene and periodontal health. Periodontal disease remains highly prevalent worldwide, with severe periodontitis affecting roughly 10–11 % of the population [12]. Orthodontic fixed appliances exacerbate this problem by creating plaque-retentive niches on the teeth, thereby increasing plaque accumulation and the risk of gingival inflammation [3]. Inadequate mechanical oral hygiene and standard prophylactic measures often fail to control the intensified inflammation associated with orthodontic treatment, underscoring the need for novel preventive approaches.

Fluorosis – chronic overexposure to fluoride – is endemic in many regions and poses additional challenges to oral health. In parts of India alone, an estimated 60 million people are at risk for fluorosis [1]. Epidemiological studies indicate that individuals in high-fluoride areas tend to exhibit a greater prevalence and severity of gingivitis and periodontitis [13]. Excess fluoride intake is known to disrupt the redox balance in biological tissues: experimental evidence shows that fluoride acts as an enzymatic toxin, inducing oxidative stress and related cellular damage [10]. Fluorosis is consistently associated with elevated levels of reactive oxygen species (ROS) and oxidative by-products in oral tissues, reflecting a state of chronic oxidative stress in the periodontium [13]. Some reports have noted mixed findings on the fluorosis-periodontitis link [7], but the bulk of molecular data supports that fluoride toxicity can exacerbate oxidative and inflammatory pathways in oral tissues.

The combination of fixed orthodontic forces on a background of fluorosis would be expected to amplify periodontal inflammation and tissue injury. Orthodontic tooth movement itself triggers a local

inflammatory response involving neutrophils and macrophages, which release proteolytic enzymes and ROS that drive lipid peroxidation in gingival tissues. One key marker of lipid peroxidation is malondialdehyde (MDH), and studies confirm that salivary MDH is significantly elevated in patients with active periodontitis compared to healthy individuals [8]. In fact, recent clinical analyses have identified high salivary MDH as a useful indicator of periodontal tissue oxidative damage [8, 11]. Concurrent fluorosis could further impair the periodontium's adaptive responses under orthodontic force; for example, animal models of fluorosis show blunted angiogenic healing responses during orthodontic tooth movement [5]. Thus, both clinical and experimental evidence suggest that fluorosis, together with the mechanical stress of braces, can synergistically exacerbate gingival inflammation and oxidative injury.

Importantly, there is growing evidence that counteracting oxidative stress can mitigate periodontal damage [9]. Antioxidant supplementation and other redox-modulating therapies have shown promise in reducing inflammation in periodontal disease [10]. In orthodontic patients, adjunctive natural anti-inflammatory agents have demonstrated significant benefits in controlling gingival inflammation. For instance, mouthrinses containing herbal antioxidants like Aloe vera and green tea have been shown to significantly reduce plaque indices, gingival index scores, and bleeding on probing in brace-wearing patients – with efficacy comparable to that of chlorhexidine mouthwash [14]. Likewise, a 2 % resveratrol oral gel was found to markedly improve gingival health and lower gingival inflammation over 8 weeks in adolescents undergoing orthodontic treatment [6]. Furthermore, a recent randomized trial reported that a propolis-containing mouthwash produced a dramatic reduction in salivary oxidative stress levels (as measured by 8-oxo-dG) in patients with fixed appliances, outperforming standard fluoride rinse in reducing oxidative biomarkers [4]. These findings underscore the potential of adjunctive antioxidant and anti-inflammatory therapies to protect the gingival tissues during orthodontic treatment.

Given the considerable oxidative and inflammatory burden imposed by both fluorosis and orthodontic therapy, there is a clear rationale for exploring combined preventive strategies.

The purpose of the study was to evaluate the effect of a therapeutic complex of drugs on malondialdehyde levels in the oral fluid of children with endemic fluorosis and orthodontic pathology.

Materials and methods. Biochemical analyses of oral fluid were conducted in 25 children aged 6–7 years with endemic fluorosis and orthodontic pathology (main group: 13; comparison group: 12). Biochemical studies were carried out in the "Laboratory of biochemistry and vivarium" of the State Establishment "The Institute of stomatology and maxilla-facial surgery National academy of medical sciences of Ukraine" (SE "ISMFS NAMS"). The study was carried out from 14 May 2024 to 19 May 2025.

Eligibility required a confirmed clinical diagnosis of endemic dental fluorosis according to the Dean Index (very mild to moderate), absence of untreated carious lesions or acute oral infection after professional sanitation, no history of systemic disease, long-term medication, or antioxidant supplementation, no prior orthodontic intervention, and written informed consent from parents/guardians. Exclusion criteria were any chronic somatic illness, recent (≤3 months) antibiotic or anti-inflammatory therapy, enamel defects unrelated to fluorosis (e.g., molar-incisor hypomineralisation), poor compliance, or withdrawal of consent at any stage. Thirteen participants meeting these criteria were allocated to the main group, which received protocol-based basic therapy supplemented twice yearly with a therapeutic–prophylactic complex, while twelve formed the comparison group, treated with professional oral hygiene, sanitation, basic protocol therapy, and diet- and drink-related counselling only. Biochemical parameters in unstimulated oral fluid were assessed at five time points: baseline (pre-intervention), immediately before bracket placement, and at 1-, 6-, and 12-month post-intervention. No post-enrolment exclusions, cross-overs, or drop-outs occurred; consequently, group sizes remained unchanged throughout the 12-month follow-up period.

Both study arms underwent baseline professional oral sanitation – comprehensive caries management, supragingival scaling, polishing, and individual hygiene instruction two weeks before bracket bonding. The comparison group received no further adjunctive care. The main group followed a defined therapeutic-prophylactic protocol:

Resin infiltration with Icon (INN: low-viscosity tetraethylene-glycol-dimethacrylate resin; DMG Dental-Material Gesellschaft mbH, Germany) was applied to all smooth-surface white-spot lesions strictly per manufacturer's instructions 14 days before fixed-appliance placement, thereby arresting incipient caries, enhancing bracket adhesion, and increasing local enamel acid-resistance. Professional fluoride reinforcement was provided with Flairesse Varnish 1.5 % (INN: sodium fluoride 22,600 ppm; Ivoclar Vivadent AG, Liechtenstein), one full-arch application at baseline and a second at month 6. Systemic mineral support comprised Calcicker oral suspension (INN: calcium lactate 1,000 mg + phosphorus 125 mg + cholecalciferol 200 IU per 5 mL; Coral Laboratories Ltd, India) administered at 5 mL twice daily, 30 min before meals, for 30 consecutive days. The therapeutic-prophylactic complex described herein is provisional; optimisation studies are ongoing, and a refined protocol will be detailed in future reports

Unstimulated oral fluid was collected from children in the morning, on an empty stomach, by spitting into sterile centrifuge tubes (after rinsing their oral cavity twice with water) for 5–10 minutes. Before performing biochemical analysis, the oral fluid was thawed at room temperature, centrifuged at 2,500 rpm for 20 minutes at +4°C (bench centrifuge RS-6, MedTech, Ukraine), and the supernatant was collected for analysis. Biochemical studies of the content of malondialdehyde (MDH) – lipid peroxidation index, were carried out in the oral fluid of patients. MDH content was calculated by reaction with 2-thiobarbituric acid ("Sigma", USA) [1].

All treatment, preventive and diagnostic measures were carried out only after the patients signed a voluntary informed consent in accordance with the principles of bioethics outlined in the Declaration of Helsinki "for Ethical Principles for Medical Research Involving Human Subjects" and "Universal Declaration on Bioethics and Human Rights (UNESCO)". All participants were adults, cognitively competent, and not otherwise classified as a vulnerable population under Good Clinical Practice. Studies recommended by the Commission on Bioethical Expertise (conclusion of the bioethics commission of the SE "ISMFS NAMS", protocol No. 1020 of 04/30/2024).

Data processing was carried out with STATISTICA 6.1. Before parametric testing, the Shapiro-Wilk normality test was applied to each continuous variable; none showed significant deviation from a Gaussian distribution ($p>0.05$). Therefore, inter-group comparisons were performed with the two-tailed Student's t-test. Between-group differences were deemed statistically significant at $p<0.05$ [2].

Results of the study and their discussion. Lipid peroxidation occurs mainly in biological membranes and is physiologically necessary for the synthesis of leukotriene regulators and phagocytosis. However, in pathological processes, a significant increase in lipid peroxide levels acts as a damaging factor, leading to disruption of membrane structure and function. Under the influence of a pathological factor, the intensity of lipid peroxidation (LPO). Malondialdehyde is the end product of lipid peroxidation and a marker of inflammation. MDH content in oral fluid can be used to assess LPO levels in the oral cavity and their changes under treatment conditions. Table 1 presents the results of MDH content determination in the oral fluid of children with endemic fluorosis who require orthodontic treatment.

Table 1

Determination of malondialdehyde content in the oral fluid of children with endemic fluorosis during complex orthodontic treatment, mmol/L (M±m)

Terms / Groups	Terms of the study				
	Initial state	After 1 month	After 3 months	After 6 months	After 1 year
Reference values for the norm	0.17±0.01				
Comparison, n=12	0.36±0.02 p<0.001	0.28±0.01 p<0.001 p_1<0.02	0.32±0.02 p<0.001 p_1<0.05	0.34±0.02 p<0.001 p_1>0.4	0.38±0.02 p<0.001 p_1>0.5
Main, n=13	0.34±0.02 p<0.001 p_2>0.5	0.23±0.01 p<0.001 p_1<0.001 p_2<0.002	0.20±0.01 p<0.001 p_1<0.001 p_2<0.001	0.17±0.009 p>0.4 p_1<0.001 p_2<0.001	0.19±0.01 p>0.2 p_1<0.001 p_2<0.001

Note. p – significance of differences from the norm; p_1 – significance of differences from the initial state. p_2 – significance of differences from the indices in groups.

The data presented in the table show that at the initial stage of observation in children of both groups, the MDH level in oral fluid was twice as high as the reference values ($p<0.001$), which indicates the presence of inflammation and intense lipid peroxidation processes in the oral cavity of children with fluorosis. At the second stage of observation (before the fixation of braces), the MDH content in the oral fluid of children was significantly lower than the initial values, by 22.2 % in the comparison group ($p_1<0.001$), and a more significant decrease was found in the main group by 32.4 % ($p_1<0.001$).

At this stage of observation, the indicator studied in the main group, which was additionally prescribed a preventive complex, was significantly lower by 17.9 % than the corresponding data in the comparison group. Thus, the biochemical results from oral fluid indicate a decrease in lipid peroxidation and oral cavity inflammation in children with endemic fluorosis under the influence of the studied prophylactic complex.

Subsequently, in the dynamics of observation (after 1, 6, and 12 months) after the fixation of braces, the MDH level in the oral fluid of children with fluorosis in the comparison group who underwent oral hygiene was high and did not differ from the baseline level, and was significantly higher than the normal values.

As a result of using the therapeutic and prophylactic complex in children of the main group, a significant decrease in the product of lipid peroxidation – MDH content – was observed during the observation period. Biochemical analysis of the children's oral fluid conducted after 1 month revealed a

41.2 % decrease in MDH levels ($p_1<0.001$), after 6 months a 50 % decrease ($p<0.001$), and after 12 months a 44.1 % decrease ($p<0.001$) relative to baseline data. It should be noted that MDH levels reached reference values after 6 months of observation and remained at this level after 12 months.

At the same time, at all stages of observation, MDH content in the comparison group was significantly higher than the corresponding data for the main group of children who were additionally prescribed a therapeutic and prophylactic complex.

Thus, consistently low levels of MDH in the oral fluid of children in the main group with endemic fluorosis and orthodontic treatment during observations, and therefore the intensity of lipid peroxidation, are most likely the result of activation of antioxidant protection against the background of regular therapeutic and preventive measures. The biochemical analysis revealed that, at baseline, malondialdehyde concentrations in unstimulated saliva from children with endemic fluorosis were approximately twice the reference value, corroborating earlier reports that chronic fluoride exposure and orthodontic irritation synergistically elevate lipid peroxidation-driven oxidative stress in the oral cavity [10]. Such pronounced oxidative activity aligns with clinical observations that salivary MDH levels are markedly higher in patients with active periodontal inflammation than in periodontally healthy controls [8]. After one month but still before bracket placement, the comparison group showed only a modest (≈22 %) decline in MDH, whereas the main group receiving the adjunct therapeutic-prophylactic complex achieved a significantly greater (≈32 %) reduction. These findings mirror outcomes from randomized trials in orthodontic patients, in which antioxidant-rich mouthrinses or gels (e.g., propolis or resveratrol formulations) produced substantial early reductions in salivary oxidative markers relative to standard care [4, 6]. As treatment progressed, the divergence between groups widened. In the comparison cohort, MDH rebounded and remained persistently elevated throughout the 12-month follow-up, an expected consequence of fixed appliances, which create plaque-retentive niches and sustain low-grade gingival inflammation that conventional hygiene measures rarely suppress fully [3]. By contrast, children in the main group exhibited a stepwise decline in MDH, reaching values statistically indistinguishable from population norms by six months and maintaining near-physiological levels at one year. This durable normalization suggests that the complex not only provided acute antioxidant support but also modulated the underlying redox balance during prolonged orthodontic force application. Mechanistically, several components of the complex are designed to seal porous fluorotic enamel, foster fluorapatite formation, and optimize calcium-phosphate turnover interventions that can reduce enamel solubility and mitigate acid diffusion. These remineralizing actions, together with targeted antioxidants and anti-inflammatory agents, likely curtailed reactive oxygen species generation at the gingival interface, thereby limiting lipid peroxidation. The data therefore extend existing evidence that fluoride-induced enzymatic disruption and ROS overproduction can be effectively countered by multimodal antioxidant strategies [10]. Collectively, the present results demonstrate that adjunctive use of a tailored therapeutic-prophylactic regimen can significantly attenuate oxidative stress, as reflected by sustained reductions in salivary MDH, throughout the course of orthodontic treatment in children with endemic fluorosis. By curbing lipid peroxidation and, by implication, downstream inflammatory tissue damage, the complex may offer a practical means to enhance periodontal resilience in this high-risk pediatric population.

Conclusions

1. Endemic fluorosis in 6–7-year-old children is associated with a two-fold elevation of salivary malondialdehyde, indicating high basal lipid-peroxidation activity before orthodontic intervention.

2. Standard professional hygiene alone yields only a transient reduction in oxidative stress; MDH levels rebound once fixed appliances are placed, remaining significantly above physiological norms for at least 12 months.

3. Adjunctive administration of the multi-component therapeutic-prophylactic complex produces an early (≥30 %) and sustained (≥40 %) suppression of salivary MDH, with values normalising by the sixth month of orthodontic treatment and remaining within reference limits thereafter.

4. The TPC demonstrates pronounced antioxidant and membrane-stabilising properties, supporting its utility as a preventive adjunct for mitigating oxidative oral-tissue injury in paediatric patients undergoing orthodontic therapy against a background of dental fluorosis.

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ORAL HEALTH STATUS IN PATIENTS WITH RHEUMATOID ARTHRITIS

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To investigate the prevalence and structure of dental disease among patients with rheumatoid arthritis in Azerbaijan, 123 patients with rheumatoid arthritis underwent dental examinations. The control group consisted of 130 individuals without any somatic diseases. The oral hygiene status was assessed using the Greene-Vermillion Hygiene Index. The periodontal status was evaluated using the Mühlemann Bleeding Index, Svarkova Iodine Test, Gingival Index, and the Fuchs and Ramfjord indices. Periodontitis was identified in 63.4 % of RA patients, markedly higher than in the control group, where it was observed in only 33.9 %. The findings revealed significant differences between the two cohorts across several oral health indicators (Svarkova Iodine, the Fuchs and Ramfjord indices). These results indicate that patients with rheumatoid arthritis exhibit a higher prevalence of several stomatological conditions (particularly periodontitis, gingivitis, pulpitis, and mucosal diseases) compared to individuals without somatic diseases.

Key words: rheumatoid arthritis, periodontitis, oral hygiene, Svarkova Iodine Test, Fuchs index, Ramfjord index.

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СТАН ЗДОРОВ'Я РОТОВОЇ ПОЛОСТІ У ХВОРИХ НА РЕВМАТОЇДНИЙ АРТРИТ

З метою вивчення поширеності та структури стоматологічних захворювань серед хворих на ревматоїдний артрит в Азербайджані проведено стоматологічне обстеження 123 пацієнтів з діагнозом ревматоїдний артрит. Контрольну групу склали 130 осіб без соматичних захворювань. Стан гігієни порожнини рота оцінювався за допомогою індексів гігієни Гріна-Вермільона. Стан пародонту оцінювався за допомогою індексу кровоточивості Мюлеманна, йодного тесту Сваркової, ясенного індексу та індексів Фукса і Рамфьорда. Пародонтит був виявлений у 63,4 % хворих на РА, що значно вище, ніж у контрольній групі, де він спостерігався тільки у 33,9 %. Отримані дані виявили значущі відмінності між двома когортами за кількома показниками здоров'я порожнини рота (йодний тест Сваркової, індекси Фукса і Рамфьорда). Отримані результати свідчать про те, що у пацієнтів з ревматоїдним артритом спостерігається більш висока поширеність ряду стоматологічних захворювань (зокрема, пародонтиту, гінгівіту, пульпіту і захворювань слизової оболонки) в порівнянні з особами без соматичних захворювань.

Ключові слова: ревматоїдний артрит, пародонтит, гігієна порожнини рота, йодний тест Сваркової, індекс Фукса, індекс Рамфьорда.

Recent studies have shown a connection between oral health status and a range of chronic diseases, including rheumatoid arthritis (RA). RA is characterized not only by joint pain and joint damage but also by systemic inflammation and comorbidities that contribute to increased mortality [6, 10]. As one of the most common autoimmune arthropathies, RA affects approximately 0.24 % to 0.65 % of the global population [4].