Study of Mineral Composition and Morphology of Hard Tissues of Teeth in Patients with Thyroid Gland Pathology in Russia, Belgorod Region

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Abstract: Discovering the dependence of changes in the hard tissues of intact teeth in patients with thyroid disorders. The changes in macro-and trace elements composition of dental hard tissues in healthy subjects and patients with Thyroid Gland Pathology (TGP) were uncovered using scanning electron microscopy (FE Quanta 200 3D, the Netherlands). Reduction of the Ca/P ratio in the hard teeth tissues of patients with TGP compared to the control group. Teeth enamel of the control group contains 4% more calcium than the primary group. The changes in morphology structure of hard teeth tissues were studied in patients with TGP compare to healthy patients. The diameter of the enamel prisms and dentinal tubules in teeth of patients with TGP is different than in the control group. Cement characterized by spongy structure in patients with TGP.

Key words: Thyroid gland pathology, hypothyroidism, teeth, enamel, micro-elements, macro-elements, scanning microscope

INTRODUCTION

Endocrine pathology has a high prevalence in the community. According to World Health Organization, 34% of the world population suffer from the deficiency of iodine (De Benoist et al., 2008). According to the American Association of Thyroid Problems (ATA) about 20 million Americans have thyroid disease. Lack of iodine and as a result the goiter (25-80%) is found in Western and Eastern Siberia (Tyumen Region, Bashkortostan, Tatarstan, Krasnoyarsk Territory, the Republic of Tuva, Yakutia, Buryatia).

The possibility of developing thyroid diseases is 5-8 times higher in women than in men. In 2010, the US had identified about 45,000 cases of thyroid cancer, nearly 2000 patients died of thyroid cancer. As of 2008, over 450,000 patients is living with the cancer. According to data in the research analysis (Peshkova and Pavlova, 2011), the prevalence of hyperthyroidism in the Russian Federation was 18.4 per 100,000.

The absence or lack of iodine disturbs the synthesis of thyroid hormones, resulting in developing of hypothyroidism (Young, 1989). Hypothyroidism in Russia occurs in about 19 out of 1,000 women and one out of 1,000 men. Epidemiological studies revealed prevalence of the disease in selected population groups reaches 10-12%.

Thyroid gland is an important regulator of metabolism and it affects all body functions. Problem of the relationship of TGP and oral-status relatively recently studied (Brook and Brown, 2008; Cerchiai et al., 2006; Chi et al., 2010, Gorodetskaia and Korenevskia, 2010, Pavlova et al., 2014) some of general changes in the oral cavity with hypothyroidism such as macroglossia, taste disturbance, delayed eruption of primary and permanent teeth, delayed wound healing, enamel hypoplasia (Chandna and Bathla, 2011; Ionescu et al., 2004). People with endemic goiter observed poor condition of the gums and more severe periodontitis.

The experiments on rats affected with hypothyroidism under stress has revealed that gum recession and atrophy of alveolar bone, tooth mobility (Pavlova et al., 2004).

In contrast to hypothyroidism, symptoms of an overactive thyroid gland less pronounced and more specific. The following symptoms were detected in hyperthyroidism: increase the development of dental caries, periodontal disease, increasing thyroid tissue which takes place mainly in the rear side surface of the tongue, accelerated eruption of teething, maxillary and/or mandibular osteoporosis (Skalny, Burket, American Thyroid Association). Hyperthyroidism can lead to change in macro-and trace elements composition of the dental hard tissues. The hyperthyroidism causes demineralization of the bone tissue, leading to reduction of calcium and phosphorus levels in the skeleton structure which includes tooth enamel (Pavlova et al., 2014).

The goal of the research is studying changes of hard tissues of teeth because of thyroid gland pathology; determining changes in the structure of hard intact and carious teeth tissues with modern diagnostical methods.

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MATERIALS AND METHODS

The materials for the study were 32 samples of intact teeth from people who live in Belgorod region, Russia. They formed two groups: control (healthy patients without thyroid gland pathology) and primary (patients with thyroid gland pathology). The samples were washed with saline to remove blood residues and soft tissues. Samples were cut by longitudinal or transverse cutting of teeth. The surface of the samples was washed with gel-etching for enamel and dentin. Samples were monitored via a scanning electron microscope with X-Ray analysis and a function of non-contact detection of macro-and trace elements (©FEI Quanta 200 3D® and ©FEI Quanta 600 FEG®).

X-ray analysis provides the estimation of micro volumes of material. Special electron-optical system forms a thin electron probe, bombarding a small (about 1-2 μ) section of the test area. Microspectral composition of exact area is analyzed using a spectrograph with a bent crystal. Electron probe inspects a given surface area of the analyzed sample and the monitor displays a magnified image and distribution of chemical elements. The absolute sensitivity is 10^{-13}-10^{-15} g. The error in elemental analysis is 0.2-0.25% (concentration). We studied the macro-and trace elements of hard teeth tissues: carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, sulfur, potassium, calcium, fluoride, silicon, chloride.

In order to study of the samples, scanning probe (atomic force) microscopy was used. After viewing the targeted areas, the photos were reviewed and morphometric analysis was conducted. The study was performed in the modes of continuous or intermittent contacts on the device ©Negra-Aura® (NT-MDT, Zelenograd, Russia) using commercial Si-or SiN-cantilever (NSG01, NT-MDT, Russia) in the atmosphere and low vacuum. The study of the surface of fresh cleavage as substrates were used mice (muscovite) or highly oriented pyrolytic graphite (pyrographite).

Processing and construction of the AFM images was performed using the software <NOVA® (NT-MDT, Russia) and <ImageAnalysis® (NT-MDT, Russia).

RESULTS AND DISCUSSION

The enamel surface topographical analysis were examined with scanning probe microscopy and revealed that the samples with visually not altered enamel on the teeth with caries process have changes of microlayer. The control group has a fairly smooth surface contour, the pores are present as a place of partial opening of enamel prisms (size 0.5±0.18-2.5±0.25 μ). In the presence of a pathological process, the pores’ size increased to 1.2±0.2-2.8±0.31 μ. At the same time in a unit volume 5x5 M detected the existence of several open pores which is unusual for the control group. The presence of calcium, phosphorus, oxygen, potassium, sodium, magnesium, fluoride and nitrogen in certain proportions in hard tissues intact and carious teeth was found while studying the microelement composition (Table 1). Teeth enamel of the control group (42.2±1.57%) contains 4% more calcium than the primary group (38.15±2.16%). The amount of calcium in the enamel with caries decreased 4 times (10.52±2.14%) in the dentin, 7 times (5.7±2.38%). Normal Ca/P ratio is 1.67-2 when the crystal Hydroxyapatite (HAP) is able to resist degradation. The study found that in patients with TGP in the hard tissues of the tooth affected by caries process, it is reduced to 1.1 (Table 2).

This ratio indicates the status of the enamel and resistance to cariogenic factors. When it falls to the threshold values (below 1.33), the destruction of hydroxyapatite crystals starts which is indicative of the transition physiological demineralization in the pathological process. Teeth of patients with TGP contain 18.88% phosphorus that is less than teeth of patients without TGP (20.01±0.27%). There has been a significant oxygen increase in the content of 3.64% in the teeth of

<p>| Table 1: Mineral composition of enamel and dentin of intact and caries affected teeth in healthy patients |
|-------------------------------------------------|----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Elements</th>
<th>Intact teeth</th>
<th>Dentin</th>
<th>Enamel</th>
<th>Dentin</th>
<th>Enamel</th>
<th>Dentin</th>
<th>Enamel</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>35.7±1.12*</td>
<td>37.65±2.15*</td>
<td>68.1±2.5*</td>
<td>36.85±1.23*</td>
<td>45.2±2.67*</td>
<td>43.7±1.46*</td>
<td></td>
</tr>
<tr>
<td>Na</td>
<td>0.58±0.16</td>
<td>0.76±0.15</td>
<td>0.83±0.22</td>
<td>1.17±0.12</td>
<td>0.34±0.19</td>
<td>0.66±0.2</td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>0.02±0.003</td>
<td>0.028±0.003</td>
<td>0.015±0.004</td>
<td>0.017±0.003</td>
<td>0.019±0.005</td>
<td>0.021±0.002</td>
<td></td>
</tr>
<tr>
<td>Mg</td>
<td>0.11±0.05*</td>
<td>0.26±0.008</td>
<td>1.968±0.75*</td>
<td>0.57±0.08*</td>
<td>0.215±0.05*</td>
<td>1.33±0.05*</td>
<td></td>
</tr>
<tr>
<td>Si</td>
<td>5.05±0.24</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>20.01±0.27*</td>
<td>19.83±0.34*</td>
<td>9.67±1.4*</td>
<td>19.85±0.73*</td>
<td>4.61±0.8*</td>
<td>17.70±1.0*</td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cl</td>
<td>0.85±0.18*</td>
<td>0.71±0.19*</td>
<td>0.18±0.07*</td>
<td>0.55±0.06*</td>
<td>0.00*</td>
<td>0.21±0.23*</td>
<td></td>
</tr>
<tr>
<td>K</td>
<td>0.45±0.05</td>
<td>0.38±0.15</td>
<td>0.56±0.11</td>
<td>0.46±0.08</td>
<td>0.22±0.17</td>
<td>0.41±0.13</td>
<td></td>
</tr>
<tr>
<td>Ca</td>
<td>42.2±1.57*</td>
<td>40.37±1.11*</td>
<td>10.52±2.14*</td>
<td>40.53±1.14*</td>
<td>5.7±2.38*</td>
<td>34.95±2.81*</td>
<td></td>
</tr>
</tbody>
</table>
patients with thyroid disorders (39.84±1.26%) compared to the teeth of patients without TGP (35.76±1.12%). Analysis of the results constitutes a reduction of the Ca/P ratio in the hard tissues of patients with TGP compared to the control group. In dentin demineralization process is more pronounced: the dentin in control group: 40.37±1.11% not visually affected dentin: 34.95±2.84%. The process may be associated with the fact that the amount of calcium in the dentin is less than enamel, so, expressed in the demineralization of dentin greater extent. Caries process significantly increases the content of Oxygen (O) in the enamel twice and in dentin 1.2 times, indicating that the intensity of metabolic processes in specific tissues of the tooth. The ratio of calcium to oxygen is in inverse relation to each other, showing the presence or absence of compensatory changes.

The presence of Silicon (Si) in the enamel in the caries area is 5.05±2.4% and in the dentin is a sharp increase to 41.95±3.36%. According to Skalny (2003), high silicon content indicates a disturbance of water-salt metabolism (Volzke et al., 2005). According to Liflyandsky (2004), silicon promotes the synthesis of collagen and participates in bone morphogenesis. Our assumption is that a sharp increase in the silicon content in the area of the caries process can be associated with life-reactivity cariogenic bacteria in the secondary dentin in response to irritation. There is a lack of Sulfur (S) in hard tissues of healthy teeth while in the enamel and the dentin of teeth with caries process identified content item equal to 3.14±1.23 and 1.57±0.19%. Analysis of the results in the study of macro-and trace elements composition in the dentin found statistical differences between the patients with thyroid disorders and control group. The calcium level in dentine was significantly lower by 7.54% in the primary group than the control (40.37±1.11%). There is statistically significant reduction at 3.14% of phosphorus in tested group compared to the control group (19.83±0.34%). There is a higher oxygen content compared to the control group at 9.45% (37.65±2.15%). There is no presence of silicon in dentin of healthy patients while in the teeth of patients with TGP its content is 0.74±0.28% (0.74). The increased silicon content indicates a lesion of water-salt metabolism. Silicon promotes the synthesis of collagen and participates in bone morphogenesis. Significant differences noted in the study of sodium: enamel of the primary group contains amount of sodium (1.09±0.21%) two times higher than in teeth of the control group (0.58±0.16%). The chlorine content in the teeth of patients with thyroid disorders (0.25±0.11%) is 3.4 times less than in teeth of healthy patients (0.85±0.18%). A slight presence of sulfur (0.06±0.03%) was found in the enamel of teeth of primary group and that mineral was absent in teeth of control. Also, enamel of control does not have silicon but the primary group contains 1.02±0.33% of that mineral. The effects of changes in the percentage of sulfur in the hard tissues of teeth are not thoroughly explored. We hypothesize that TGP may be the cause of the sulfur appearance in the enamel and dentin. This change reflects the presence of pathological processes because the exchange of sulfur is controlled by the same factors that regulate protein metabolism (thyroid hormones).

Exploring the macro-and trace element composition of the intact enamel and with caries in patients with thyroid diseases identified elements that define the pathological changes in the outbreak: there is an increase in the oxygen content of enamel caries in the area 1.7 times in connection with processes deorganisation and demineralisation. Reduction of calcium content 2.3 and 2.6 times of sodium enamel caries zone indicates the presence of pathological processes.

In healthy teeth, dentin silicon is not found but in the teeth of patients with thyroid pathology its content is 0.74±0.28%. Comparative analysis of the content of magnesium in the dentin shows a 3.5 times increase in the teeth of patients with thyroid disorders. Our theory is that dysregulation of magnesium exchange is caused by overactive thyroid gland. All the data above is proving of
the influence of thyroid disorders in the hard tissues of teeth. The photos were obtained by using scanning electron microscopy for the study of the morphology of dental hard tissues. A cross section of an intact tooth, produced at a magnification of 10,000 times on the unit (device) «FEI Quanta 200 3D» shows that the enamel prisms have the accurate shape, densely adjoin to each other resembling a faceted cylindrical fiber (Fig. 1).

The enamel prisms have S-shaped curves, originating from enamel-dentine junction and ending at the surface of the tooth, diverging radially the entire thickness of the enamel which corresponds to the normal. The diameter of the enamel prisms varies from 3.5-8.0 M an average diameter of 4.86 M. There are many scratches on the enamel surface from the tooth brush (Fig. 2).

Enamel and dentin attached to each other tightly and the border resembles a zip-line that provides a strong connection of these tissues (Fig. 3). In the image of enamel with caries: interenamel prisms spaces were expanded, breaking the shape of the enamel prisms that change the correct position due to their thickening and loosening. Areas with hypomineralised enamel look less transparent than the surrounding enamel. If the initial dental caries on the enamel surface portions represent “white spots” then later these portions become darker. Such changes are due to the penetration of pathogens and dyes by loosening interprisms substance. Dentin in their structure resembles coarse-fibered bone. On micro-section samples of the control group dentin looks smooth, the layer is dense with a yellowish tinge. The dentine slice by electron microscopy revealed structural units: a basic substance and the dentinal tubules that are present in large numbers, starting from the pulp-dentin border and ending near the enamel-dentineal junction.

Transverse section of dentinal tubules shows the state of intact dentin. Tubules are clearly rounded or oval shape their diameter is approximately the same and varies from 1.5-3.5 M (Fig. 4). The average diameter of 2.01±0.26 μ. Dentinal tubules on the longitudinal section have the same width. Dentinal tubules have more electron-dense substance (peritubular dentin) which is lining the tube: white smooth rim which corresponds to the dentin having improved the electron density as a manifestation of a greater degree of mineralization. Dentinal tubules contained odontoblast processes but their bodies are located on the periphery of the pulp. Dentin tubules permeate from the junction with the pulp chamber to the enamel without interruption. Examining the state of the dentin in patients with TGP noted that the open dentinal tubules are most often found in the cervical region (the enamel-cement border). Dentinal tubules are dilated, twisted and fused together. The average tubes

![Fig. 1: The morphology of the intact tooth enamel 2.4, healthy patient. Transverse section. Enamel prisms. SEM (x5000)](image1)

![Fig. 2: Intact tooth 3.6. Scratches on the vestibular surface of the enamel. SEM (x1200)](image2)

![Fig. 3: Intact tooth 2.7, healthy patient. Longitudinal section. The border enamel and dentin. Amelodental compound. SEM (x500)](image3)
Fig. 4: The morphology of the intact tooth dentin 1.5, healthy patient. A cross section of the dentinal tubules. Tubules are rounded, about the same diameter. Tubules with a white rim of mineralized dentin. SEM (x10000)

diameter in control group is $2.01 \pm 0.26 \mu m$. In patients with hypothyroidism average tubes diameter is increased to $3.02 \pm 0.23 \mu m$ in some cases, reaching $5.9 \mu m$.

We found partially or completely obturated canals. Content of tubules in mature teeth presented by mineralized structures. In the normal state the dentinal tubules filled dental liquor. Dentinal tubules and the liquor which is circulating in them creates the conditions necessary for metabolism of organic and inorganic substances.

Results of the study of the microstructure show that the topography and surface topography of intact and carious teeth are significantly different. Macroscopic appearance of dentin caries is a cavity formed with softened dark tissue. At microscopic analysis we observed a tissue which is loss of a dense: dentin tubules are deformed. They change the diameter and shape, merge with each other and destroyed.

The analysis of enamel surface with scanning probe microscopy revealed that visually nonaltered enamel on the teeth with caries process there is a change of microrelief. The samples of the control group have a fairly smooth surface contour; the pores are present, as a place of partial opening of enamel prisms (size $0.5 \pm 0.18 \times 2.5 \pm 0.25 \mu m$). When a pathological process is present, the pores' size increases to $1.2 \pm 0.2 \times 2.8 \pm 0.31 \mu m$. In a unit volume $5 \times 5 \mu m$ detected the existence of several open pores which is untypical for the control group.

Macroscopic view of cement is different from the enamel by the presence of root roughness which is serving for the attachment of ligaments it also have yellowish tinge. Typically, cement adherent to tooth enamel tightly or cement is covered enamel but in some cases it does not reach the enamel. Intact cement is fibrous structure of collagen with uniform mineralization.

Caries cement has more clearly defined boundaries of the pathological process. Three-dimensional images, obtained using scanning probe microscopy, show the differences between cement with carious process and intact cement tooth: root surface defects appeared demineralized hilly terrain. Root surface of the tooth is covered from the neck to the top of acellular cement layer which width is $20-40 \mu m$. Cellular cement occupies a smaller area (thickness of $0.9-0.2 \mu m$). It is most active area of root growth (place of bifurcation, trifurcation, root apex). In patients with thyroid disorders, acellular layer of cement is thinner than $15-30 \mu m$.

Scanning electron microscopy revealed some changes in the morphological characteristics of hard dental tissues in patients with thyroid gland hypofunction. Examining the enamel enamel shows cracks, the number of which is increased in comparison with the control group. On the cut of enamel we can trace the cracks they may extend deep and cleft forming. Tooth enamel in patients with thyroid diseases have high fragility.

In the group of patients with TGP enamel surface is rough, the presence of open enamel prisms. The diameter of the enamel prisms varies from $4.5-8.7 \mu m$ (in patients without TGP: $3.5-8 \mu m$). Average diameter of $5.9 \pm 1.22 \mu m$ (in patients without TGP: $4.86 \pm 0.18 \mu m$). Interprism spaces are expanded. Significant structural enamel defects were found.

Dentin zone is visualized as a homogeneous structure (Fig. 5). In assessing the state of the dentin in patients with TGP noted that open dentinal tubules are
Fig. 6: Dentin after acid etching (15 sec, 32% phosphoric acid). Intact teeth of patient with TGP. SEM (x2000)

Fig. 7: Cement-dentin border. Longitudinal cut. Tooth of a healthy patient; tooth of patient with TGP. SEM (x1000)

more often found in the cervical area (enamel-cement border). In the presence of gingival recession is a loss of epithelial attachment, respectively, increases the surface with exposed dentinal tubules. Dentinal tubules expanded, alter the normal round shape to an oval, elongated and sometimes merge with each other (Fig. 6). The average diameter of the tubes of the control group 2.01±0.26 micron. In patients with hypothyroidism average diameter of tubes is increased to 3.02±0.23 micron in some cases, reaching 5.9 M.

Cement surface is covered with cracks that going deep into dentin (Fig. 7). The tooth of patient with TGP has a zone of spongy structure of cement (Fig. 8). Root of the tooth is covered by cement uneven: the edge of the cement near enamel is thinnest. Zones are formed, characterized by spongy structure.

Structural changes in the hard teeth tissues of people with thyroid disorders occur in the context of demineralization, especially calcium, phosphorus and magnesium. That is leading to the insolvency of local compensatory-defense mechanisms. Magnesium and silicon enhance the mineralization process and affect the densities of enamel.

Among patients with thyroid diseases we can see a tendency to complain of increased hypersensitivity of dental hard tissues with localization predominantly in the cervical region, followed by the formation of cavities. Patients with TGP have a tendency to circular caries. These changes are accompanied by a disturbance of mineral metabolism in the hard teeth tissues.

Based on evaluation of SEM we compared macro- and microelements content in intact and carious teeth of healthy patients and patients with TGP. The study of elements composition found that carries area shows a decrease in the content of calcium, phosphorus, fluorine as well as preserved hard tissues with a decreasing resistance of enamel and dentin. Significant differences in the content of calcium and phosphorus were detected in dentin carries area.

Zone with disturbance of the integrity of the enamel surface layer refers to a region with a weak structure where cariogenic situations arise destruction processes associated with demineralized enamel and dentin.

CONCLUSION

SEM of the hard teeth tissues of patients with TGP shows increasing of enamel abrasion with opening dentinal tubules and followed increase of their diameter. The formation of less mineralized spaces in dentin is the result of a mineral metabolism disturbance in thyroid diseases. Observed the loss of cement layer encompassing the adjacent dentin with small changes.
in the enamel layer. We found that even in visually non-altered enamel on the teeth with caries process there is a change of the microlay. Study of mineral metabolism and morphology in hard tissues arise a great interest, since the unique properties of the enamel, dentin and cement must be considered in the prevention and treatment of pathological changes in them.

REFERENCES


