

Shnaider D.S., Dienga A.E.¹, Gorokhivskiy V.N., Mirchuk B. M.², Yefremova O.V.²,

Kordonets O.L., Suslova O.V.

Odesa National Medical University, Odesa, Ukraine; ¹State Establishment “The Institute of Stomatology and Maxillofacial Surgery of the National Academy of Medical Sciences of Ukraine”, Odesa, Ukraine; ²Danylo Halytsky Lviv National Medical University, Lviv, Ukraine

ANALYSIS OF CHANGES IN THE MINERALIZATION INDEX IN THE ORAL FLUID OF CHILDREN WITH FLUOROSIS DURING COMPREHENSIVE ORTHODONTIC TREATMENT

e-mail: oksanadenga@gmail.com

This study assessed acid and alkaline phosphatase activities and the mineralisation index in oral fluid during orthodontic treatment in children with endemic fluorosis. Twenty-five children aged 6–7 years were examined: the main group (n=13) received a therapeutic and preventive complex alongside dental treatment and professional oral hygiene; the comparison group (n=12) received standard measures. Measurements were obtained at baseline, before bracket placement, and at 1, 6 and 12 months. At one year, acid phosphatase activity in the main group decreased by 56.3 %, while mean alkaline phosphatase activity increased by 78.0 % from baseline. The mineralisation index increased from 0.94 to 3.86 arbitrary units, whereas the comparison-group value was 0.79. Comprehensive supportive care was associated with favourable enzymatic trajectories; their clinical significance requires further confirmation.

Key words: dental fluorosis, children, oral fluid, acid phosphatase, alkaline phosphatase, mineralisation index, orthodontic treatment.

Шнайдер Д.С., Дєньга А.Е., Горохівський В.Н., Мірчук Б.М., Єфремова О.В.,
Кордонєць О.Л., Суслова О.В.

АНАЛІЗ ЗМІН ІНДЕКСУ МІНЕРАЛІЗАЦІЇ У РОТОВІЙ РІДИНІ ДІТЕЙ З ФЛЮОРОЗОМ В ДИНАМІЦІ КОМПЛЕКСНОГО ОРТОДОНТИЧНОГО ЛІКУВАННЯ

Дослідження присвячене оцінюванню активності кислої та лужної фосфатаз і індексу мінералізації у ротовій рідині дітей з ендемічним флюорозом упродовж ортодонтичного лікування. Обстежено 25 дітей віком 6–7 років: основна група (n=13) отримувала лікувально-профілактичний комплекс додатково до санації та професійної гігієни, група порівняння (n=12) – стандартні заходи. Показники визначали у вихідному стані, перед фіксацією брекетів і через 1, 6 та 12 місяців. В основній групі через рік активність кислої фосфатази знизилася на 56,3 %, а середня активність лужної фосфатази підвищилася на 78,0 % щодо вихідного рівня. Індекс мінералізації збільшився з 0,94 до 3,86 ум. од., тоді як у групі порівняння становив 0,79 ум. од. Комплексний супровід асоціювався зі сприятливою ферментативною динамікою; її клінічне значення потребує подальшого підтвердження.

Ключові слова: флюороз зубів, діти, ротова рідина, кисла фосфатаза, лужна фосфатаза, індекс мінералізації, ортодонтичне лікування.

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Endemic dental fluorosis remains a relevant concern in paediatric dentistry because disturbances of enamel formation arise during tooth development and may influence subsequent dental function. A Ukrainian review by L. F. Kaskova et al. demonstrated considerable variation in fluorosis prevalence between populations, associated with differences in fluoride exposure and diagnostic approaches [1]. In clinical practice, this requires consideration of the individual fluoride exposure history, the severity of hard-tissue changes and the need for further dental treatment. Particular attention is warranted in children whose fluorosis coexists with malocclusion requiring orthodontic correction.

Current concepts of fluorosis pathogenesis encompass disturbances of ameloblast function, enamel matrix maturation and cellular signalling. K. Zhang et al. summarised evidence concerning the involvement of cellular stress, apoptosis and altered gene expression in the development of fluorotic defects [15]. In an experimental study by F. J. Aulestia et al.,

fluoride exposure disrupted calcium signalling and mitochondrial function in enamel-forming cells [5]. These findings provide a rationale for examining tissue metabolism, but do not justify equating an established enamel defect with ongoing fluoride toxicity at every subsequent stage of childhood.

Orthodontic treatment introduces additional conditions capable of modifying oral homeostasis. Fixed appliances hinder mechanical cleaning of tooth surfaces, while orthodontic tooth movement involves periodontal tissue remodelling. According to the systematic review by S. Cerroni et al., fixed orthodontic appliances may be accompanied by deterioration in periodontal indices, highlighting the importance of hygiene monitoring throughout treatment [9]. In children with existing enamel changes, preventive support should address both the hard tissues and the response of surrounding tissues to prolonged orthodontic loading. Assessment of tooth position alone therefore does not encompass the entire biological response to treatment.

Experimental observations support the possibility that fluorosis modifies tissue responses. X. Ding et al. identified a reduced gingival angiogenic response and lower expression of components of the VEGF/P13K/AKT/eNOS signalling pathway in rats undergoing orthodontic tooth movement against a background of chronic fluorosis [10]. These findings apply to a specific experimental model and cannot directly describe the course of treatment in children. Nevertheless, they support investigation of local biochemical processes in patients with coexisting conditions, because an identical mechanical intervention may act on tissues with different baseline functional characteristics.

Oral fluid provides an accessible biological medium for repeated, non-invasive assessment of these processes [4]. It contributes to acid–base balance, mineral transport and protection of tooth surfaces, while its composition reflects contributions from salivary glands, oral tissues and microorganisms [11, 14]. S. Liu et al. identified differences in salivary microbial composition between individuals with fluorosis and healthy participants [13]. Oral fluid biochemical findings should therefore be interpreted within the overall local environment, in which mineral metabolism, biofilm status and tissue responses interact. Repeated sampling of this biological fluid is particularly suitable for paediatric cohorts.

Alkaline phosphatase (ALP) is of interest among the enzymatic parameters studied in relation to phosphate metabolism and mineralisation. However, changes in total salivary ALP activity are context dependent. S. Bevinagidad et al. assessed ALP together with calcium, phosphorus, pH, enamel white spot lesions and oral hygiene indices in orthodontic patients; increasing enzyme activity was accompanied by higher plaque and gingival inflammation indices [7]. An isolated increase in ALP therefore does not invariably indicate a favourable clinical outcome. Its interpretation requires comparison with baseline activity, other enzymatic parameters and the clinical condition.

Acid phosphatase (ACP) and its isoforms are also investigated in relation to tissue turnover, including resorptive processes. The systematic review by L. Kakali et al. examined markers of bone formation and resorption in gingival crevicular fluid during orthodontic tooth movement, while identifying substantial methodological heterogeneity and risk of bias [12]. A. P. Cazzolla et al. studied specific salivary markers, including tartrate-resistant acid phosphatase, and demonstrated the potential value of monitoring their individual trajectories [8]. Total ACP in oral fluid and its bone-associated isoform are not interchangeable analytes, a distinction that must be considered when comparing studies.

Combined measurement of ALP and ACP allows the direction of changes in two enzymatic components to be characterised. In the present study, their ratio was used as the mineralisation index (MI), summarising biochemical trajectories within the selected observation model. This approach can

establish whether an increase in one parameter accompanies a decrease in the other and whether these changes persist after bracket placement. However, the enzyme ratio is an indirect measure that requires further comparison with enamel and periodontal characteristics; it does not replace clinical or instrumental assessment of mineralised tissues.

Prevention of demineralisation during fixed orthodontic treatment remains a subject of clinical investigation. The Cochrane review by P. E. Benson et al. identified limited certainty of evidence for individual fluoride delivery methods intended to prevent new demineralised lesions [6]. This highlights the need to evaluate specific preventive programmes, their components, schedules and outcomes. In a study a therapeutic and preventive complex used during orthodontic treatment in children with fluorosis was accompanied by a sustained increase in catalase activity [3]. These observations support further investigation of biochemical responses to supportive treatment, although an antioxidant response does not automatically establish the state of phosphatase activity.

Investigation of coordinated changes in acid and alkaline phosphatase activities during orthodontic treatment in children with fluorosis therefore remains relevant. Assessment at baseline, after the preparatory stage and at 1, 6 and 12 months after bracket placement can distinguish an early response from the persistence of changes at later time points. Comparing standard preventive measures with the adjunctive therapeutic and preventive complex can identify differences in enzymatic trajectories and clarify the potential role of MI in laboratory monitoring of this paediatric population.

The purpose of the study was to assess changes in acid and alkaline phosphatase activities and the mineralisation index in oral fluid in children with endemic fluorosis receiving a therapeutic and preventive complex during orthodontic treatment.

Materials and methods. A comparative clinical and biochemical study with repeated oral fluid measurements was conducted in 25 children aged 6–7 years with endemic fluorosis and orthodontic pathology. Biochemical investigations were performed in the Laboratory of Biochemistry and Vivarium of the State Establishment “The Institute of Stomatology and Maxillofacial Surgery of the National Academy of Medical Sciences of Ukraine” (SE “ISMFS NAMS”). Biochemical follow-up covered the preparatory stage and 12 months after bracket placement. The study was carried out from 3 June 2024 to 27 June 2025.

According to the therapeutic and preventive measures received, the children belonged to two groups:

- the comparison group comprised 12 children who received dental treatment to eliminate oral disease and professional oral hygiene as part of preparation for and support during orthodontic treatment;

– the main group comprised 13 children who received the developed therapeutic and preventive complex (TPC) in addition to dental treatment to eliminate oral disease and professional oral hygiene.

The main cohort additionally underwent a staged therapeutic-prophylactic complex designed to prevent enamel demineralisation, enhance remineralisation, regulate calcium–phosphate metabolism, and support connective tissue metabolism during orthodontic treatment. The regimen comprised a preparatory phase before bracket placement and measures during appliance treatment. Dental treatment to eliminate oral disease and professional oral hygiene were common components of care in both groups. The overall biochemical response to the complex was assessed; the effects of its individual components were not compared separately in this analysis.

Pre-orthodontic phase (days –14 to 0): Icon® resin infiltration system (INN: triethylene glycol dimethacrylate-based infiltrating resin with 15 % hydrochloric acid etchant and ethanol drying agent; DMG, Germany) – a single professional infiltration procedure performed according to the manufacturer's instructions 14 days before bracket bonding to arrest initial carious lesions, improve bracket adhesion, and increase enamel acid resistance.

Orthodontic treatment phase: Flairesse® Varnish (INN: sodium fluoride 5 %, equivalent to 22,600 ppm fluoride, with xylitol; DMG, Germany) – professional application to the enamel surfaces once every 6 months to promote remineralisation; CalciCare® oral suspension (INN: calcium carbonate 625 mg, equivalent to elemental calcium 250 mg, and cholecalciferol 125 IU per 5 mL; Indoco Remedies Ltd., India) – 5 mL orally twice daily before meals for 30 days to support calcium–phosphate metabolism, tooth mineralisation, and bone formation; Calcit® dental gel (INN: composition not verified; manufacturer not specified) – topical applications to the enamel surfaces for 14 days; Pemovo® tablets (INN: pure eggshell membrane 500 mg, a natural source of collagen, hyaluronic acid, glucosamine, and chondroitin; Asfarma Medical Dental Ürünler ve İlaç Sanayi Ticaret A.Ş., Turkey) – one tablet orally once daily with water in children older than 6 years to provide supplementary connective tissue constituents.

Biochemical assessments were performed at five time points: at baseline; after preparatory therapeutic and preventive measures immediately before bracket placement; and at 1, 6 and 12 months after bracket placement. Baseline values provided the basis for assessing subsequent changes within each group. Assessment before bracket placement characterised the response to preparatory measures; the one-month assessment reflected early changes after the start of appliance treatment; and the six-month and one-year assessments characterised persistence or change in the biochemical response over longer follow-up. Between-group comparisons were made at corresponding time points.

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 set forth 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 Odesa National Medical University, protocol No. 18 of 12/06/2023).

Unstimulated oral fluid was used for laboratory analysis. Samples were collected in the morning in a fasting state after two rinses with water. Children spat through a funnel into graduated centrifuge tubes for 5 minutes. The collected volume was determined from the tube graduations; if it was below 3 mL, collection was extended to 10 minutes. During collection, tubes were placed in a container with ice.

Before biochemical testing, oral fluid was centrifuged at 2500 rpm for 20 minutes at +4 °C. After separation of the sediment, the supernatant was used for analysis.

Acid phosphatase (ACP) and alkaline phosphatase (ALP) activities were measured in the supernatant. Results were expressed in $\mu\text{kat/L}$ and analysed separately for each enzyme. The mineralisation index (MI) was additionally assessed as the ratio of phosphatase activities: $\text{MI} = \text{ALP}/\text{ACP}$. Because both activities are expressed in the same units, their ratio is dimensionless; it is reported in arbitrary units in the table. MI was analysed alongside the activity of each enzyme.

Data processing was carried out with STATISTICA 6.1. Prior to 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. When pair-wise contrasts were required (Control $\times$ Comparison, Control $\times$ Intervention, Comparison $\times$ Intervention), the family-wise type-I error rate was controlled with the Bonferroni adjustment. Between-group differences were deemed statistically significant at $p < 0.003$ [2].

Results of the study. Phosphatase activities and mineralisation index values are presented in Table 1. At baseline, both groups exhibited elevated ACP and reduced ALP activities relative to the reference values.

Baseline ACP activity was $1.19 \pm 0.07 \mu\text{kat/L}$ in the comparison group and $0.96 \pm 0.05 \mu\text{kat/L}$ in the main group, respectively 3.05-fold and 2.46-fold higher than the reference value of $0.39 \pm 0.02 \mu\text{kat/L}$ ($p < 0.001$). Before bracket placement, ACP decreased to 1.00 ± 0.05 and $0.65 \pm 0.04 \mu\text{kat/L}$, corresponding to reductions of 16.0 % and 32.3 % from baseline. The reported values were $p_1 < 0.001$ for the main group and $p_1 < 0.05$ for the comparison group.

Table 1

Acid and alkaline phosphatase activities and mineralisation index in oral fluid in children with fluorosis during orthodontic treatment (M±m)

Parameter	Group	Baseline	Before bracket placement	At 1 month	At 6 months	At 12 months
Acid phosphatase activity, $\mu\text{kat/L}$ Reference value 0.39±0.02	Comparison n=12	1.19±0.07 p<0.001	1.0±0.05 p<0.001 p ₁ <0.05	1.04±0.06 p<0.001 p ₁ >0.6	1.17±0.08 p<0.001 p ₁ >0.8	1.25±0.07 p<0.001 p ₁ >0.7
	Main n=13	0.96±0.05 p<0.001 p ₂ >0.8	0.65±0.04 p<0.001 p ₁ <0.001 p ₂ <0.001	0.55±0.03 p<0.001 p ₁ <0.001 p ₂ <0.002	0.36±0.02 p>0.6 p ₁ <0.001 p ₂ <0.001	0.42±0.02 p>0.6 p ₁ <0.001 p ₂ <0.001
Alkaline phosphatase activity, $\mu\text{kat/L}$ Reference value 1.50±0.13	Comparison n=12	0.95±0.05 p<0.001	1.10±0.06 p<0.001 p ₁ >0.1	1.05±0.07 p<0.001 p ₁ >0.4	1.18±0.08 p<0.05 p ₁ >0.3	0.99±0.06 p<0.001 p ₁ >0.8
	Main n=13	0.91±0.05 p<0.001 p ₂ >0.25	1.22±0.08 p>0.1 p ₁ <0.02 p ₂ >0.25	1.36±0.09 p>0.4 p ₁ <0.02 p ₂ <0.001	1.73±0.08 p>0.2 p ₁ <0.001 p ₂ <0.001	1.62±1.0 p>0.8 p ₁ <0.001 p ₂ <0.001
Mineralisation index ALP/ACP, arbitrary units Reference value 3.84	Comparison n=12	0.79	1.10	1.0	1.0	0.79
	Main n=13	0.94	1.87	2.47	4.80	3.86

Note. p, comparison with the reference value; p₁, comparison with baseline; p₂, between-group comparison.

Subsequently, ACP activity in the comparison group was 1.04±0.06, 1.17±0.08 and 1.25±0.07 $\mu\text{kat/L}$ at 1, 6 and 12 months, respectively. The reduction at one month was 12.6 % relative to baseline, whereas the final value was 5.0 % higher than baseline. The reported p₁ values did not indicate statistically significant differences from baseline at these time points; ACP remained above the reference level (p<0.001).

In the main group, ACP activity decreased to 0.55±0.03, 0.36±0.02 and 0.42±0.02 $\mu\text{kat/L}$ at 1, 6 and 12 months. These changes represented reductions of 42.7 %, 62.5 % and 56.3 % from baseline (p₁<0.001). Activity was lower than in the comparison group by 47.1 % at one month (p₂<0.002), 69.2 % at six months and 66.4 % at one year (p₂<0.001). At the final two time points, the table reports p>0.6 relative to the reference value.

Baseline ALP activity was 0.95±0.05 $\mu\text{kat/L}$ in the comparison group and 0.91±0.05 $\mu\text{kat/L}$ in the main group, respectively 36.7 % and 39.3 % below the reference level (p<0.001). Before bracket placement, the means increased to 1.10±0.06 and 1.22±0.08 $\mu\text{kat/L}$, corresponding to increases of 15.8 % and 34.1 %. The between-group difference at this time point was not statistically significant (p₂>0.25).

In the comparison group, ALP activity was 1.05±0.07, 1.18±0.08 and 0.99±0.06 $\mu\text{kat/L}$ at 1, 6 and 12 months; the reported p₁ values did not indicate significant differences from baseline. In the main group, the corresponding means were 1.36, 1.73 and 1.62 $\mu\text{kat/L}$, exceeding baseline by 49.5 %, 90.1 % and 78.0 %. The reported values were p₁<0.001 at 6 and 12 months and p₁<0.02 at one month. At all three time points, ALP was higher than in the comparison

group by 29.5 %, 46.6 % and 63.6 %, respectively (p₂<0.001 according to the source table).

Baseline MI was 0.79 arbitrary units in the comparison group and 0.94 in the main group, against a reference value of 3.84. In the comparison group, values before bracket placement and at 1, 6 and 12 months were 1.10, 1.00, 1.00 and 0.79. In the main group, MI was 1.87, 2.47, 4.80 and 3.86, respectively, representing 1.99-fold, 2.63-fold, 5.11-fold and 4.11-fold increases from baseline. At 1, 6 and 12 months, the ratios of main-group MI to comparison-group MI were 2.47, 4.80 and 4.89. These comparisons are descriptive.

The findings demonstrate different trajectories of phosphatase activity under two approaches to preventive support during orthodontic treatment. In both groups, reduced baseline ALP was accompanied by elevated ACP, resulting in MI values substantially below the reference value. Following preparatory measures, the means changed in a favourable direction, but subsequent trajectories diverged. In the main group, progressive ACP reduction accompanied ALP elevation, whereas early changes in the comparison group did not produce a sustained approach of the enzyme ratio towards the reference level. This coordinated response is the most informative feature of the findings.

Discussion. The baseline enzymatic imbalance can be considered in the context of disturbed local homeostasis in children with fluorosis and orthodontic pathology. Experimental evidence concerning the effects of excessive fluoride on calcium signalling and mitochondrial function provides a biological basis for this interpretation [5]. However, current fluoride intake, fluoride concentrations in biological media and cellular

signalling markers were not measured in our study. The abnormalities therefore do not isolate the independent contribution of fluoride exposure or demonstrate direct fluoride-mediated enzyme inhibition in the examined children. They reflect the overall status of the study cohort.

The decline in ACP before bracket placement indicates that part of the biochemical response developed during the preparatory stage. ACP decreased by 32.3 % in the main group and by 16.0 % in the comparison group. Subsequently, main-group activity was 0.36 ± 0.02 $\mu\text{kat/L}$ at six months and 0.42 ± 0.02 $\mu\text{kat/L}$ at one year. The proximity of these means to the reference value is consistent with attenuation of the baseline enzymatic abnormality. However, absence of a significant difference from a reference value does not formally establish equivalence or confirm normalisation in every participant.

Interpretation of ACP reduction requires a distinction between total enzyme activity and specific markers of bone resorption. A. P. Cazzolla et al. evaluated the tartrate-resistant isoform alongside other bone turnover markers, unlike the total ACP measurement used here [8]. Our data therefore do not demonstrate direct osteoclast inhibition and cannot independently establish preservation of alveolar bone. The best-supported interpretation is a reduction in the measured phosphatase activity in oral fluid; clinical and instrumental comparisons are required to clarify its tissue origin.

The main-group increase in ALP was greatest at six months, when the mean rose from 0.91 to 1.73 $\mu\text{kat/L}$, an increase of 90.1 %. At one year it was 1.62 $\mu\text{kat/L}$, remaining 78.0 % above baseline. This differed from the comparison group, whose final value of 0.99 $\mu\text{kat/L}$ was close to the baseline value of 0.95 $\mu\text{kat/L}$. The combination of ALP elevation and ACP reduction indicates a favourable change in the enzyme ratio. Nevertheless, certainty regarding the final ALP value depends on verification of the uncertainty estimate reported for this parameter in the source table.

The context-dependent nature of ALP is supported by S. Bevinagidad et al., who observed increased activity during orthodontic treatment together with higher clinical inflammation indices [7]. This explains why ALP should not be regarded as a universally favourable marker. In our study, the favourable interpretation rests on the direction of change from an initially reduced level and the simultaneous change in ACP. Direct hard-tissue assessments are necessary to confirm enamel remineralisation, because enzyme activity in mixed oral fluid does not measure the mineral content of enamel.

MI most clearly reflected the between-group difference at later time points. In the main group it increased from 0.94 to 4.80 arbitrary units at six months and was 3.86 at one year; the corresponding comparison-group values were 1.00 and 0.79. The increase in the main group resulted from both a rising numerator and a falling denominator. An identical ratio can therefore theoretically have different

biochemical origins and should be assessed alongside both underlying enzymes. An MI above the reference value at six months should likewise not be interpreted as mineralisation exceeding physiological levels.

The decline in MI from 4.80 to 3.86 between the six-month and one-year examinations was 19.6 %. It accompanied a modest ACP increase and ALP decrease relative to six months, although both parameters remained markedly different from baseline. No statistical assessment of this comparison between time points was provided, so this is a descriptive change rather than demonstrated loss of effect. Persistence of the one-year MI near the reference value supports interest in the duration of the biochemical response and in determining appropriate intervals for supportive measures in subsequent studies.

The favourable combined trajectory may relate to support of the local oral environment during comprehensive preventive care. Evidence of microbial changes in fluorosis [13] and Ukrainian observations of a catalase response to therapeutic and preventive measures [3] suggest microbial and redox processes as possible accompanying mechanisms. Their relationships with ALP, ACP or MI were not tested in the present analysis. Enhanced antioxidant defence, membrane stabilisation and biofilm changes therefore remain possible explanations rather than established mediators of the complex's effect.

In the comparison group, return of MI to 0.79 at the final examination indicates limited persistence of the initial response under the conditions of this observation. This should not be generalised as evidence that professional oral hygiene is ineffective. The systematic review of periodontal status during fixed orthodontic treatment supports regular hygiene monitoring [9], while the review of demineralisation prevention demonstrates that outcome appraisal depends on the particular intervention and certainty of evidence [6]. As detailed adherence data and clinical indices were unavailable for this sample, their contributions cannot be separated.

The observed differences support further evaluation of the complex in a study with clearly described participant allocation, a standardised intervention and repeated measurements. The methodological heterogeneity in orthodontic biomarker research identified by L. Kakali et al. [12] highlights the importance of harmonising sampling conditions and analytical methods. For our model, comparison of MI with changes in demineralised lesions and periodontal indices is particularly necessary. Until such data are available, these findings should be regarded as evidence of a favourable association between comprehensive supportive care and the enzymatic response, rather than definitive evidence of an effect on mineralised tissue structure.

Limitations. The study involved a relatively small cohort of children with fluorosis undergoing orthodontic treatment, which should be considered when extending the findings to other patient groups. The assessment focused on oral fluid biochemical

markers; their relationship with clinical changes in enamel and periodontal tissues merits further investigation. In addition, the one-year follow-up

allowed assessment of biochemical changes during treatment, while their longer-term stability remains to be established.

Conclusions

1. In children with fluorosis and orthodontic pathology, baseline ACP activity was 2.46–3.05 times the reference value and ALP activity was 36.7–39.3 % lower; MI was 0.79–0.94 arbitrary units against a reference value of 3.84.

2. Adjunctive TPC use was accompanied by ACP reductions of 42.7 % at one month, 62.5 % at six months and 56.3 % at one year relative to baseline ($p_1 < 0.001$). A sustained ACP reduction was not established in the comparison group.

3. In the main group, mean ALP activity at 6 and 12 months exceeded baseline by 90.1 % and 78.0 %, respectively. ALP elevation occurred together with ACP reduction, producing a favourable trajectory in their ratio.

4. Main-group MI increased from 0.94 to 4.80 arbitrary units at six months and was 3.86 at one year; corresponding comparison-group values were 1.00 and 0.79. At one year, MI in the main group was 4.11 times its baseline value and 4.89 times the comparison-group value; these ratios are descriptive.

5. The findings support further investigation of comprehensive preventive care and combined monitoring of ALP, ACP and MI. Confirmation of a clinical remineralising effect requires verification of the primary statistical data and comparison of biochemical trajectories with direct enamel and periodontal outcomes.

Further research should focus on the long-term evaluation of acid and alkaline phosphatase activities and the mineralisation index in the oral fluid of children with fluorosis undergoing comprehensive orthodontic treatment in order to determine whether favourable biochemical changes correlate with enamel remineralisation, the prevention of demineralised lesions and the clinical condition of periodontal tissues during treatment and subsequent follow-up.

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ORCID: Shnaider D.S. <https://orcid.org/0009-0001-5936-6547>, Dienga A.E. <https://orcid.org/0000-0002-1633-1344>, Gorokhivskiy V.N. <https://orcid.org/0000-0002-8767-2939>, Mirchuk B.M. <https://orcid.org/0000-0002-5210-9337>, Yefremova O.V. <https://orcid.org/0000-0002-5149-2151>, Kordonets O.L. <https://orcid.org/0000-0002-1225-9779>, Suslova O.V. <https://orcid.org/0000-0002-4631-3809>.

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