MIXED SALIVA TRACE ELEMENT COMPOSITION IN CHILDREN WITH DENTOALVEOLAR ANOMALIES THROUGH APPARATUS-INVOLVED TREATMENT

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ABSTRACT

Lab-diagnostic methods have been employed to study immunoglobulin E and microelements in the non-stimulated oral liquid in children aged 4.5 yrs. – 8 yrs. using removable orthodontic appliances. It has been shown that an appropriate index of correlation connection between the immune system stress and the level of microelements is the growth of the copper/zinc ratio gradient in case of increasing concentration of iron and tungsten in the mixed saliva. It has been proven there is a need for conducting allergen-specific immune-modifying therapy to bring to normal the humoral immunity factors in order to improve the anti-microbial protection of the oral cavity.

KEYWORDS — microelements, correlation analysis, salivodiagnostics, antigenic stress, sensibilization.

TOPICALITY

The current stage in the evolution of medical technologies has witnessed specific significance in laboratory-diagnostic research into the role that macro- and microelement imbalance plays in the health of the child population. Trace element metabolism has been proven to depend significantly on the immune status as well as on climate-geographic, environmental, genetic, bio-social, and chronobiological factors that determine the body resistance at large. The effect of chemical elements is specified by the concentration intervals allowing normal metabolism. The intensity of metabolic reactions depends on the adjustment ability and the macroorganism capacity, which are coded and "approved" in the genotype [1,4,5].

Based on the threshold concentration theory by V.V. Kowalski we can say that functions in an organism can be regulated only under specific limits of the geochemical environment variability. Below the level of the minimum threshold concentration (lack of assimilation or insufficient intake of chemical elements), and above the upper threshold concentration (excessive supply of chemical elements) the homeostatic regulation function will be disturbed due to reduced resistance and joint activity of adjustment mechanisms [2,10].

Childhood has been shown to possess significant features in the immune and trace element status, as well in the tissues responsiveness to various xenogeneic materials. Note to be made that through a lengthy mechanical impact on the abutment teeth periodontium tissues, removable orthodontic appliances cause realignment of the entire dentoalveolar set; this comes along with altered microbiocenosis in the oral cavity, which is an important pathogenic mechanism disturbing the oral cavity homeostatic balance [6,7].

Scientific literature contains serious proof to the fact that the presence of IgE in any biological liquid may be viewed as a sign of an allergic reaction [3,8,9]. Given that it appears reasonable to investigate the correlation links between the presence of the allergic component (IgE) and the level of microelements in the oral cavity in children undergoing treatment for their dentoalveolar issues involving removable orth-

odontic appliances from base materials pertaining to different classes, while following the dynamics of their use. The data obtained from the correlation analysis – taken as an integral index for homeostatic balance through various stages of orthodontic treatment – would allow not only predicting allergy components and hypersensitivity, yet could also help reveal the efficiency of adjustment mechanisms for stabilizing immunological and trace element parameters in the mixed saliva.

Purpose of the research – to study the correlation links between the level of microelements and IgE in the mixed saliva of children undergoing treatment for dentoalveolar anomalies employing removable orthodontic devices of various base materials.

MATERIALS AND METHODS OF RESEARCHING

The modern international classification ISO 1567:1999 (Dentistry – Materials for base prostheses) was used to select three types of base materials used to manufacture removable orthodontic appliances. The 1st type material was polymethylmethacrylate-based (PMMA) cold-cured plastic Meliodent RR (Heraus Kulzer, Germany), which is an acrylic-based copolymer. Powder – fine-dispersed, suspension PMMA, containing the initiator – benzoyl peroxide and the activator – disulfanil; liquid – methacrylic acid methyl ether containing the activator – dimethylparatoluidine. The orthodontic appliances were made through hydropolimerization on gypsum base in the Ivomat IP3 (Ivoclar-Vivadent) polymerizer. The 2nd type material was hot-cured PMMA plastic ProBase Hot (Ivoclar-Vivadent, Lichtenstein), which is an acrylic graft-copolymer. Powder – fine-dispersed, suspension and the graft copolymer of methacrylic acid methyl ether; liquid – methacrylic acid methyl ether containing the cross-linking agent – diphenylpropane. The orthodontic devices were produced through compression pressing in the water polymerizer Acrydig 4 (F. Manfred). The 3rd type material used was the base material Versyo (Heraus Kulzer, Germany), which is a cross-linked composite acrylic plastic with a structure of interpenetrating polymer networks. The monomer system is a mix of multifunctional radicals with a high molecular weight containing no PMMA. The content of the non-organic filler (SiO_2) – 8%, particle size – 0.6–0.8 μm. The orthodontic appliances were made using the gypsum-based photo curable technology with a prior polymerization in the Heralight polymerizer (Heraus Kulzer) and the final polymerization in the Heraflash (Heraus Kulzer). All the materials were polymerized observing the cycle parameters as indicated by the manufacturer.

The investigation of the microelement content and IgE in the non-stimulated oral liquid (NOL) was done in 67 children aged 4.5–8 years with satisfactory and good oral hygiene. The patients were divided into a control a three major groups for out-patient observation. The control group was 18 children with orthognathic occlusion undergoing regular check-up and needing no orthodontic treatment.

Group 1 included 16 patients with abnormal teeth position and no dentition defect who had their 20 orthodontic appliances made of the 1st type material.

Group 2 was 18 patients with abnormal teeth position while their 22 orthodontic devices were made of the 2nd type material.

Group 3 included 15 patients who had abnormal teeth position and who had 19 orthodontic appliances made for them of the 3rd type material.

The tested appliances were permanently used for two months. All the participants were instructed regarding the standard tooth brushing methods in view of their age and the respective device maintenance requirements. The hygiene skills in the children were monitored using the hygiene index (Fedorov-Volodkina, 1972).

To study the level of microelements and that of IgE each of the patients had their NOL samples taken in a clinical setting, on an empty stomach at 8–9 o'clock, four times (prior to the treatment; 14 days after; 30 after; 60 days after the start of the orthodontic treatment). The patients were asked to restrain from any salivation inducing activities – eating; gum chewing; teeth brushing, and mouth rinsing. The patients in all the groups under investigation received a prior professional teeth cleaning. To collect NOL each patient was seated with their head down for some time; swallowing saliva was not allowed. When investigating the microelement content mixed saliva (0.7 ml) was taken straight from the oral cavity; the samples were placed in test-tubes (volume – 10 ml) (method by R.V. Karaseva, 2006) to be further stored under 0 to +4° C. When analyzing the IgE content the patient had to spit the accumulated NOL (5–7 ml) out into a sterile graded chilled glass test-tube. After that the mixed saliva was centrifuged for 15 minutes at 8.000 rev/min. The NOL supernatant fraction was separated in plastic tubes and stored under –30° C. When analyzing the immune and element content of the NOL lab-diagnostic methods were used to check all the patients' level of microelements to be found in the removable orthodontic appliances (Mn, Cu, Co, Mo, Ni, Ti, Fe, Zn, Cr, W), as well as the IgE rate was established in each case.

The study of the microelement content in mixed saliva was done through the methods for Inductively

Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) on the device Optima 2000 DV (Perkin Elmer, USA) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) on ELAN-9000 (Perkin Elmer, USA) following the methodology as approved by the Ministry of Healthcare, Russian Federation (S.I. Ivanov et al., 2003; L.G. Podunova et al., 2003).

Method: this method is based on the oxidation-acid "wet" mineralization of the biosubstrate samples through the sample preparation and its further analysis for the required chemical elements with atomic emission spectroscopy employing high frequency inductively coupled argon plasma as the source of excitation. The ICP-AES method implies excitation of atom emission spectrum in the inductively coupled argon plasma and automatic registration of the position and intensity for the spectrum lines, which correspond particular elements.

All the biosubstrate samples went through preparation following the Methodological Recommendation approved by the Ministry of Healthcare and Social Development of the Russian Federation (Detection of Chemical Elements in Biological Environments and Preparations Through Inductively Coupled Plasma Atomic Emission Spectroscopy and Inductively Coupled Plasma Mass Spectrometry) (2003). The samples were analyzed following the Organizational Standards for Measuring Methodology 01-2009 (СТО МВИ 01-2009).

The substrate sample (0.7 cm^3) was weighed on analytical scales in fluoro-plastic insulation test-tubes. Each tube was placed in a thermo block heated up to 115 °C, and then steamed dry. After that each tube was filled with 2 cm^3 of high purity nitric acid and then sample volume (chilled down to room temperature) was brought up to 10 cm^3 with distilled deionized water to be further mixed. The test-tube was covered with the self-adhesive protective film Parafilm(R) M, placed in the automatic sampler socket, after which measuring was conducted on the spectrometer Specord-M40 (CARL ZEISS JENA, Germany) under the following parameters: power output – 1300 Watts; cooling stream – 15 l/min; auxiliary stream – 0.2 l/min; bearing stream – 0.85 l/min; sample feed rate – 1.5 l/min. The following element spectrum lines were used for the measures (λ): Mn – 257.610 nm, Cu – 327.393 nm, Co – 267.716 nm, Mo – 202.031 nm, Ni – 221.648 nm, Ti – 334.940 nm, Fe – 238.204 nm, Zn – 206.200 nm, Cr – 267.716 nm, W – 292.464 nm.

The prepared samples were introduced into the spectrometer, just like the sample element atomic emission, was done in the automatic mode according to the operations manual. The emission intensity, once the light penetrated the monochromator grating and the optic scheme, was registered with a photosensitive device where the photocurrent was measured and processed in the spectrometer computer system. The analytical signals from the spectrometer Specord-M40 were processed through the respective software. The measuring result was approved as the arithmetic mean value of two parallel estimates, the divergence between them not exceeding the recurrence limit ($r \geq 14\%$).

The IgE index in the biological material was determined following the A.I. Karpischenko method (1998). Method: monoclonal antibodies (moAb) directed towards two different antigenic areas of IgE molecules (immunoglobulin concentration determined with calibrating graphs). In each couple one of the moAb was used to sensitize the tray surface and provided for coupling at the solid phase of IgE from the analyzed samples. A second moAb, coupled with peroxidase, interacted with another antigenic determinant coupled at the solid phase of the IgE molecule. The non-coupled components of the samples as well as the excess of the labeled moAb were removed from the solid phase by repeated washing with buffer solution containing Twin-20. The peroxidase activity at the solid phase was assessed based on the decomposition of orthophenylenediamine substrate and hydrogen peroxide. The decomposition product turned chromogen molecules into colored derivative substance the amount of which was proportionate to fermentative activity. The reaction was terminated with sulphuric acid. The result was registered on the microtray reader Infinite F 50 (Tecan), using the MagellanTM software for immune-enzyme assay with a vertical path in transmission density units with a wave length of 492 nm.

RESULTS AND DISCUSSION

The trace element analysis in the NOL in the control group showed the presence of the following microelements: Cu, W, Fe, Zn, Cr. The statistically meaningful fluctuations of the microelement content in the NOL were: Cu – from 0.64±0.03 to 0.75±0.04 mg/l; W – from 0.48±0.03 to 0.61±0.03 mg/l; Fe – from 1.23±0.06 to 1.38±0.07 mg/l; Zn – from 0.61±0.03 to 0.72±0.03 mg/l; Cr – from 0.47±0.03 to 0.60±0.03 mg/l. We took the averaged values (Cu – 0.70±0.04 mg/l; W – 0.55±0.03 mg/l; Fe – 1.31±0.07 mg/l; Zn – 0.67±0.03 mg/l; Cr – 0.53±0.03 mg/l for a conditional norm, which offers the best description for the parameters of the mixed saliva microelement status in children.

Table 1 contains the numbers for the microelement content in the NOL of the 1st Group's patients through various parts of the orthodontic treatment.

The microelement content in the NOL of the patients in the 2nd Group through various stages of the

orthodontic treatment is shown in Table 2.

In Table 3 you can see the microelement content detected in the NOL in Group 3 through various stages of the treatment.

The published data on the scientific research miss information on the microelement content in the mixed saliva, as well as potential changes at various stages of orthodontic treatment in children. The results obtained from the investigation of the mineral composition of the NOL before and after the removable appliances were used, showed that the patients demonstrated significant alterations in the chemical elements content. Thus, all the patients showed statistically reliable increase in the concentration of the microelements that are part of the removable orthodontic appliances (Mn, Cu, Co, Mo, Ni, Ti, W, Fe, Zn, Cr), while many of these (Mn, Co, Mo, Ni, Ti) had not been found in the mixed saliva prior to the orthodontic treatment.

The evaluation of the results of the laboratory-diagnostic research makes it safe to say that of all the microelements found in the mixed saliva the most significant growth (if compared to the initial data) was registered in iron (129.3±5.2% – 168.8±6.8%) and tungsten (156.1±6.4% – 224.2±9.2%). It has been proven that in the etiology of gingivitis a significant role is played by microorganisms, in particular staphylococci that are to be found in the dental deposit, the subgingival space, and in the saliva, and which need iron to function. Excess of iron inhibits the bacteriostatic effect of lactoferrin, leukocyte chemotaxis and phagocytosis, macrophage phagocytosis, transformation of lymphocytes, as well as the bactericide role of antibodies and complement. The death of staphylococci under the influence of polymorphonuclear leukocytes is inhibited with free (protein-bound) iron but not hemoglobin or catalase. Besides, mixed saliva also accepts erythrocytes, which, while decomposing, release non-protein iron this increasing its general level in the given environment. The combination of the factors in question facilitates progressive growth of the microflora and the development of allergic (inflammation) processes in the oral cavity.

Zinc is known to play an important role in the cell and humoral immunity. Zinc deficit increases susceptibility to infection, reduces the production of g-interferon and interleukine-2, the lytic activity of natural killers, and their relative content in the T-lymphocyte population. An analysis of the results showed that in all groups the patients going through the initial stage of orthodontic treatment showed a reliable decrease of Zinc concentration in the saliva (8.2±0.4% – 21.1±0.9%) while the level of Copper went up (16.2±0.8% – 27.6±1.1%), if compared to the control group and the data obtained prior to the treatment. In 60 days of orthodontic treatment an increase of Copper due to Zinc deficit in mixed saliva provides for an increased gradient in the ratio Copper/Zinc in all the groups under control: Group 1 – from 1.07 to 1.70; Group 2 – from 1.15 to 1.58; Group 3 – from 1.10 to 1.40. The gradient increase in the Copper/Zinc ratio in NOL promotes the permeability of mucous tunic epithelium for bacterial flora, which stimulates the activity of inflammatory processes in the oral cavity.

The IgE index in the NOL at various stages of orthodontic treatment in the groups can be seen in Table 4.

The control group patients just like those in the groups under out-patient observation, revealed no IgE in their NOL before the start of the orthodontic treatment. A comparative analysis of the IgE content in the NOL of the patients belonging to the groups that were under investigation, done after 2 months of orthodontic treatment, allows concluding that the

Table 1. *Microelements in the NOL through various stages of orthodontic treatment, patients of Group 1 (mg/l) (M±m)*

Element	Maximum concentration limit in water	Term of investigation			
		Prior to treatment	After 14 days	After 30 days	After 60 days
Mn	0.1	Not detected	0.28±0.01	0.34±0.01	0.37±0.02
Cu	1.0	0.76±0.04	0.82±0.04	0.93±0.05	0.97±0.05
Co	0.1	Not detected	0.08±0.01	0.09±0.01	0.11±0.01
Mo	0.25	Not detected	0.10±0.01	0.09±0.01	0.10±0.01
Ni	0.1	Not detected	0.14±0.01	0.16±0.01	0.17±0.01
Ti	0.1	Not detected	0.14±0.01	0.18±0.01	0.19±0.01
W	0.05	0.58±0.03	1.34±0.07	1.53±0.08	1.88±0.09
Fe	0.03	1.38±0.07	2.82±0.14	3.56±0.17	3.71±0.18
Zn	5.0	0.71±0.04	0.68±0.03	0.63±0.03	0.57±0.03
Cr	0.05	0.61±0.03	0.82±0.04	0.76±0.03	0.93±0.05

Table 2. *Microelements in the NOL through various stages of orthodontic treatment, patients of Group 2 (mg/l) (M±m)*

Element	Maximum concentration limit in water	Term of investigation			
		Prior to treatment	After 14 days	After 30 days	After 60 days
Mn	0.1	Not detected	0.18±0.01	0.23±0.01	0.21±0.01
Cu	1.0	0.73±0.03	0.76±0.03	0.81±0.04	0.92±0.04
Co	0.1	Not detected	0.09±0.01	0.08±0.01	0.09±0.01
Mo	0.25	Not detected	0.08±0.01	0.09±0.01	0.09±0.01
Ni	0.1	Not detected	0.12±0.01	0.14±0.01	0.15±0.01
Ti	0.1	Not detected	0.18±0.01	0.16±0.01	0.15±0.01
W	0.05	0.51±0.02	1.12±0.06	0.96±0.05	1.31±0.07
Fe	0.03	1.28±0.07	2.05±0.11	2.34±0.12	2.86±0.14
Zn	5.0	0.63±0.03	0.61±0.03	0.60±0.03	0.58±0.03
Cr	0.05	0.51±0.03	0.73±0.03	0.67±0.03	0.56±0.03

Table 3. *Microelements in the NOL through various stages of orthodontic treatment, patients of Group 3 (mg/l) (M±m)*

Element	Maximum concentration limit in water	Term of investigation			
		Prior to treatment	After 14 days	After 30 days	After 60 days
Mn	0.1	Not detected	0.19±0.01	0.21±0.01	0.24±0.02
Cu	1.0	0.74±0.03	0.74±0.03	0.77±0.03	0.86±0.04
Co	0.1	Not detected	0.09±0.01	0.08±0.01	0.09±0.01
Mo	0.25	Not detected	0.09±0.01	0.09±0.01	0.08±0.01
Ni	0.1	Not detected	0.13±0.01	0.15±0.01	0.16±0.01
Ti	0.1	Not detected	0.15±0.01	0.17±0.01	0.17±0.01
W	0.05	0.54±0.03	0.95±0.05	1.28±0.07	1.41±0.08
Fe	0.03	1.23±0.06	2.27±0.11	2.62±0.13	2.82±0.14
Zn	5.0	0.67±0.04	0.65±0.03	0.63±0.04	0.61±0.03
Cr	0.05	0.56±0.03	0.83±0.04	0.75±0.03	0.73±0.03

Table 4. *IgE in NOL at various stages of orthodontic treatment (IU/l) (M±m)*

Term of investigation	Control group	Group 1	Group 2	Group 3
Prior to treatment	Not detected	Not detected	Not detected	Not detected
After 14 days	Not detected	1.72±0.09	1.65±0.09	1.36±0.06
After 30 days	Not detected	2.68±0.14	2.33±0.12	1.54±0.08
After 60 days	Not detected	3.24±0.16	2.86±0.15	2.07±0.09

highest increase (88.4±3.5%) was in case of using appliances of cold-cured base plastic, while light-cured base plastic materials offered the lowest increase in the values (52.2±2.1%).

The published scientific data provide no complete information on the dynamics of the change in the IgE level in NOL at various stages of orthodontic treatment in children. Detecting IgE in the NOL of the patients who were somatically healthy and in whom this immunoglobulin was not detected at the initial check-up, sends us suggesting that this presence is due to the treatment involving appliances and the orthodontic devices in the oral cavity. We believe that when orthodontic appliances are constantly washed by the oral liquid then there is a continuous bilateral ion exchange between the mixed saliva and the chemical substances that are part of the device. The chemical microelements in the removable appliances (Mn, Cu, Co, Mo, Ni, Ti, Fe, Zn, Cr, W) diffuse into the mixed saliva, while being antigens that cause sensitizing.

The data available in the respective literature nowadays prove that the chemical elements in removable appliances play a significant role in the development of IgE-dependent pathogenic mechanisms. A specific issue in the development of immunological reactions involving the cytophilic IgE is the ability to get fixed on the surface of mast cells and basophiles, which can be accounted for by a large number of receptors to the Fc-fragments of IgE, which are found on these cells. In case of further coupling of the IgE, which is fixed on mast cells or basophiles, with an antigen (microelement), there appears degranulation of these cells with a release of histamine and other vasoactive substances leading to an allergy reaction.

Our view is that the maximum level of IgE in the NOL that is found on Day 60 is related to the saturation of mixed saliva with microelements, and a rise of an allergy component. Proof to it is that at first the removable appliances were kept in the oral cavities for some limited time (1–1.5 hours), this period of time to go up further on (by Day 14 – up to 4–5 hours), and on Day 60 only they started to use the devices for 16–18 hours per day.

The scientific data available as well as the results of our own research projects make it possible to state that in children using removable orthodontic appliances, at the initial stage, the most significant increase in the level of iron (168.8±6.8%), tungsten (224.2±9.2%), the copper/zinc ratio gradient (58.9±2,4%) as well as IgE (88.4±3.5%) was shown in case of cold-cured base plastics. The serious strain that the immune system suffers in case of using cold-cured materials can be explained, as we see it, by high water solubility, the presence of peroxidates in the base material, as well as a significant level of the residual monomer. The lowest increase in iron (129.3±5.2%), tungsten (156.1±6.4%), the copper/zinc ratio gradient (27.3±1.2%), and IgE (52.2±2.1%) was detected in case of light-cured base plastic materials; we believe this could be explained by low water solubility and complete absence of the residue monomer and peroxidates in the base composite plastic.

FINDINGS

1. Comparative evaluation of mixed saliva microelement composition in children through various stages of orthodontic treatment with removable devices manufactured of various base materials is an informative and prognostically meaningful test in determining the intensity of allergic process, which also offers a proper view of the physiological condition at large.
2. Correlation analysis allows the most complete understanding of the dynamics as well as interrelation between the microelement content and E-immunoglobulin in children's mixed saliva; this shows mobilization of adjustment mechanisms aiming at the body's increased functional activity through treatment with orthodontic devices.
3. Using removable orthodontic appliances for children results in a strain to the immune system due to antigen stress, which, in turn, is explained by a statistically proven increase in the mixed saliva of virtually all the chemical microelements that are to be found in the orthodontic appliances.
4. A proper indicator of correlation dependence between the level of microelements and the strain in the immune system is an increased copper/zinc ratio gradient, which is the case in the event of a higher concentration of tungsten and iron in the oral liquid. The level of other microelements that play smaller roles in producing antigens does not change significantly, which is to be taken into account when making a comprehensive assessment of the impact that orthodontic treatment has on the patient's immunological defense mechanisms.
5. The most significant increase in the concentration of iron, tungsten, the copper/zinc ratio gradient, as well as IgE in children at the initial stage of their orthodontic treatment, was seen in case of using cold-cured base plastic appliances.
6. The mixed saliva microelement analysis can be justified if used as a diagnostic test for evaluating the general condition at various stages of the orthodontic treatment. This would allow "tailor-made" scheme for correcting the mineral metabolism in order to increase the efficiency of therapy for dentoalveolar anomalies and deformations in children with allergies in the anamnesis.
7. It has been shown that when orthodontic appliances are used to treat children it is advisable to carry out allergen-specific immune-modifying therapy to improve the humoral immunity factors in order to strengthen the oral cavity antimicrobial protection.
8. The issue of the allergy component (IgE) and disturbed oral liquid homeostasis in case of using removable orthodontic appliances to treat children has not been widely covered and requires further investigation.

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