Changes of the tooth enamel following the application of a new prevention method in children suffering from cerebral palsy and gastro-esophageal reflux disease

Aurelia Spinei, Alina Monica Picos, Petra Nicoara, Andrei Picos, Iurie Spinei

Abstract. Aim: The purpose of the paper consisted in studying the structural and chemical composition changes of the tooth enamel occurred after simultaneous application of laser therapy and deep fluoridation of enamel at children suffering from cerebral palsy (CP) associated with the gastro-esophageal reflux disease (GERD). Material and methods: 36 children suffering from CP associated with GERD were taken into the study, with ages between 13 and 15. Depending on the prevention measures applied, the children were divided in 3 groups: L1 included 12 children who benefitted from deep fluoridation of the tooth enamel; in L2, we applied the physical-chemical method of preventing the caries and dental erosions (laser therapy and deep fluoridation of the enamel), the control group (L3) included 12 children whose teeth were brushed using fluoride toothpastes. For research on the structure and chemical composition of the tooth enamel, 24 teeth extracted for orthodontic purpose have been studied using the scanning electron microscopy - SEM and radio spectral microanalysis (REM) was performed using the method of energy dispersive X-ray spectroscopy. Results: Following the application of the exogenous methods for the prevention of caries and dental erosions, the frequency of carious lesions increases. At 75% of children, the frequency of dental erosions increases. In the deep layers of the enamel, we found deposits of CaF2 of energy dispersive X-ray spectroscopy. Results: Following the application of the exogenous methods for the prevention of caries and dental erosions, in groups L1 and L2 on the surface and in the deep layers of the enamel, we found deposits of CaF2 and MgF2, and CuF2 micro-globules, considerable reduction of carbon concentration, normalization of the relation between Ca/P and significant increase of fluorine content. The physical-chemical method for the prevention has contributed to the increase of the F concentration 1.63 times in the superficial layers and 2.08 times in the deep layers of the enamel, as compared to the method of deep fluoridation of the enamel. Conclusion: simultaneous application of laser therapy with nanofluorides at children suffering from CP ensures an effect of maximum protection.

Key Words: dental enamel, CP, GERD, deep fluoridation, laser 0.85-0.98 μm, SEM.

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Introduction

Cerebral palsy (CP) represents a burden for the society and could not be eliminated despite the progress achieved in the field of medicine (Panteliadis 2004; Johnson 2002; Poddar 2013). Cerebral palsy at children (CP) includes a group of non-progressive neurological disorders defined by an inadequate control of movement and posture. It is estimated that in the USA over 100,000 children under the age of 18 suffer from a neurological disability due to CP (Popescu 2001). In France and in England, 25% of the patients with CP suffer from considerable motor deficiency, being unable to walk on their own and 30% suffer from intellectual disability (Panteliadis 2004; Popescu 2001; Johnson 2002). Prevalence of CP (medium and severe forms) is 1.5-2.5 out of 1000 of living newborns, higher in the developing countries. At children with neuromotor disabilities, we frequently encounter eating (feeding) difficulties, including deglutition disorder, vomiting and GERD. Tsamtsouris et al (2003) have detected a prevalence of 75% of GERD at children suffering from cerebral palsy. The long-term action of the gastric acids attacks on tooth enamel may cause dental erosion. The severity of the erosion has been correlated with the duration of the disease, frequency of the vomiting, the low pH of the saliva, type of eliminated acids and last, but not least, the quantity and quality of the saliva. Thus, the study demonstrated the increased prevalence of dental erosion at children suffering from cerebral palsy, which can be associated with GERD (Su et al 2003). GERD and other intrinsic factors, by significant decrease of saliva pH under the critical level, disturb the balance between the demineralization and remineralization processes in the tooth enamel, causing the dissolution of the hydroxyapatite crystals in the tooth enamel. When the saliva pH decreases under 2.0 and the oral hygiene is in good condition, we observe a significant increase of the frequency of dental erosion (Marsicano et al 2013; Firouzei et al 2011; Poddar 2013), while in case of less significant decrease of saliva pH and abundant dental plaque deposits, the frequency of carious lesions increases. At 75% of
the children suffering from CP we identified the decrease of the functional resistance of enamel under the action of acids, thus the enamel resistance test value is 68.29±4.27%, which indicates the low acid resistance of the tooth enamel, which is an essential risk factor in the occurrence of caries and dental erosions (Okushko 2011). Thus, the results of a large number of studies indicated an increased prevalence of dental caries, the occurrence of the multiple tooth decay and dental erosions at children suffering from cerebral palsy associated with GERD.

In our previous study (Spinei 2014), using the scanning electron microscopy SEM and IR spectroscopy, we established the following structural and chemical composition features of tooth enamel in children with severe neuromotor disabilities:

- the electron optical structure of enamel surface is characterized by the presence of areas with a large amount of pores and micro-cracks;
- increased concentration of CO32- ions located in the B-type area;
- increasing share of the organic component of enamel in relation to the mineral component, and respectively, decreasing percentage weight of P, Ca, Cl, Mg, Na and hydroxyapatite content;
- increased concentration of carbonate-apatite, low intensity of phosphate peaks and a significant growth in the organic components.

So, as a result of this study, we determined the structural features of the enamel at the molecular and macroscopic level, caused by mineralization disorders occurred during tooth formation and during post-eruptive mineralization at children with severe neuromotor disabilities. Thus, based on the result of the literature sources analysis and based on our personal researches, we can say that at children suffering from CP associated with GERD, due to the mineralization disorders of the rough dental tissues, as well as due to considerable decrease of the saliva remineralization ability, associated with frequent and long decrease of the saliva pH, leads to the considerable decrease of the enamel resistance to acid attack, followed by prevailing demineralization processes on a longer period of time, mass dissolution of hydroxyapatite crystals and formation of extended areas of demineralized enamel, thus representing an increased risk for the occurrence of dental erosion and new carious lesions.

Therefore, in order to prevent dental caries at children with CP associated with GERD it is necessary to apply prevention measures in a rational and efficient manner, targeting the individual risk factors, and also considering the behavioral particularities of disabled children. Thus, prevention of dental troubles at children with disabilities associated with digestive diseases is an important element in the dental care, for persons with special needs, having an impact that is both medical and social, ensuring the health of the oral cavity, but also contributing to the improvement of these children’s life quality.

The purpose of the paper consisted in the study of changes in the structure and chemical composition of the tooth enamel, occurred after the simultaneous application of laser therapy and deep fluoridation of the enamel at children suffering from cerebral palsy associated with the gastro-esophageal reflux disease.

**Material and methods**

This research was preceded by a clinical study conducted on a sample of 309 conventionally healthy children for three years, where we evaluated the caries protective effect of different exogenous methods meant for dental caries prevention. The methods that have been found to be most effective were applied in this research.

In order to achieve the established objective, 36 children suffering from CP were included in the study, with ages between 13 and 15. We performed the clinical examination of the patients, including the assessment of the dental caries prevalence by estimating the DMFT index. The assessment of the erosive wear was performed on the palatine/lingual sides, on the vestibular and incisal/occlusal sides, noting down a single value for each sextant, corresponding to the dental side having the greatest erosion degree. The final score of the BEWE index was obtained by adding up the values of all sextants based on which the erosive wear was calculated. In addition to this, we assessed the enamel resistance to acids, using the enamel resistance test following the method proposed by Okushko (2011), the pH of saliva (GC), salivary flow rate (GC), the buffering capacity of saliva (GC) and the remineralization potential of saliva, assessed by estimating the remineralization speed of saliva using the method elaborated by Leontiev et al (2007).

The children included in the study suffer from spastic quadriplegia associated with GERD. Due to the existing deficiencies, the children are not able to manage independently, and even to perform the oral cavity hygiene on their own. For the purpose of performing the oral hygiene systematically, the medium qualified medical staff, the auxiliary staff and other persons who ensure medical care to these children.

Depending on the caries preventive measures applied, the children were divided into 3 groups:

- **L1** – included 12 children who received deep dental enamel fluoridation, carried out as follows: after cleaning the oral cavity 1-3 drops of solution Gluftored No. 1 have been applied on teeth surface for 60 seconds, after which 1-3 drops of suspension Gluftored No.2 were applied on the same surfaces. Gluftored preparation, Vladmiva (Russia), is intended for deep fluoridation of the enamel and includes a set of two components: 1 fl 10 ml enamel sealant, and 1 fl 10 ml suspension. The enamel sealant No.1 contains a set of nanofluorides: 7.0 g of magnesium fluorosilicate, 0.3 g of copper fluorosilicate, 0.1 g of sodium fluoride (as stabilizer) and distilled water. The suspension No.2 contains: 19.3 g highly dispersed calcium hydroxide, 4.1 g copper hydroxide, 0.6 g of calcium hydroxycuprate, calcium sulfate dihydrate, methyl cellulose and distilled water.

- **L2** – consisted of 12 children to whom the physicochemical method of preventing dental caries has been applied (laser therapy and deep enamel fluoridation). The suggested method was carried out as follows: after cleaning the oral cavity the tooth surfaces were irradiated with laser light of 0.85-0.98 μm, and the exposure time was 60 seconds. Then, 1-3 drops of Gluftored solution No.1 were applied on the surface of the teeth and simultaneously laser light in the same regime was irradiated. After that, 1-3 drops of suspension Gluftored No.2 were applied and infrared laser light was irradiated for 60 seconds on the same surfaces (Brevet MD 582) (Spinei 2013).
To conduct this method a laser device was used, Optodan ALST-01, Vend, Russia, with Gallium arsenide-based semiconductor (GaAs) with an impulsive generation regime, 0.85-0.98 µm wavelength, 2000 -3000 Hz frequency and 5 W impulse power. For groups L1, and L2, 2-3 preventive sessions have been performed based on the previously estimated risk of caries. L0, the control group included 12 children who had undergone tooth brushing with fluoride toothpaste in which the F concentration was appropriate to children’s age. The study duration was 3.5 months.

Of the total number of 36 children included in the study, the extraction of upper premolars was recommended to 12 (33.33%) subjects. We studied the structure and chemical composition of different regions of dental enamel. Was investigated 72 pieces of enamel prepared from 24 extracted teeth following orthodontic indications (Table 1). Scanning electron microscopy SEM was performed with TESCAN VEGA TS 5130 MM equipment and further radio spectral microanalysis (RSM) was performed by method of energy-dispersive x-ray spectroscopy using EDX detector of materials (Oxford Instruments energy-dispersive x-ray system) (UK). This review was conducted in the National Centre for Research and Testing of Materials of the Technical University of Moldova.

The study was approved by the Research Ethics Committee of SUMP “Nicolae Testemițanu” and UMF Iuliu-Hatieganu and was conducted in accordance with ethical requirements, and with the written consent of the children’s parents or their legal representatives.

Data analysis was performed using Microsoft® Excel® 2013 and IBM® SPSS Statistics 22.0 software using the functions and modules of these programs.

Results

At the initial clinical examination, performed on 36 children suffering from spastic quadriplegia associated with GERD the dental decay was detected at 63.89% of them, the incidence of the DMFT index being 2.56±0.33. Dental erosion was detected at 47.22% of the total number of children, and the incidence of erosive wear was 3.58±0.7. The increased risk of erosive wear was detected at 2 (5.56%) children, the average risk - at 4 (11.11%) and the low risk, respectively - at 14 (38.38%) children. This clinical situation was aggravated by the increased risk of caries prevention session elucidated deposit of CaF2, MgF2 and CuF2 crystals in the composition of deposited sediment (Fig. 1).

As a result of the deep fluoridation method (L1) and the combined method - laser therapy and deep fluoridation (L2) in the pieces of enamel prepared from extracted teeth after the last caries prevention session, we found a highly dispersed grained sediment on some areas of the enamel surface. The radio-spectral microanalysis performed simultaneously allowed us to estimate the atomic relationship of Ca, P, F, Mg, Cu, which confirms the presence of CaF2, MgF2 and CuF2 crystals in the composition of deposited sediment (Fig. 1).

Enamel surface in the control group (Fig. 2) is characterized by the presence of micro-cracks, protrusions and other microstructures in the form of holes, which extend into micro-ducts of up to 2 µm in diameter, forming a relief like a “honey comb”. On enamel fracture surfaces we observed micro tunnel-shaped ducts that penetrate into the deeper layers of enamel and change the direction of adamantine prisms).

The SEM and radio spectral microanalysis of the enamel pieces prepared from extracted teeth within 1 month after the last caries preventive session elucidated deposit of CaF2, MgF2 and CuF2 micro-globules with dimensions not more than 5 nm on enamel surface in groups L1 and L2, as opposed to the lack of globules in the control group. In group L2 we found a large number of micro-globules on the enamel surface, as compared to L1 (Fig. 3).

During the observation period, no significant pH changes were estimated, changes of the buffering capacity, or of the remineralization capacity of saliva.

As a result of applying the prevention measures, we found that the enamel increased its resistance to acids, by 29.06±2.8% (p<0.005), while in the control group, it remained unchanged.

During the observation period, no significant pH changes were estimated, changes of the buffering capacity, or of the remineralization capacity of saliva.

Figure 1. SEM picture of external enamel surface of an intact tooth extracted after the last laser therapy session and simultaneous deep fluoridation. Depositing of highly dispersed granular sediment. Spectrum 1 - the result of spectral analysis in the highly dispersed granular sediment.
increased compared with the control group 1.21 times in L1 and in L2, respectively - 1.28 times, reaching thus normal values. As a result of deep fluoridation of enamel the F concentration constituted 0.27±0.06 (p<0.001) in superficial layers and 0.12±0.02 (p<0.001) in the deep ones and increased 7.5 times on the enamel surface researched and 8.57 times in deep layers compared to the results obtained after tooth brushing with fluoride toothpaste. Simultaneous application of laser irradiation under low intensity impulses in the range of infrared wavelengths and nanofluorides contributed to the increase of the F concentration 12.22 times in the surface layers of enamel, which constituted 0.44±0.05 (p<0.001) and 0.25±0.03 (p<0.001) in deep layers, thus increased 17.85 times, as compared to the control group.

Comparative analysis of caries protective effect of preventive methods applied in L1 and L2 revealed statistically significant differences between the concentration of organic substances and minerals both in the superficial and deep layers of enamel.

Table 1. Distribution of study groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Children</th>
<th>Extraction of enamel</th>
<th>Total</th>
<th>%</th>
<th>Total</th>
<th>%</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1</td>
<td>12</td>
<td>33.33</td>
<td>4</td>
<td>11.11</td>
<td>8</td>
<td>33.33</td>
<td>24</td>
<td>33.33</td>
</tr>
<tr>
<td>L2</td>
<td>12</td>
<td>33.33</td>
<td>4</td>
<td>11.11</td>
<td>8</td>
<td>33.33</td>
<td>24</td>
<td>33.33</td>
</tr>
<tr>
<td>L0</td>
<td>12</td>
<td>33.33</td>
<td>4</td>
<td>11.11</td>
<td>8</td>
<td>33.33</td>
<td>24</td>
<td>33.33</td>
</tr>
<tr>
<td>total</td>
<td>36</td>
<td>100</td>
<td>12</td>
<td>33.33</td>
<td>24</td>
<td>100</td>
<td>72</td>
<td>100</td>
</tr>
</tbody>
</table>

Figure 2. SEM picture of external enamel surface of an intact tooth. Spectrum 1 - the result of spectral analysis in the holes of micro-ducts. Spectrum 2 - the result of the spectral analysis on the surface of hydroxyapatite crystals

Figure 3. SEM picture of external enamel surface of an intact tooth extracted 1 month after the last laser therapy session and simultaneous deep fluoridation. Depositing of micro-globules. Spectrum 1 - the result of spectral analysis in the area of micro-globules
Table 2. The composition of macro and micro-elements in children’s dental enamel as a result of application of exogenous methods for preventing dental caries (atom %, M±SD)

<table>
<thead>
<tr>
<th>The studied area of enamel</th>
<th>The composition of macro and micro-elements in children’s dental enamel (atom %, M±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
</tr>
<tr>
<td>granular sediment *</td>
<td>L₁</td>
</tr>
<tr>
<td></td>
<td>L₂</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
</tr>
<tr>
<td>superficial layer **</td>
<td>L₁</td>
</tr>
<tr>
<td></td>
<td>L₂</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
</tr>
<tr>
<td>deep layer **</td>
<td>L₁</td>
</tr>
<tr>
<td></td>
<td>L₂</td>
</tr>
<tr>
<td></td>
<td>L₃</td>
</tr>
<tr>
<td></td>
<td>P₁</td>
</tr>
<tr>
<td></td>
<td>P₂</td>
</tr>
<tr>
<td></td>
<td>P₃</td>
</tr>
<tr>
<td></td>
<td>P₄</td>
</tr>
</tbody>
</table>

* immediate results within 3,5 month after the last caries preventive session
** results after the last caries preventive session
Thus, as a result of laser therapy along with deep enamel fluoridation the C concentration decreased by 3.53% on enamel surface and by 3.99% in its deeper layers as compared to the topical application of nanofluorides (p₁₂<0.001). The Ca/P relationship in the L₂ exceeds the estimated value of this indicator 1.05 times in group L₁ (p₁₂<0.001). The physicochemical prevention method helped increase the F concentration 1.63 times in the surface layers of enamel and respectively 2.08 times in the deep layers, ensuring a greater caries preventive effect compared to the chemical method for dental caries prevention (p₁₂<0.001). We give particular importance to the growth of F, Mg and Cu concentration in the deeper layers of enamel, which confirms the deposit of CaF₂, MgF₂ and CuF₂ crystals in the deeper layers of enamel.

Discussions

According to the results of Knappwost (1993, 1999) researchs, the concentration of Ca²⁺ and PO₄³⁻ ions in saliva is usually relatively constant and stays at a level that is sufficient to ensure the remineralization process. The concentration of OH-ions can be reduced 1000 times, e.g. to pH=4 in the interdental area. Remineralization-decalcification processes are regulated by OH ions. The insufficiency of these ions can be compensated by intake of fluoride ions, which are structurally analogous (Knappwost 1993; Knappwost et al 1999).

The caries preventive effect of deep fluoridation agents is due to high fluoride concentration at the saliva/enamel interface. In addition, the past decades have shown that this is not the only mechanism with caries protective action of the preparation developed by A. Knappwost. Thus, the studies convincingly demonstrated that the bivalent copper ions are involved in the attachment of hydroxyapatite crystals to the keratin and collagen fibers. The presence of copper ions ensures not only the growth of enamel remineralization effect but also contributes to the destruction of micro-organisms in the biofilm and prevents the colonization of tooth surface with cariogenic microorganisms (Knappwost 1993; Knappwost et al 1999).

The caries protective action of laser irradiation in the range of infrared wavelengths is explained by the quantum theory of chemical bonds through the action of photons on the crystal lattice of the enamel. Thus the photon stream generated by laser irradiation activates the protein matrix of dental hard tissues and contributes to the acceleration of ion exchange and of diffusion in enamel. The research conducted by Mintz and Berg (1995) revealed that irradiation of infrared rays has an activating effect on protein molecular structures, leads to a conformational change in enamel matrix proteins, which enhances the metabolism and diffusion, and contributes to a better penetration of fluorine ions in the enamel. So, as a result of LIR irradiation we get normal homeostasis and the increase of enamel resistance to the action of cariogenic factors.

Upon the interaction of the laser irradiation in low intensity pulsed mode in the range of infrared wavelengths in the tooth enamel a number of complex changes takes place in the structure of the crystal lattice following which these occur: enamel remineralization, condensation of the crystal lattice, and reduction of its permeability. As a result, enamel micro-hardness grows and its solubility decreases, thereby providing high caries-resistance (Kunin et al 2000).

The action of LIR irradiation on tooth pulp is manifested predominantly by the stimulation of the secretory function of odontoblasts and microcirculation, which ultimately induces the enhancement of metabolic processes in dental tissues. The effect of LIR irradiation on oral cavity microflora manifests itself by reduced pathogenicity of cariogenic strains including...
Streptococcus mutans. Consequently, the cariogenic potential of dental biofilm drops. At the same time, specific humoral immune protection mechanisms activate, and stimulation of non-specific defense mechanisms takes place.

LIR irradiation helps normalize saliva secretion (regardless of initial hyper or hypo-salivation), including normalization of pH, of organic and mineral fraction ratio, the micellar structure and normalization of Ca and P concentration ratio, thus ensuring active remineralization of enamel. In addition, under the influence of LIR irradiation, adjustment or increase of immune properties of saliva and oral liquid take place (Fried et al. 2002).

The combined physicochemical method that we propose consists of LIR irradiation and simultaneous application of medication for deep fluoridation of enamel, the use of which triggers the caries preventive effect of both manipulations, creates optimal conditions for penetration of fluoride ions not only at the surface, but also into the enamel depth and their incorporation in the crystal lattice of enamel. The deep fluoridation protects keratin fibers from proteolysis, which manifests itself by significant reduction of enamel solubility and permeability, the increase in enamel micro-hardness; moreover, it causes the reduction of acidogenic microorganisms and of speed of bacterial plaque accumulation, and LIR irradiation contributes to the increase and prolongation of mineralization effect of nanofluorides, thus ensuring a considerable caries protection effect. The application of Gluftored preparation for deep fluoridation of enamel surface and simultaneous LIR irradiation is simple and quick to perform, does not cause discomfort to patients, which allows the use of this method for patients with psycosomatic disabilities. High efficiency of the method proposed and the achievement of the long-lasting caries protective effect in a short time, helps reduce the number of prevention sessions, saving time and financial resources of the practitioner. That method facilitates the penetration of fluoride ions on the surface, but also into the depth of dental enamel, which ensures higher effectiveness of dental caries prevention. Thus, the combined action of IR laser irradiation and application of nanofluorides creates optimal conditions for penetration of fluoride ions in enamel, which enhances the caries protective effect. Therefore, LIR irradiation contributes to the growth and prolongation of mineralization effect of nanofluorides. Therefore, the laser irradiation and simultaneous application of nanofluorides could be an optimal solution to accelerate the metabolic processes in the hard tissues of the tooth, which is particularly important for people with high caries risk.

Conclusions
As a result of using the physicochemical method and deep enamel fluoridation we discovered the depositing of CaF₂, MgF₂, and CuF₂ micro-globules on the surface and deeper layers of enamel, a considerable reduction in carbon concentration, normalization of Ca/P ratio, and significant increase in fluoride content. The physicochemical prevention method helped increase the F concentration 1.63 times in the surface layers of enamel and respectively 2.08 times in the deep layer, ensuring a greater caries preventive effect compared to deep fluoridation of enamel. Laser irradiation of dental enamel under low intensity pulsed regime in the range of infrared wavelengths and the simultaneous application of nanofluorides secures a maximum caries protective effect in children with severe disabilities.

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