Original paper

Study of the morphological structure and chemical composition of the dentin of intact teeth and teeth with cervical pathology

Badanie budowy morfologicznej i składu chemicznego zębiny w zębach zdrowych i objętych zmianami chorobowymi w obrębie szyjek zębowych

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Key words: dentine, non-carious cervical lesions, dental caries, scanning electron microscope.

Słowa kluczowe: zębina, niepróchnicowe zmiany przyszyjkowe, próchnica zębów, skaningowy mikroskop elektronowy.

Abstract

Introduction: The microstructure and chemical composition of tooth dentin reflect the complex processes associated with pathological conditions. Therefore, the study of their features is relevant in understanding the tactics of diagnosis and prevention of cervical lesions.
Aim of the research: A comparative analysis of the morphological structure and chemical composition of the dentin of intact teeth, teeth with cervical caries, and a wedge-shaped defect.
Material and methods: The study included 29 clinically extracted teeth of both jaws and their longitudinal sections, from patients aged 25–54 years, using a JSM-6490 LV focused-beam electron microscope (scanning) with system of energy-dispersive X-ray microanalysis. The chemical composition of 235 dentine areas in the incisal region (tubercle), equator, and cervical area was determined as a percentage of the weights of carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulphur, chlorine, zinc, potassium, and aluminium.
Results: The differences in the content of oxygen, sodium, and zinc in the dentin of all studied anatomical regions were determined, depending on the state of hard dental tissues ($p \leq 0.05$). The teeth with cervical caries had less oxygen and more zinc while the teeth with a wedge-shaped defect had less sodium ($p \leq 0.05$). An inverse correlation was revealed between carbon and phosphorus, and carbon and calcium in the area of the incisal region (tubercle); direct correlation between phosphorus and calcium ($p \leq 0.05$) at the equator and in the cervical region was also revealed.
Conclusions: The heterogeneity of mineral dentin content in the studied zones is probably because of the load on various areas and their morphological characteristics.

Streszczenie

Wprowadzenie: Mikrostruktura i skład chemiczny zębiny odzwierciedlają złożone procesy zachodzące w przebiegu stanów chorobowych. Z tego względu ich analiza ma istotne znaczenie dla prawidłowej strategii diagnostyki i zapobiegania uszkodzeniom szyjek zębowych.
Cel pracy: Analiza porównawcza budowy morfologicznej i składu chemicznego zębiny w zębach zdrowych oraz zębach z próchnicą w obrębie szyjek i ubytkami klinowymi.
Materiał i metody: Badaniem objęto 29 zębów po ekstrakcji klinicznej z obu szczęk, a także ich przekroje podłużne. Zęby pochodziły od pacjentów w wieku 25–54 lat. Analizę przeprowadzono przy użyciu skaningowego mikroskopu elektronowego JSM-6490 LV z wiązką skupioną, z systemem mikroanalizy rentgenowskiej z dyspersją energii. Skład chemiczny 235 obszarów zębiny w okolicy brzegu siecznego (guzka) i równika oraz szyjki określono jako procent masy węgla, tlenu, wapnia, fosforu, sodu, magnezu, siarki, chloru, cynku, potasu i aluminium.
Wyniki: Określono różnice w zawartości tlenu, sodu i cynku w zębinie wszystkich badanych okolic anatomicznych w zależności od stanu tkanek twardych zęba ($p \leq 0{,}05$). W zębach z próchnicą szyjek stwierdzono niższą zawartość tlenu i wyższą zawartość cynku, natomiast w zębach z ubytkiem klinowym mniej sodu ($p \leq 0{,}05$). Zaobserwowano odwrotną zależność między zawartością węgla i fosforu oraz węgla i wapnia w okolicy brzegu siecznego (guzka), a także bezpośrednią zależność pomiędzy zawartością fosforu i wapnia ($p \leq 0{,}05$) w okolicy równika i szyjki.
Wnioski: Niejednorodna zawartość substancji mineralnych zębiny w badanych obszarach wynika prawdopodobnie z różnic w obciążeniu poszczególnych stref oraz ich cech morfologicznych.

Introduction

Lesions located around the cervical circumference of the teeth are becoming more common; they range from erosion-induced cervical hypersensitivity to secondary lesions under existing restorations. The reasons for the loss of hard dental tissues vary from abrasion and erosion to abfraction in this area [1]. Caries, a wedge-shaped defect, enamel erosion, and cracks are often detected among cervical pathologies [2, 3]. Cervical caries occurs in 20–30% of diagnosed cases [2], mainly in patients aged 30–50 years [4]. The prevalence of a wedge-shaped defect increases with age [5–7], and it reaches its maximum by the age of 50–70 years [4].

The microstructure and chemical composition of hard dental tissues reflect complex processes associated with age-related changes in the body and the features of damage of both carious and non-carious origin to hard tissues [8]. Identifying the patterns within these processes will allow a deeper understanding of the mechanisms of their development and will help to develop tactics for the diagnosis and prevention of diseases associated with changes directly in the enamel and dentin [8]. Therefore, it has become urgent to determine the features of the morphological structure, the number, and the distribution of the chemical elements in the dentin of the teeth with cervical pathology.

Aim of the research

The aim of the study was to perform a comparative analysis of the morphological structure and chemical composition of the dentin of clinically intact teeth, teeth with cervical caries, and a wedge-shaped defect. Also we aim to identify a possible correlation between macro- and microelements.

Material and methods

We examined 29 clinically extracted teeth of both jaws and their longitudinal sections (12 clinically intact ones, 10 with wedge-shaped defects, 7 with cervical caries) of patients aged 25–54 years using a JSM-6490 LV focused-beam electron microscope (scanning) with a system of energy-dispersive X-ray microanalysis INCA Penta FET×3 (OXFORD Instruments, England) according to the previously described method [9]. The teeth were extracted for orthodontic indications. We calculated local mass fractions of chemical elements using the method of the peak to background ratio taking into account matrix corrections for atomic number, fluorescence, and absorption, measured in normal mass percentage (normal mass%). The mineral composition of 235 dentin areas in the incisal region (tubercle), equator, and cervical area were determined as percentages of the weights of carbon, oxygen, calcium, phosphorus, sodium, magnesium, sulphur, chlorine, zinc, potassium, and aluminium. Replication measurements were averaged in one sample before statistical analysis. Dentin was examined at approximately the same distance from the enamel-dentin border. The study was conducted at the base of the Donetsk Institute of Physics and Technology of the National Academy of Sciences of Ukraine. The work was performed in accordance with the principles of the Helsinki Declaration of the World Medical Association "Ethical Principles of Medical Research with the Involvement of a Human Being as a Research Object", Order No. 690 of the Ministry of Health of Ukraine (dated 23 September 2009) and approved by the Bioethics Commission of the Donetsk national medical university. Prior to engaging in the study, all participants were provided with written informed consent.

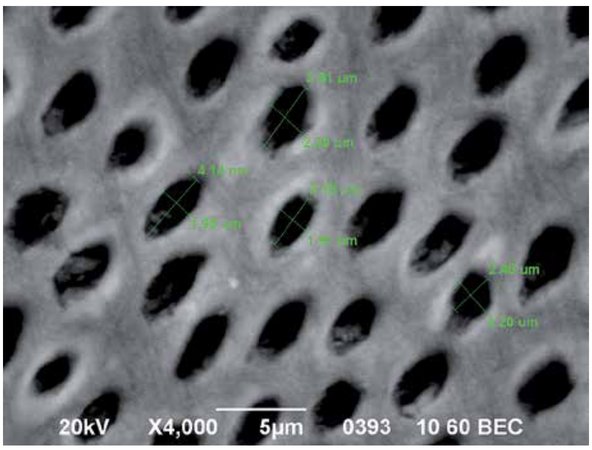

Figure 1. Electron microscopic image of the dentin of a clinically intact 3.5 tooth (4000×)

Statistical analysis

Statistical analysis was performed using the Statistica 12.0 computer program (3BA94C4ED07A). To check the presence of the relationship between the variables, correlation analysis was carried out (Pearson's parametric correlation method) based on the determination of the parametric Brave-Pearson coefficient (r) with the confidence level of 95%. The reliability of the obtained results was assessed using Student's t-test, and the correlation between the indicators – based on Student's t-test using Z-test (Fisher's Z-test). The differences were considered statistically significant at $p \leq 0.05$. The significance of the differences between the groups was assessed based on the analysis of variance.

Results

Electron microscopic examination of the dentin of intact teeth showed well-defined functioning dentinal tubules, the average diameter of which was 2.68 ±0.22 µm (Figure 1). The destruction of the dentinal tubules and an increase in their average diameter to

2.94 ±0.27 µm were observed in the caries process (Figure 2).

When studying the morphological structure of the dentin in the focus of a wedge-shaped defect, partial (Figure 3) or complete (Figure 4) obliteration of the dentinal tubules was observed; their diameter varied depending on the study area. The average diameter of the dentinal tubules was 2.51 ±0.51 µm on the upper (coronal) surface forming a wedge-shaped defect, 2.86 ±0.47 µm – on the lower (near-gingival) surface, 2.49 ±0.35 µm ($p > 0.05$) – at the junction of the surfaces.

The results of determining the dentin chemical composition in the incisal region (tubercle) are presented in Table 1. The differences were found in the content of oxygen, sodium, aluminium, chlorine, potassium, and zinc ($p \leq 0.05$).

We detected more sodium, aluminium, chlorine, and zinc in the teeth with cervical caries: sodium – 3 times greater than in the teeth with a wedge-shaped defect, and by 39% greater when compared with intact teeth (there was 2.1 times less sodium in the samples with a wedge-shaped defect than in intact samples); aluminium and zinc – by 2.5 times and 23 times, respectively, than in the other 2 groups, in which it was the same; chlorine – 2.8 times greater than in the group of teeth with a wedge-shaped defect, and 79% greater compared with intact teeth (there was 36% less chlorine in the samples with a wedge-shaped defect than in intact samples). Conversely, the oxygen content was 13% less in the dentin of the teeth with cervical caries compared to the group with a wedge-shaped defect, and it was 11% less compared with intact teeth. The level of magnesium was higher by 26% in the dentin of the teeth with a wedge-shaped defect compared with the teeth with cervical caries ($p \leq 0.05$). The amount of sulphur was 43%higher in the samples with non-carious cervical pathology compared with the other 2 groups. There was more potassium and calcium in the dentin of clinically intact teeth: potassium – 2 times more than in the other 2 groups, calcium – 5% more than with teeth with a wedge-shaped defect.

In the dentin of the incisal region (tubercle) an inverse correlation of moderate and high strength between phosphorus and carbon, calcium and carbon was found, $p \leq 0.05$.

The results of the dentin chemical composition determination in the equatorial area are presented in Table 2. Differences were found in the amount of carbon, oxygen, sodium, magnesium, phosphorus, calcium, and zinc, depending on the state of hard dental tissues ($p \leq 0.05$).

We determined more carbon, sodium, phosphorus, calcium, and zinc in the teeth with cervical caries: carbon – 16% more than in the group with a wedge-shaped defect, and 11% more compared with intact teeth; sodium 32% more than in the group with a wedge-shaped defect, and 11% more than in

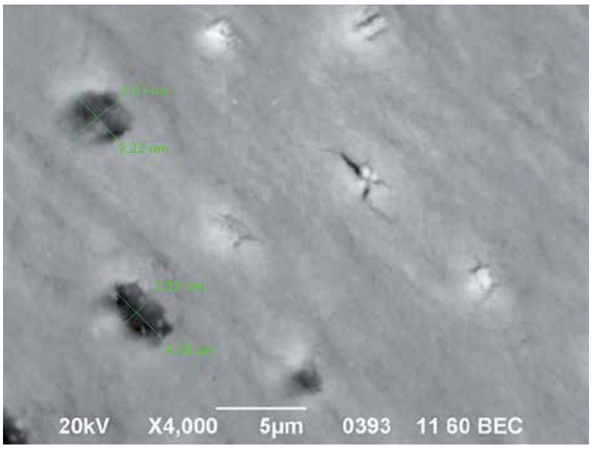

Figure 2. Electron microscopic image of 3.3 tooth dentin in the area of cervical caries (4000×)

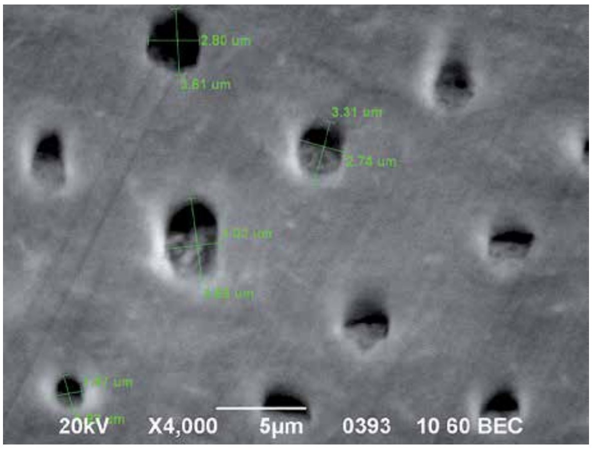

Figure 3. Electron microscopic image of the dentin surface of a 2.4 tooth with wedge-shaped defect (4000×)

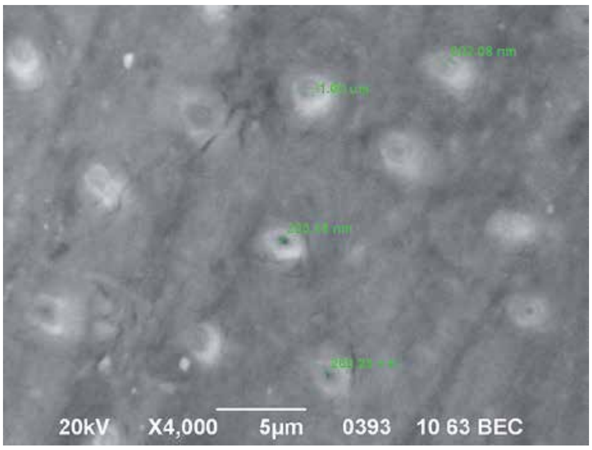

Figure 4. Electron microscopic image of the dentin surface of a 1.5 tooth in the area of the junction of the surfaces of a wedge-shaped defect (4000×)

Table 1. The dentin chemical composition of the incisal region (tubercle)

Chemical element (normal mass %)	Teeth with wedge-shaped defects ($\bar{x}$ ± m)	Teeth with cervical caries ($\bar{x}$ ± m)	Clinically intact teeth ($\bar{x}$ ± m)	*P*-value
C	27.52 ±1.57	29.23 ±5.18	26.97 ±3.69	0.127
O	36.54 ±0.67$^{\$}$	31.96 ±3.93$^{\#}$	35.81 ±1.89$^{\$}$	< 0.001*
Na	0.36 ±0.06$^{\#\$}$	1.07 ±0.67	0.77 ±0.25	< 0.001*
Mg	0.44 ±0.08$^{\$}$	0.35 ±0.23	0.41 ±0.16	0.191
Al	0.02 ±0.02$^{\$}$	0.05 ±0.06$^{\#}$	0.02 ±0.04$^{\$}$	0.010*
P	12.46 ±0.54	12.17 ±1.07	12.21 ±0.28	0.401
S	0.10 ±0.05$^{\#\$}$	0.07 ±0.06	0.07 ±0.03	0.092
Cl	0.09 ±0.04$^{\#\$}$	0.25 ±0.21$^{\#}$	0.14 ±0.05$^{\$}$	< 0.001*
K	0.02 ±0.02$^{\#}$	0.02 ±0.03$^{\#}$	0.04 ±0.04$^{\$}$	0.019*
Ca	22.46 ±1.07$^{\#}$	23.74 ±2.83	23.61 ±1.78	0.102
Zn	0.05 ±0.06$^{\$}$	1.14 ±1.37$^{\#}$	0.05 ±0.07$^{\$}$	< 0.001*

*p – the statistical significance of differences; *the difference between the groups is statistically significant, p ≤ 0.05; #the difference from the dentin of intact teeth is statistically significant, p ≤ 0.05; $the difference from the dentin of the teeth with cervical caries is statistically significant, p ≤ 0.05.*

Table 2. The dentin chemical composition of the equator

Chemical element (normal mass %)	Teeth with wedge-shaped defects ($\bar{x}$ ± m)	Teeth with cervical caries ($\bar{x}$ ± m)	Clinically intact teeth ($\bar{x}$ ± m)	*P*-value
C	23.37 ±3.68$^{\$}$	27.13 ±4.26$^{\#}$	24.45 ±3.16$^{\$}$	< 0.001*
O	36.68 ±1.61$^{\$}$	30.59 ±5.63$^{\#}$	37.00 ±2.33$^{\$}$	< 0.001*
Na	0.67 ±0.46$^{\$}$	0.89 ±0.31	0.80 ±0.21	0.048*
Mg	0.33 ±0.09$^{\#}$	0.38 ±0.30$^{\#}$	0.58 ±0.08$^{\$}$	0.002*
Al	0.04 ±0.05	0.03 ±0.03	0.04 ±0.05	0.824
P	12.87 ±0.53$^{\#}$	13.11 ±1.16$^{\#}$	12.02 ±0.32$^{\$}$	< 0.001*
S	0.11 ±0.08	0.09 ±0.05	0.08 ±0.04	0.124
Cl	0.13 ±0.11	0.15 ±0.11	0.11+0.09	0.551
K	0.04 ±0.04$^{\#}$	0.03 ±0.03	0.02 ±0.02	0.103
Ca	25.65 ±2.22	26.83 ±2.46$^{\#}$	24.91 ±1.02$^{\$}$	0.007*
Zn	0.21 ±0.49$^{\$}$	0.80 ±0.58$^{\#}$	0.06 ±0.11$^{\$}$	< 0.001*

*p – the statistical significance of differences; *the difference between the groups is statistically significant, p ≤ 0.05; #the difference from the dentin of intact teeth is statistically significant, p ≤ 0.05; $the difference from the dentin of the teeth with cervical caries is statistically significant, p ≤ 0.05.*

the group with intact hard tissues; phosphorus 2% more than in the group with a wedge-shaped defect, and 9% more than intact teeth (there was 7% more phosphorus in the teeth with a wedge-shaped defect compared with intact teeth); calcium 5% more than in teeth with a wedge-shaped defect, and 7.7% more than intact teeth; zinc 3.8 times more than in teeth with a wedge-shaped defect, and 13 times more when compared with intact teeth. Conversely, the oxygen content was 17% less in the dentin of teeth with cervical caries than in the group of teeth with a wedge-shaped defect, and 18% less than in intact teeth. The amount of potassium was 2 times higher in the dentin of the teeth with a wedge-shaped defect than in clinically intact teeth ($p \leq 0.05$). The amount of magnesium was 53% higher in the dentin of intact teeth than in the samples with cervical caries, and by 75% higher than teeth with a wedge-shaped defect.

The correlation was determined in the dentin of the equator: the inverse type – between carbon and oxygen, chlorine and oxygen, the direct type – between phosphorus and calcium. There was a correlation between calcium and oxygen: direct – in the groups with a wedge-shaped defect and intact hard tissues (r = 0.7181); inverse – in the groups with cervical caries and intact hard tissues (r = –0.6656) and in the groups with cervical pathology (r = –0.4967).

The results of determining the dentin chemical composition of the cervical region of clinically intact teeth and teeth with cervical pathology are presented in previous works [9]. The following correlation was determined in this anatomical zone: inverse type – between carbon and phosphorus, carbon and calcium, magnesium and sodium; direct type of high strength – between phosphorus and calcium. A correlation was found between calcium and oxygen: direct correlation – in the groups with cervical caries and intact hard tissues (r = 0.6199), inverse correlation in the groups with a wedge-shaped defect and intact hard tissues (r = –0.8891) and in the groups with cervical pathology (r = –0.3611). The correlation was revealed between chlorine and oxygen: direct – in the groups with a wedge-shaped defect and intact hard tissues (r = 0.4738), inverse – in the groups with cervical caries and intact hard tissues (r = –0.4921) and in the groups with cervical pathology (r = –0.3883).

Discussion

The morphological structure of the dentin of clinically intact teeth revealed round, approximately the same diameter dentinal tubules, which confirms other researchers' results [10]. The dentin is subject to reactive transformation, the nature of which is directly dependent on the special features of both internal adverse factors and external ones [11]. Therefore, its morphological structure in non-carious and carious processes has significant differences [8]. Areas of demineralization were identified in the dentin of the teeth with a wedge-shaped defect, and they were observed in 69% of the cases of non-carious cervical lesions according to Wada *et al.* [12]. Depending on the area of the study, other researchers found different degrees of obliteration of the dentinal tubules in teeth with wedge-shaped defects [13, 14]. Obliteration of the dentinal tubules along the entire length leads to the formation of the homogeneous surface, which can be detected over the entire area of exposed dentin [7]. Walter *et al.* identified "dead tracts" in the dentin of teeth with non-carious cervical lesions in 88% of the cases (62% of which were located directly in the lesion focus), sclerosed dentin was diagnosed in the lesion focus of the teeth in 48%, and tertiary dentin of the teeth in 60% [15]. Tkachenko *et al.* found hypermineralized dentin in the affected area with stenosis and tubular obliteration in the teeth with a wedge-shaped defect [8].

Dilated dentinal tubules were identified in the dentin of the specimens with cervical caries, and according to Petrenko it histologically corresponds to the complete absence of peritubular dentin, which is replaced by the intertubular substance [11]. Pavlova *et al.* also identified deformed and dilated dentinal tubules with a carious process [10] where there was a partial deposition of petrification which did not block their internal lumen completely. Such dentinal tubules can be referred to as "dead tracts". However, according to Petrenko, such a name is not entirely appropriate because they are the only structures in carious dentin that retain the ability to drain altered dentin. Thus, the author suggests calling them "drainage tracts". This is an alteration of dentin but not sclerosis, which is characterized by the increase in the tissue density [11]. The average diameter of the dentinal tubules was larger in the teeth with cervical caries, but the revealed differences were statistically insignificant ($p > 0.05$).

The quantitative composition of the chemical elements in hard dental tissues is not constant. Significant differences were determined in the content of oxygen, sodium, and zinc in the dentin of all studied anatomical regions depending on the state of hard dental tissues ($p \leq 0.05$). There was significantly less oxygen and more zinc in the teeth with cervical caries, and less sodium ($p \leq 0.05$) – in the teeth with a wedge-shaped defect. There was more zinc in the dentin of the teeth with cervical caries: in the area of the incisal region (tubercle) – by 23 times, at the equator – by 3.8 times and 13 times, in the cervical region – by 22 times, when compared with the teeth with non-carious pathology and intact hard tissues, respectively ($p \leq 0.05$). The accumulation of zinc in hard dental tissues reduces the efficiency of remineralization processes [16]. The amount of aluminium of the teeth with cervical caries was significantly higher by 2.5 times in the area of the incisal region (tubercle) and by 14 times in the cervical region compared with the teeth with a wedge-shaped defect and clinically intact hard tissues. And its accumulation by hard dental tissues is not as important as the inhibition of the metabolism of calcium and phosphorus [17].

Inverse correlation was determined between carbon and phosphorus, carbon and calcium in the area of the incisal edge (tubercle) and the cervical area of the samples; there was direct correlation between phosphorus and calcium ($p \leq 0.05$) at the equator and in the cervical region, which confirms other researchers' results [18]. Distinctive features of the correlation of calcium with oxygen in the dentin of the equator and the cervical region depending on the state of hard tissues were revealed. The correlation between calcium and oxygen was direct in the dentin of the equator in the groups with a wedge-shaped defect and clinically intact hard tissues, and inverse in the groups with cervical pathology and in the groups of the teeth with cervical caries and intact hard tissues. The correlation between calcium and oxygen was direct in the cervical region in the groups with cervical caries and clinically intact hard tissues, and inverse in the groups with cervical pathology and in the groups of the teeth with a wedge-shaped defect and intact hard tissues.

Conclusions

The development of a pathological process is an external manifestation of internal changes in the morphological structures. The assumption about the differences in the chemical composition of the dentin of clinically intact teeth and those with cervical pathology in the incisal region (tubercle), equator, cervical zone ($p \leq 0.05$) has been confirmed, which is probably due to the load of different areas and their morphological characteristics. The strength and types of identified correlations between chemical elements in the dentin confirm the difference in the processes occurring in different types of cervical pathology. The imbalance of mineral components in the dentin can be considered as one of the aetiological factors in the appearance and development of cervical lesions of hard dental tissues.

Conflict of interest

The authors declare no conflict interest.

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