

Particularities of the chemical composition of dental enamel in children with neuromotor disabilities and gastro-esophageal reflux disease

¹Aurelia Spinei*, ²Alina Monica Picos*, ¹Ina Romanciuc, ²Andrei Picos, ¹Iurie Spinei

¹ Departement of Maxillo-facial surgery, pedodontics and orthodontics, “Nicolae Testemitanu” State University of Medicine and Pharmacy, Chişinău, Republic of Moldova; ² Departement of Prosthetic Dentistry, “Iuliu Hatieganu” University of Medicine and Pharmacy, Cluj-Napoca, Romania.

*These authors contributed equally to this work

Abstract. Objective: The purpose of the hereby paper consisted in the study of particularities of the chemical composition of dental enamel in children suffering from severe neuromotor disabilities associated with gastro-esophageal reflux disease (GERD). Material and methods. Using the Fourier transform spectroscopy method (FTIR) we have studied the structure and the chemical composition of various areas of the dental enamel, namely 64 pieces of enamel prepared from 32 teeth extracted from children aged between 13 and 17, following orthodontic indications. Results: We established that in the enamel of the children suffering from severe neuromotor disabilities associated with GERD the weight of the organic component of enamel is increased, the content of hydroxyapatite content is reduced, it contains carbon substituted hydroxyapatite with low intensity of peak phosphorus and significant increase of organic components. The detected particularities of the chemical composition of dental enamel indicate the considerable reduction of the enamel resistance to acid attack, thus leading to a high risk of dental erosion or new carious lesions. Conclusions: The FTIR spectroscopy method of dental enamel offers new opportunities in the study of pathogenic mechanisms involved in the initiation and evolution of carious lesions and dental erosions, to make a prediction of these disorders, to elaborate measures for the prevention of tooth decay and evaluate their efficiency.

Key Words: dental enamel, neuromotor disabilities, GERD, FTIR spectroscopy.

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Corresponding Authors: A. M. Picos, email: alinapicos@yahoo.com

Introduction

Infrared (IR) spectroscopy is based on the interaction of electromagnetic radiation in the IR domain, with molecules forming a substance. It lies in measuring the wavelength and the intensity of the infrared light by a sample. If the electromagnetic radiation interacts with molecules, in addition to stimulating electrons, the molecule may also take energy under the form of rotation energy and vibration energy of the molecule (Koutsopoulos 2002; Rey et al 1989; Fan et al 2009). The application of infrared (IR) microscopes operating in the reflecting mode and have a high intensity of synchrotron radiation acting as IR source, allows the analysis of the hard dental tissues state, as well as the content of organic compounds (Rey et al 2007; Bader et al 2001; Simmer et al 2011; Thompson et al 2013; Gutierrez-Salazar et al 2003; Tiznado-Orozco et al 2009).

At this time, there is much interest in the research of the crystalline structure of the biological and synthetic apatite. The interest for this issue is justified by the need to carefully study the micro-morphological structure of the tooth in order to illustrate the etiologic factors, the mechanisms of pathogenesis, the prognosis of dental decay and non-carious disorders, as well as the

evaluation of the structural changes of dental enamel occurred after the application of measure for the prevention of tooth decay. It is well known that the non-organic phase of the dental enamel represents the carbonate-substituted hydroxyapatite (Rey et al 1989; Elliott et al 2002; Fleet, Liu 2005, Fleet 2013). The carbonate ions composing the dental enamel apatite are located in two anionic centers (sites). The carbonate-ion (CI) substitution takes place mostly in the PO_4^{3-} sites (CI type B) and rarely in the OH- sites CI type A (Fleet & Liu 2005). The total amount of carbonate determined in the enamel represents 3% mass, and around 11% of this amount is found in type A sites. Previous researches have shown that in the temporary teeth and decayed teeth, the CI concentration is considerably increased, compared to permanent teeth, which have a different degree of maturity and intact teeth (Fan et al 2009; Simmer et al 2012; Rey et al 1991; Goldberg et al 1995). Consequently, the research of structure particularities of tooth enamel, assessment of the CI amount, has an important role in assessing the characteristics of the mineral phase of the enamel. We also established the fact that the intact enamel presents various texture features, in persons with different level of resistance to acid factors attacks. Thus, when the resistance to decay decreases, the porosity of the

enamel increases. The qualitative analysis of the enamel composition proved that in children with high resistance to carious attacks, the concentration of Ca, P, Cu, Pb, Mg, Mn, Ir, Na, Co, S is higher and the concentration of Fe, Zn, B, K, C, Si, Sr; Pt (Fleet & Liu 2005; Rey *et al* 1991; Peroos *et al* 2006) is lower. The result of a great number of studies indicated an increased prevalence of dental decay, occurrence of multiple surface cavities and dental erosions at children suffering from severe neuromotor disabilities associated with gastro-esophageal reflux disease GERD. Considering the highest degree of dental decay and the continuous increase of dental erosion prevalence, assessed at children suffering from severe neuromotor disabilities and GERD, the study of the dental enamel composition is important for further elaborating new precise clinical methods of prognosis of cavities and dental erosions, and evaluating the efficiency of prevention measures applied at children with this pathology. The purpose of the hereby paper consisted in the study of particularities of the chemical composition of dental enamel at children suffering from severe neuromotor disabilities associated with GERD. If particularities exist, associations with dental pathology may exist in these patients.

Materials and methods

In order to accomplish the objective, the clinical descriptive study was performed on a sample of 100 children, aged between 13 and 17. In the research group we included 50 (50%) children suffering from severe neuromotor disabilities, of them 25 (25%) have been diagnosed with GERD, the duration of the disease representing 1.6 ± 0.8 years. Due to the existing deficiencies, the children included in the study are not capable to manage independently, and even perform the oral cavity hygiene on their own. The control group, consisting of children (50%) without any disabilities, and 25 (25%) of the suffering from GERD around 1.5 ± 0.4 years.

We performed the clinical exam of the patients, assessing the dental caries frequency by estimating the DMFT index. The assessment of the erosive wear was performed using BEWE score on the palatine/lingual sides, vestibular and incisal/occlusal sides, writing 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 summing up the values of all the sextants, based on which we estimated the risk of erosive wear. In addition to this, we assessed the resistance of the enamel to acids, using the enamel resistance test, following the method suggested by Okushko (2011).

Of the total number of 100 children included in the study, according to the indications of the orthodontist, the extraction of the upper premolars was recommended at 16 patients. We studied the structure and the chemical composition of various regions of the dental enamel, looking at 64 pieces of enamel prepared from 32 teeth extracted following orthodontic indications (Table 1). For the research, we selected only the first premolars on the upper arch. The enamel pieces were dried at 110°C . The enamel powder was obtained in amount of 30-40 mg, using a fine drill from the surface, up to 1mm deep in the enamel.

The researches with infrared laser spectroscopy within the limits of $400\text{-}4000\text{ cm}^{-1}$ were performed by applying the Infrared Spectrometer IRAffinity-1 (© Japan). In order to obtain the IR spectra, the enamel powder was pressed as tablet. For standard,

we used naphthalene, as this substance has no reaction on the analyzed pieces, in normal conditions, it does not absorb humidity, and has a narrow band of absorption at the frequency of 780 cm^{-1} , in the adjacent area of the analyzed sector. This allows the minimization of errors when comparing the relative intensity in the experimental spectra. When obtaining the most informative spectral bands we found a compromise between the concentration of apatite and the standard in the tablet (so that the absorption does not exceed 75% - the interval in which the law of Lambert-Ber acts) and obtaining the maximum intensity of the analytical lines and comparison lines. During the experiments, we applied the optimum percentage of the pill's mass for the analyzed piece and for the standard, which represented 2.5% and 0.833% respectively. From each enamel piece we made 3 tablets, measuring 20mm in diameter. Then, using the method of the base line, we calculated the average ratio of the IR laser radiation absorption intensity of the analytical lines in the enamel pieces, which had a different level of resistance to acids, and different intensity of absorption of external standard lines, at a frequency of 780 cm^{-1} . The relative intensity of the CO_3^{2-} ions was determined using the spectral band of 873 cm^{-1} (type B substitution) with a higher absorption than the spectral band of 878 cm^{-1} .

The study was approved by the Ethics Committee for Research of USMF "Nicolae Testemițanu" and "Iuliu Hațieganu"UMF, being conducted according to the ethical requirements, with the written consent of children's parent or legal representatives. The analysis of the data was performed using Microsoft® Excel® 2013 and IBM® SPSS Statistics 22.0 software, using their functions and modules.

Results

At the initial clinical exam of 100 children aged between 13 and 17 years, we found that all children suffering from severe neuromotor disabilities are affected by dental decay, compared to the frequency of dental decay, which represents 56% at healthy children and 84% at children suffering from GERD. The caries experience index (DMFT) is 2.96 ± 0.33 at children suffering from severe neuromotor disabilities and 3.52 ± 0.12 at children suffering from severe neuromotor disabilities associated with GERD. At children that are conventionally healthy (group L_{2a}) we found a decrease of the DMFT index, of 2.47 times, compared to children suffering from severe neuromotor disabilities (group L_{1a}), while at children in L_{2b} group, this indicator is 1.22 times lower compared to L_{1b} group.

Dental erosions were found at 56% of the children suffering from GERD and 64% of the total number suffering from neuromotor disabilities associated with GERD, the incidence of the erosive wear being 5.16 ± 0.12 at children in group L_{1b} and 3.4 ± 0.15 in group L_{2b} . The average risk of erosive wear was found at 4 (16%) children with GERD included in the research group and 4 (16%) children suffering from GERD from the control group, low risk - at 12 (48%) children suffering from neuromotor disabilities associated with GERD and 7 (28%) children suffering from GERD of the control sample, respectively (Table 2). We assessed the resistance of dental enamel to acids by determining the index through the enamel resistance test, elaborated by V. Okushko. Thus, the highest resistance to acids of the tooth enamel and the minimum value of the index ($31.16 \pm 2.39\%$) was

Table 1. Distribution of the study groups

| Group | Number of children | | | | no. of extracted teeth | | no. of enamel pieces studied | |
|-----------------------------------------------------------------------|--------------------|-----|-------------------------|-----|------------------------|-----|------------------------------|-----|
| | total | | Extraction of premolars | | abs. | % | abs. | % |
| | abs. | % | abs. | % | | | | |
| L _{1a} Children with severe neuromotor disabilities | 25 | 25 | 4 | 25 | 8 | 25 | 16 | 25 |
| L _{1b} Children with severe neuromotor disabilities and GERD | 25 | 25 | 4 | 25 | 8 | 25 | 16 | 25 |
| L _{2a} Healthy children | 25 | 25 | 4 | 25 | 8 | 25 | 16 | 25 |
| L _{2b} Children with GERD | 25 | 25 | 4 | 25 | 8 | 25 | 16 | 25 |
| Total | 100 | 100 | 16 | 100 | 32 | 100 | 64 | 100 |

Table 2. Affection by tooth decay and erosive wear at children

| Groups | L ₁ children with neuromotor disabilities | | | | L ₂ children without disabilities | | | |
|-------------------------------------------------|------------------------------------------------------|-----------|--------------------|-----------|----------------------------------------------|----------|--------------------|-----------|
| | GERD | | | | GERD | | | |
| | L _{1a=25} | | L _{1b=25} | | L _{2a=25} | | L _{2b=25} | |
| Indicator | % | M±SD | % | M±SD | % | M±SD | % | M±SD |
| DMFT | 100 | 2.96±0.33 | 100 | 3.52±0.12 | 56 | 1.2±0.21 | 84 | 2.88± |
| BEWE | 8 | 0.12±0.02 | 64 | 5.16±0.12 | 0 | 0 | 56 | 3.4±0.15 |
| BEWE+DMFT | 8 | - | 64 | - | 0 | - | 4 | - |
| Risk of erosive wear (final BEWE score): | | | | | | | | |
| No risk < 2 | 100 | 0.12±0.01 | 36 | 0.22±0.03 | 0 | 0 | 56 | 0.14±0.01 |
| Low risk (3-8) | 0 | 0 | 48 | 6.67±0.24 | 0 | 0 | 28 | 5.4±0.22 |
| Average risk (9-13) | 0 | 0 | 16 | 12.25±1.4 | 0 | 0 | 16 | 9.25±1.1 |
| High risk >14 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

found at healthy children. At children with severe neuromotor disabilities the enamel resistance index represents 69.27±2.81%, at children suffering from GERD, 53.38±3.14% respectively, while the lowest value of enamel resistance to acids was found at children suffering from severe neuromotor disabilities associated with GERD, the enamel resistance index was 84.22 ± 2.18% and indicating the increased risk of demineralization of dental hard tissues.

In the next stage of the study, after the extraction of 32 upper premolars, according to the indications of the orthodontist, we applied spectroscopy through vibration with Fourier transform, to describe and identify the mineral and organic elements of 64 pieces of enamel. We analyzed 128 absorption spectra. The interpretation of the spectral bands was performed according to the data of Tarnowski et al (2002) and Ager et al (2006), being presented in table 3.

Most of the spectra used are similar in terms of absorption peak form and position. Figures 1-4 show the typical absorption spectra of the enamel pieces at healthy children, suffering from GERD, the severe neuromotor disabilities and the association between neuromotor disabilities and GERD.

Based on the evaluations made on groups L_{1a}, L_{1b} and L_{2b} we determined the position of bands ν₁ of the phosphate ion in the area 959.014 – 960.049 cm⁻¹ (Table 3), which allows us to state that the mineral phase of all studied samples represent carbonate-apatite type B. The relative concentration of the phosphate ion is lower in the enamel of children suffering from severe

neuromotor disabilities, representing 956.014±2.4 and at patients suffering from severe neuromotor disabilities associated with GERD, 945.012±1.3 respectively. This indicates the reduction of the hydroxyapatite phosphate network's regularity and the irregular nature of the phosphate network, which could be induced by the type A ionic substitutions (carbonate ions substitute hydroxyl ions) or the presence of phosphate in the amorphous calcium. Only at healthy children (L_{2a}) we detected the ν₁ band, in the area 962-964 cm⁻¹, which points out the presence of highly crystallized hydroxyapatite.

The IR spectra of the tooth enamel allow determining the oscillations spectra located in the area 1020,6 cm⁻¹ – the most intense absorption band, associated with valent oscillations ν₃ (PO₄³⁻) of the phosphate ion, as well as the stretching vibrations ν₄ (PO₄³⁻) located within the limits of 597.6 cm⁻¹ and 559.8 cm⁻¹, corresponding to the inorganic compound. The IR absorption spectra obtained from the enamel areas of children from groups L_{1a}, L_{1b} and L_{2b} are significantly different from the IR absorption spectra of the enamel areas of healthy children. Thus, the intensity of the peaks corresponding to the inorganic compound is reduced, while the peaks corresponding to organic elements of the tooth enamel or have an increased intensity.

The width of the PO₄ peak bands indicates the degree of crystallizations of hydroxyapatite. Thus the intact enamel at healthy children, this indicator represents 17.61, which confirms the high degree of crystallization of hydroxyapatite. In the enamel of teeth extracted from children suffering from GERD, this

Table. 3. Identification of IR spectra

| Wave no. (cm ⁻¹) | Fragment, vibration |
|---------------------------------|---------------------------------------------------------------------------------------------------|
| 430 | PO ₄ ³⁻ ν ₄ (P-O deformation vibration) |
| 580 | PO ₄ ³⁻ ν ₄ (P-O deformation vibration) |
| 855 | Proline benzene ring |
| 876 | Hydroxyproline benzene ring |
| 950-964 | PO ₄ ³⁻ ν ₁ (P-O valence symmetric vibration) |
| 1001-1003 | Phenylalanine benzene ring trend |
| 1030 | PO ₄ ³⁻ ν ₃ (P-O valence symmetric vibration) |
| 1045 | PO ₄ ³⁻ ν ₃ (P-O valence symmetric vibration) |
| 1065-1070 | CO ₃ ²⁻ ν ₁ type B substitution (C-O vibration in valence plain) |
| 1245-1270 | Amide III, C-N-H valence vibration |
| 1445 | CH ₂ (deformation vibration) |
| 1555-1565 | Amide I, C-C-H valence vibration |
| 2880-2935, 3070 | Vibration C-H |
| 1610-1620 | Y8a (vibration of tyrosine lateral chain) |
| 1665 | Amide I, C-C-H valence vibration |
| 2880-2935, 3070 | Vibration C-H |
| 3350 | Vibration C-N |

indicator increases by 0.22, at children suffering from severe neuromotor disabilities - by 0.812, and in the enamel of children suffering from severe neuromotor disabilities associated with GERD – increases by 1.036, which indicates the essential reduction of the degree of crystallization of hydroxyapatite, probably produced by the substitution of the phosphate ion by the carbonate ion.

In the result of the researches conducted using the FTIR spectroscopy, by applying the method of external standard and the baseline method, we assessed the absorption intensity ν_2 of CO₃²⁻ ions oscillation of dental enamel with different degree of acid-resistance at the frequency of 873 cm⁻¹ (ab dimension), adapted to the relative absorption intensity of the spectral band of the standard (naphthalene) at the frequency of 780 cm⁻¹ (cd dimension). Each indicator was obtained by assessing the average values of the results obtained from data of 3 spectral series analyzed simultaneously.

We calculated the percentage of CO₃²⁻ type B ions of dental enamel with low level of resistance to acids, compared to the content of CO₃²⁻ ions located in the type B area in the enamel with high acid-resistance level (Table 5). Thus, the concentration of CO₃²⁻ type B ions in the dental enamel at children suffering from severe neuromotor disabilities associated with GERD represents 20% compared to the content of these ions in the dental enamel of healthy children. Using the data obtained by Michel et al, 1995, we assessed the absolute concentration of CO₃²⁻ ions located in the type B area. Thus, we found that in the enamel

with high level of resistance to acids, this indicator represents 2.67%, and in case of low resistance - 3.2%.

The essential differences of the chemical composition of enamel pieces prepared from the teeth extracted from persons with different acid-resistance level are found in the areas specific for the carbonate ion. In the IR spectra of the dental enamel in groups L_{1a}, L_{1b} and L_{2b} we determined specific bands for CO₃²⁻ ions, which represent the interval 865-885 cm⁻¹. At healthy children (group L_{2a}) the intensity of CI bands is lower, therefore, the concentration of the CO₃²⁻ ions is lower in the enamel pieces and considerably higher in the quantity of the mineral phase.

The analysis of dental enamel using the IR spectroscopy in the hereby study allowed us to establish the particularities of the chemical composition of dental enamel in children suffering from GERD, severe neuromotor disabilities, and association of severe neuromotor disabilities with GERD. On the IR spectra in groups L_{1a}, L_{1b} and L_{2b} we detected bands specific to organic elements. Thus, the emergence of a band in area 1300-1280 cm⁻¹ was explained by the presence of the organic group (CH₂). This leads to the increase of the weight of organic element in the enamel, in relation with the mineral element and respectively, the decrease of the hydroxyapatite content, and eventually – the considerable decrease of enamel resistance to acids. The conducted research has explained the increase of peak absorption intensity in groups L_{1a}, L_{1b} of amides I - ν (C = O) and II - δ (NH), the significant increase of peak absorption intensity of amide III - δ (NH), ν (CN) due to association of primary vibration bands of covalent links of group C-N and secondary vibrations deformation bands of group N-H. In the tooth enamel of healthy children (group L_{2a}) the absorption peaks of amide III is insignificant. Moreover, the intensity of absorption maximums is increased, corresponding to the vibration modes of organic substance ν (CH₂), ν (C = O), δ (CH₂), ν₃ (CO₃²⁻) and water δ (OH).

Because the absorption bands of amide I, amide II and amide III are interdependent on the presence of organic chemical compounds in the tooth enamel, particularly amino acids, the values of the absorption bands peak surface or the integral intensity may reflect the concentrations of these substances. The values of integral intensities of the absorption band peaks for the enamel of healthy children represent: ν₃ (PO₄³⁻) – 18.4, for amide I – 0.026, for amide II – 0.041, for amide III – 0.004. For the enamel of children suffering from GERD, severe neuromotor disabilities and association of severe neuromotor disabilities with GERD, the values of integral intensities represent: ν₃ (PO₄³⁻) – 17.2, for amide I – 0.7, for amide II – 0.1 and for amide III – 0.1. The data obtained clearly prove a reduction of the integral intensity band of ν₃ (PO₄³⁻) phosphate anion, which is specific for the inorganic component of the tooth enamel and the increase of the integral intensity of amides when the tooth enamel demineralization processes are predominant.

The CO₃/PO₄ relation is increased by 0.02 in children suffering from GERD, in children suffering from severe neuromotor disabilities – it increases by 0.075, and in children suffering from severe neuromotor disabilities associated with GERD – it increases by 0.101 compared to the value of this indicator assessed in healthy children. These results suggest a decrease of crystallization caused by the substitution of the phosphate ion with the carbonate ion.

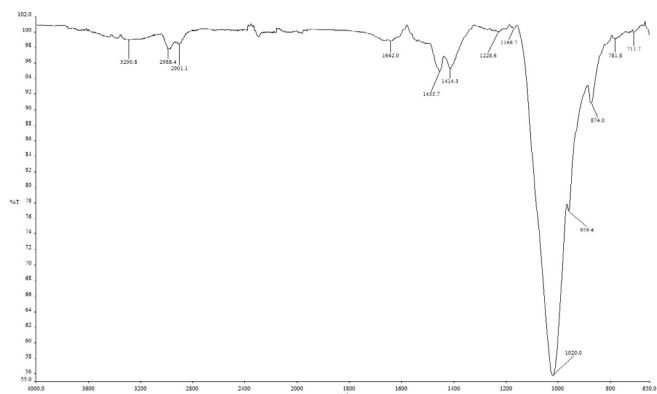


Figure 1. The IR spectrum of the enamel sample of an intact tooth extracted from a healthy child

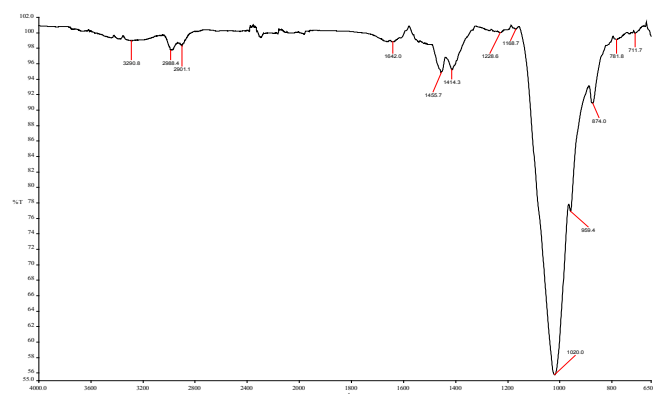


Figure 2. The IR spectrum of the enamel sample of an intact tooth extracted from a child suffering from GERD

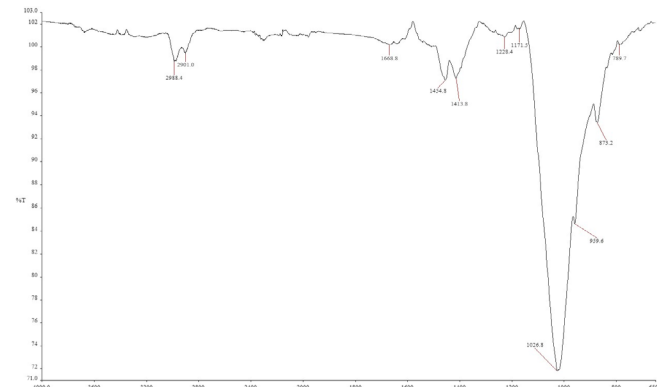


Figure 3. The IR spectrum of the enamel sample of an intact tooth extracted from a child suffering from severe neuromotor disabilities

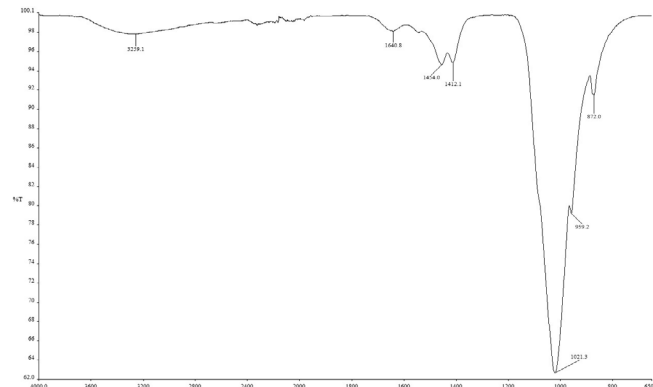


Figure 4. The IR spectrum of the enamel sample of an intact tooth extracted from a child suffering from severe neuromotor disabilities associated with GERD

Table 4. Parameters of the IR spectra

| Analyzed fragment | Children with disabilities | | Children without disabilities | |
|-----------------------------------------------------------------------------------|--------------------------------|-----------------------------------------|-------------------------------|------------------------|
| | Severe neuromotor disabilities | Severe neuromotor disabilities and GERD | Conventionally healthy | GERD |
| | L _{1a} (n=16) | L _{1b} (n=16) | L _{2a} (n=16) | L _{2b} (n=16) |
| Bands position PO₄³⁻ n₁ (cm⁻¹) | 956.014±2.4 | 945.014±1.3 | 960.049±2.6 | 959.049±2.2 |
| Bands width PO₄³⁻ n₁ at ½ height | 18.422±0.12 | 18.648±0.14 | 17.61±0.11 | 17.83±0.13 |
| CO₃/PO₄ Relation | 0.301±0.01 | 0.327±0.1 | 0.226±0.02 | 0.246±0.01 |
| Mineral/ organic matrix relation | 3.293±0.1 | 3.07±0.2 | 7.118±0.3 | 6.968±0.2 |
| Absorption peak of amide I | 0.68±0.003 | 0.7±0.01 | 0.026±0.001 | 0.04±0.002 |
| Absorption peak of amide II | 0.09±0.001 | 0.1±0.002 | 0.041±0.03 | 0.051±0.002 |
| Absorption peak of amide III | 0.09±0.002 | 0.1±0.001 | 0.046±0.02 | 0.059±0.003 |

The mineral/organic matrix relation is reduced by 0.15 in the tooth enamel in children suffering from GERD, by 3.825 in children suffering from severe neuromotor disabilities and by 4.048 in the enamel of children suffering from severe neuromotor disabilities associated with GERD. The significant reduction of the mineral/organic matrix is specific for the initiation of demineralization processes. The low content of mineral substances found in the enamel of apparently intact teeth, extracted from children suffering from severe neuromotor disabilities could be

the consequence of a disturbance of the demineralization process of hard dental tissues following a disorder in the mineral metabolism. In children suffering from severe neuromotor disabilities and GERD association, the demineralization processes are predominant, being caused by troubles of the demineralization process, as well as by the considerable reduction of the saliva pH, which confers a higher risk for the formation of carious lesions, as well as for dental erosions.

Table 5. Results obtained on the intensity of ν_2 absorption of CO_3^{2-} ions oscillation of dental enamel in children at the frequency of 873 cm^{-1} (ab dimension), adapted to the relative absorption intensity of the spectral band of the standard (naphthalene) at the frequency of 780 cm^{-1} (cd dimension)

| No. of experiment | Dental enamel in healthy children | Dental enamel in children suffering from severe neuromotor disabilities associated with GERD | Relation between dental enamel in healthy children / dental enamel in children suffering from severe neuromotor disabilities % |
|-------------------|-----------------------------------|----------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| | ab/cd | ab/cd | |
| 1 | 0.62 | 0.71 | 114.5 |
| 2 | 0.46 | 0.59 | 128 |
| 3 | 0.4 | 0.47 | 117.5 |

Discussions

The method of infrared spectroscopy allows studying the particularities of the molecular structure of organic and mineral compounds of the hard dental tissues. The interpretation of an IR spectrum implies a correlation of the absorption bands of the unknown compound spectrum with the known absorption for each type of chemical link. The peak intensity (weak, medium or intense), the form of the peak (wide or narrow) and its position (cm^{-1}) in the spectrum are very important in the interpretation of the spectra.

Area $920\text{-}980\text{ cm}^{-1}$. The band of symmetric valence vibration PO_4^{3-} (ν_1) is the most intense one in the spectrum of a mineralized tissue. The high sensibility of the environment with high mineralization degree is specific for this area: the frequency and the form of the band depend on the local environment and vary in the result of the anionic groups' substitution and the modifications of crystallinity degree. In a series of studies on the composition of natural and synthetic apatites, the frequency of band PO_4^{3-} (ν_1) is associated with the composition of adjacent areas of the mineral (Kunin et al 2000; Tarnowski et al 2002). In the FTIR spectroscopy of mineralized tissues, the surrounding areas of the apatite are divided into three groups with different frequencies of the corresponding bands. In the substituted carbonate apatite type B (carbonate ions substitute the phosphate ions in the apatite network) the phosphate band ν_1 is determined in the interval $955\text{-}959\text{ cm}^{-1}$. In the non-substituted high crystallized hydroxyapatite, band ν_1 moves to the area $962\text{-}964\text{ cm}^{-1}$. Eventually, a band of $945\text{-}950\text{ cm}^{-1}$ frequency indicates the presence of disordered phosphate network in the apatite. The causes of the modification of the apatite phosphate network have not been explained yet, but in 2002 researchers Tarnowski et al believed that this disorder is produced by the type A ion substitutions (carbonate ions substitute hydroxyl ions) or by the presence of amorphous calcium phosphate and used the term "disordered phosphate" (2002). Generally, in the bone and dental tissue, the phosphate band ν_1 represents an overlay of the three components, usually having an asymmetric form, due to the contribution of disordered phosphate vibrations and non-substituted hydroxyapatite. To evaluate the apatite degree of crystallization, the band ν_1 is assessed at $\frac{1}{2}$ of its height (Goldberg et al 1995).

Area $1065\text{-}1070\text{ cm}^{-1}$ corresponds to the carbonate ion vibration type B. In order to evaluate the phosphate/carbonate relation, the ratio between the absorption peaks intensity corresponding to the spectrum is frequently used: 959 cm^{-1} for PO_4^{3-} and 1070 cm^{-1} for a CO_3^{2-} , adding the observation that the ratio between the peak amplitude and its surface can be estimated.

Area $1400\text{-}1800\text{ cm}^{-1}$. The vibrations of the organic matrix in this area are produced by two types of links: the vibrations of -CO-NH- link, which forms the protein matrix and the vibrations associated with the lateral catenae of the amino acids. On the IR spectra, the following are clearly emphasized: the absorption peaks of the protein links, of the amidic groups -CONH₂ which have 9 vibration modes, and in the spectrum the following bands are recorded: amide I is located in area $1655\text{-}1675\text{ cm}^{-1}$ of the spectrum and represents the valence vibration C = O, amide II (1560 cm^{-1} deformed vibration N-H, valence vibration C-N); amide III ($1240\text{-}1260\text{ cm}^{-1}$ valence vibration C-N, deformed vibration N-H) (Uthgenannt et al 2007). The low intensity shoulder of the absorption peak at 1620 cm^{-1} is correlated with the vibration of the lateral catena of tyrosine Y8a. The 1450 cm^{-1} peak corresponds to the torsion deformation vibration of CH₂ in collagen (two atoms of hydrogen linked covalently to the same carbon atom are symmetrically moving). In multiple publications in the field, the vibration peaks of amide I, located in area 1665 cm^{-1} of the spectrum are used to assess the relative content of the organic matrix and to calculate the ratio between the mineral and the organic matrix (Goldberg et al 1995; Uthgenannt et al 2007).

Area $2750\text{-}3350\text{ cm}^{-1}$. The peaks in this area, are usually correlated with the vibrations of the C-H links ($2880\text{-}2935$ and 3070 cm^{-1}) C-N links (3320 and 3435 cm^{-1}) in collagen (Draper et al 2005). The 2940 cm^{-1} peak is used to assess the mineral substances/collagen relation, compared to the surface of the peaks $960\text{ cm}^{-1}/2940\text{ cm}^{-1}$.

The results of our research are comparable to the data obtained by several authors in the studies of mineral and organic components of tooth enamel (Koutsopoulos 2002; Fan et al 2009; Rey et al 2007; Bader et Shugars 2001; Simmer et al 2011; Gutierrez-Salazar et al 2003; Tiznado-Orozco et al 2009; Fleet 2013; Rey et al 1991; Goldberg et al 1995; Kunin et al 2000; Tarnowski et al 2002; Uthgenannt et al 2007; Draper et al 2005; Ager et al 2006; Bansal et al 2010; Lemor et al 2000; Rey et al 1990; Lebon et al 2010; Thompson et al 2009; Rey et al 2013).

Thus, following the analysis of 128 absorption spectra of 64 pieces of enamel, using the result of the hereby study, we established the particularities of the chemical composition, at molecular level, of the apparently intact dental enamel in healthy children, children suffering from severe neuromotor disabilities, GERD and severe neuromotor disabilities associated with GERD:

- The highest degree of mineralization of tooth enamel was found in the enamel samples taken from healthy children, having no carious lesions, confirmed by the high level of crystallization of hydroxyapatite and the minimum number substitutions of phosphate ions by carbonate ions.

- The concentration of CO₃²⁻ type B ions in the dental enamel in children suffering from severe neuromotor disabilities associated with GERD is 20%, compared to the content of these ions in the tooth enamel of healthy children.
- In children suffering from severe neuromotor disabilities associated with GERD, the weight of the organic component of enamel is increased in relation with the mineral component, while the content of hydroxyapatite is decreased, and, eventually, the resistance of enamel to acids is considerably reduced.
- The enamel samples having low acid-resistance, collected from children suffering from severe neuromotor disabilities associated with GERD, contain carbonate-substituted hydroxyapatite, with low intensity of phosphate peaks and a significant increase of organic components.

Conclusions

In the result of FTIR spectroscopy of tooth enamel in children suffering from severe neuromotor disabilities associated with GERD we established the structural particularities of the enamel, at molecular level. Thus, the mineralization disorders in the period of tooth formation and in the post-eruptive mineralization period, associated with the frequent and long-term reduction of saliva pH, lead to the considerable decrease of enamel resistance to acids, thus representing a high risk of dental erosions or new carious lesions.

The FTIR spectroscopy method applied on the tooth enamel offers new opportunities in the study of pathogenic mechanisms involved in the initiation and evolution of carious lesions and dental erosions, to perform the prediction of these disorders, in order to elaborate prevention measures and evaluate their efficiency.

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Authors

•Aurelia Spinei, Departement of Maxillo-facial surgery, pedodontics and orthodontics, “Nicolae Testemitanu” State University of Medicine and Pharmacy, 165 Stefan cel Mare si Sfânt Bd., MD-2004, Chişinău, Republic of Moldova, email: aurelia.spinei@usmf.md

•Alina M. Picos, Departement of Prosthetic Dentistry, “Iuliu Hatieganu” University of Medicine and Pharmacy, 32 Clinicilor

Street, 400006, Cluj-Napoca, Cluj, Romania, EU, email: alinapicos@yahoo.com

•Ina Romanciuc, Departement of Maxillo-facial surgery, pedodontics and orthodontics, “Nicolae Testemitanu” State University of Medicine and Pharmacy, 165 Stefan cel Mare si Sfânt Bd., MD-2004, Chişinău, Republic of Moldova,

•Andrei Picos, Departement of Prosthetic Dentistry, “Iuliu Hatieganu” University of Medicine and Pharmacy, 32 Clinicilor Street, 400006, Cluj-Napoca, Cluj, Romania, EU, email: and-picos@yahoo.com

•Iurie Spinei, Departement of Maxillo-facial surgery, pedodontics and orthodontics, “Nicolae Testemitanu” State University of Medicine and Pharmacy, 165 Stefan cel Mare si Sfânt Bd., MD-2004, Chişinău, Republic of Moldova, email: iurie.spinei@usmf.md

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