Changes in the chemical composition of mineralised teeth in children after antineoplastic treatment

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Introduction: Chemotherapy, neoplasms, and their complications linked to malabsorption, malnutrition, and metabolic disorders may lead to improper tooth development and frequent severe caries in patients during/after antineoplastic treatment and to a more frequent improper tooth development in patients undergoing chemotherapy during odontogenesis. However, the causes of these abnormalities remain unknown; there are no studies on the impact of antineoplastic treatment and its complications on the chemical composition of mineralised teeth.

Aim of the study: To compare the chemical composition of mineralised teeth extracted due to complicated caries in children after chemotherapy, and of teeth extracted due to orthodontic treatment in generally healthy children.

Material and methods: The treatment group included five teeth extracted due to complicated caries in children after antineoplastic treatment. The control group included five teeth extracted due to orthodontic treatment in generally healthy children. The chemical composition of enamel, dentine, cementum, interior of the canal, and enamel abnormalities in teeth extracted from patients after chemotherapy and in generally healthy patients were assessed with energy-dispersive X-ray spectroscopy. Results were analysed statistically.

Results: The magnesium (Mg) and zinc (Zn) mass contents in the enamel of patients after chemotherapy increased and so did the calcium (Ca) to phosphorus (P) ratio when compared to controls. Areas with abnormal enamel in patients after chemotherapy had lower concentrations of Ca and P, and higher concentrations of trace elements (Mg, Cl, and Na). The levels of the assessed elements in dentine, cementum, and inside the canal were similar in both groups of teeth.

Key words: children, chemotherapy, chemical composition of mineralised teeth.

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Introduction

Antineoplastic treatment directly and indirectly affects permanent teeth in children and adolescents.

Chemotherapy and the complications it causes, such as malnutrition, malabsorption, or metabolic disorders, may lead to improper tooth development and frequent severe caries in patients during and after antineoplastic treatment [1–7]. Dental abnormalities occur also more frequently in patients who have undergone chemotherapy during odontogenesis. Chemotherapeutics used in early childhood may also be responsible for carious lesions in mineralised dental tissues, which may promote caries. According to the literature, the calcium-to-phosphorus ratio affects the hardness of enamel. The higher the ratio, the lower the enamel mineralisation. Fagrell *et al*. and Jalevik *et al*. established that the calcium to phosphorus ratio was higher in demineralised than in healthy enamel [8, 9]. Furthermore, trace elements also affected the size of enamel prisms, which in their turn determined enamel hardness [10)]. The occurrence of trace elements was also related to the susceptibility of mineralised tissues to caries [10, 11]. The negative impact of chemotherapeutics and of the complications they cause on teeth is not fully known and there are no studies on the chemical composition of permanent mineralised teeth extracted after antineoplastic treatment. The comparative microanalysis of the chemical composition of teeth extracted from patients after chemotherapy may help to answer the question of why children after antineoplastic treatment are much more prone to developing caries than generally healthy children, and it may hint at potential treatments.

Aim of the study

The study aimed to determine the extent to which chemotherapy leads to enamel mineralisation in children who undergo antineoplastic treatments.

Material and methods

Samples analysed microscopically included:

- five permanent teeth extracted due to complicated caries, without periapical inflammatory changes, from patients who completed antineoplastic treatment (aged 14.8 ±3.2 years; group 1);
- five teeth extracted due to orthodontic treatment, from generally healthy patients (aged 15.6 ±2.3 years; group 2).

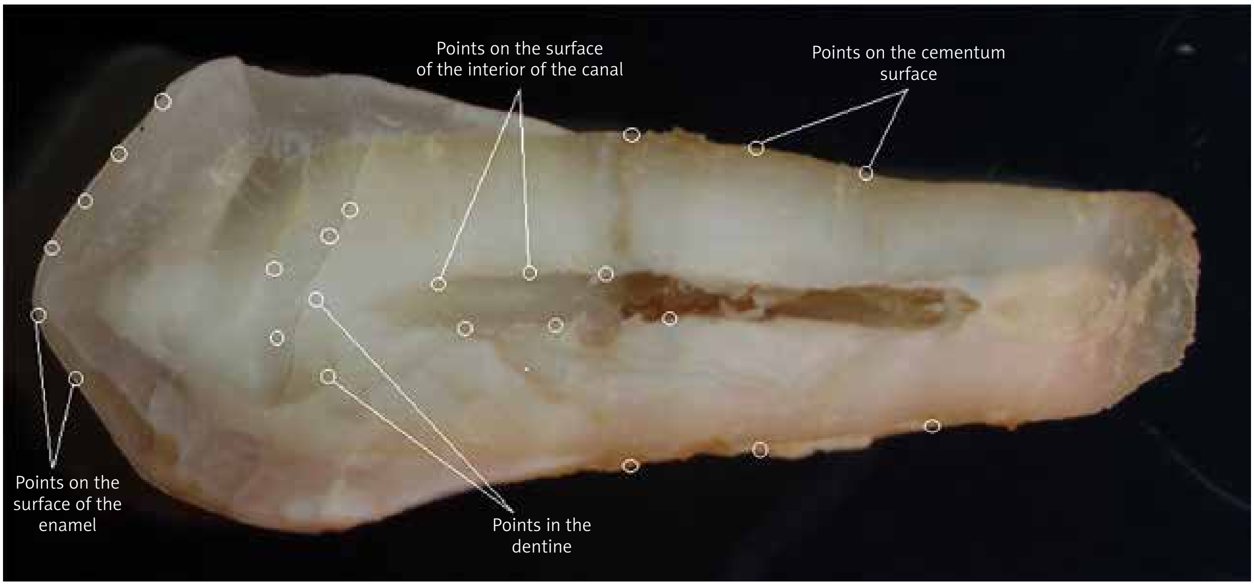

Fig. 1. Stereo microscope image of tooth with indicated element measurement points assessed with X-ray analysis

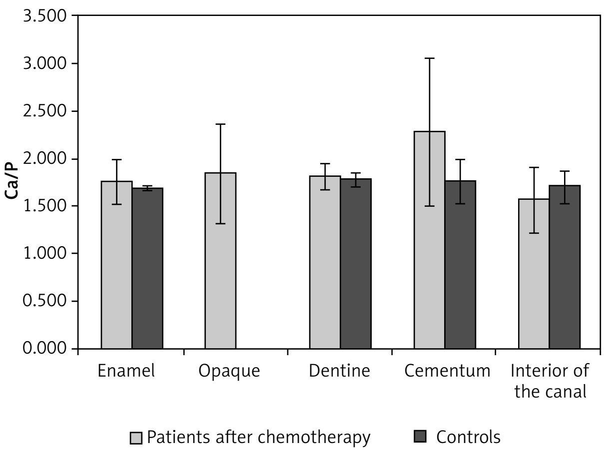

Fig. 2. The calcium to phosphorus ratio in teeth of patients after chemotherapy and controls

Table 1. Mass content of elements within enamel opacities after chemotherapy vs. macroscopically unchanged enamel in groups 1 and 2

Chemical element	Mass percent		
	Mean ± SD	*p* – opacities vs. clinically healthy enamel	
		group 1 vs. group 2	group 1 vs. group 2
Ca	24.446 ±12.282	0.1437	0.1437
P	13.206 ±5.143	0.0367*	0.0122*
Na	1.434 ±1.847	0.8340	0.8340
Cl	1.074 ±1.341	0.5309	0.1425
Mg	0.494 ±0.289	0.6761	0.4034
O	48.656 ±12.147	0.6761	0.6761

Patients received different chemotherapy regimens, including vincristine, etoposide, cisplatin, 5-fluorouracil, cyclophosphamide, and doxorubicin. Three patients started antineoplastic treatment after removing fully formed teeth, including two patients with fully formed crowns.

The extracted teeth were rinsed under running water, mechanically cleaned from soft tissues, cut lengthwise, and kept in ethanol. Dental cross-sections were visualised with a stereo microscope (SZX16, Olympus) and, after applying carbon dust with an ionic duster (JEC 530 Auto Carbon Coater, Jeol), with a scanning electron microscope (SEM; JSM-7600F Jeol). Chemical elements in the different parts of the enamel, dentine, cementum, and interior of the canal were analysed with energy-dispersive X-ray spectroscopy (EDS; Oxford X-Max) together with an electron microscope.

The analysis was performed with a magnification of 250×, at 15kV at fixed voltage measurement points (n = 6 for each area), as shown in Fig. 1.

The chemical composition was presented as mean element mass contents (arithmetic mean ± standard deviation) of elements prevailing in the assessed tooth areas in children after chemotherapy (group 1) and in generally healthy children (group 2). The calcium-to-phosphorus ratio for subsequent dental tissues was also calculated for both groups. The results were statistically analysed with the *U* Mann-Whitney test. Significance was set at $p \leq 0.05$.

Results

The microanalysis of the chemical composition of enamel within the opaque areas in children after chemotherapy showed that levels of phosphorus were statistically significantly lower than in healthy enamel. The calcium-to-phosphorus ratio in these areas was 1.851 ±0.524. It was higher in the teeth of patients after chemotherapy (1.760 ±0.232) than in those of controls (1.694 ±0.011) (Fig. 2).

The mass contents of trace elements (Cl, Mg, and Na) within the opaque areas were higher in patients treat-

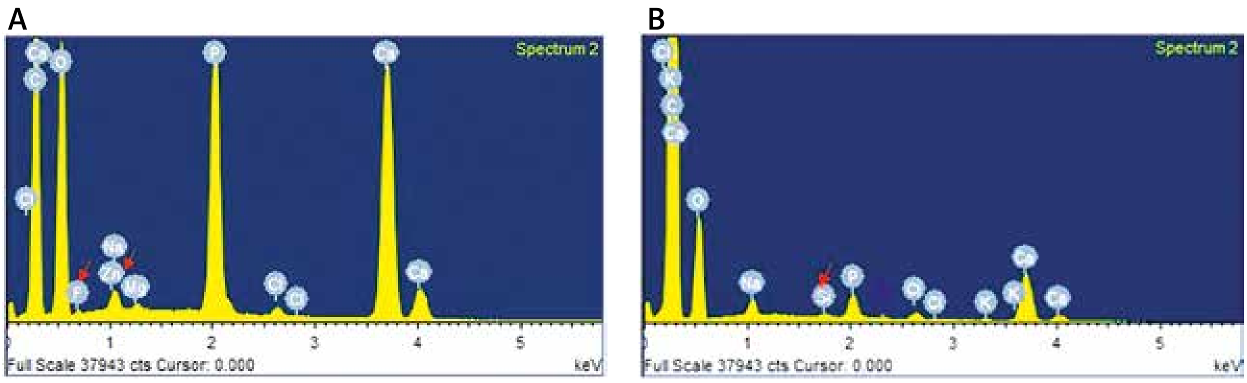

Fig. 3. Enamel spectrogram of teeth in patients after chemotherapy, indicating the prevalence of trace elements: F, Zn (**A**), and Si (**B**)

Table 2. Mass content of elements in macroscopically unchanged enamel

Chemical element	Patients after chemotherapy (group 1)	Controls (group 2)	*p*
Ca	34.676 ±0.744	35.560 ±0.283	0.0601
P	19.926 ±1.898	20.990 ±0.166	0.2101
Na	0.790 ±0.261	0.770 ±0.125	0.5309
Cl	0.634 ±0.127	0.758 ±0.073	0.0937
Mg	0.302 ±0.215	0.224 ±0.127	0.5284
O	41.498 ±0.486	41.470 ±0.265	0.8345

Table 3. Mass percent of elements in dentine

Chemical element	Patients after chemotherapy (group 1)	Controls (group 2)	*p*
Ca	36.288 ±1.574	36.104 ±0.877	0.8345
P	20.062 ±0.547	20.304 ±0.266	0.5309
Na	0.908 ±0.350	0.838 ±0.294	0.6761
Cl	0.138 ±0.171	-	0.1797
Mg	0.842 ±0.453	1.048 ±0.281	0.4034
O	41.424 ±0.355	41.640 ±0.226	0.4633

Table 4. Mass percent of elements on cementum surface

Chemical element	Patients after chemotherapy (group 1)	Controls (group 2)	*p*
Ca	32.936 ±14.143	34.242 ±2.437	0.6974
P	14.472 ±6.211	19.482 ±1.002	0.2963
Na	1.016 ±0.643	2.058 ±1.143	0.2963
Cl	0.386 ±0.355	-	0.0720
Mg	0.700 ±0.368	1.428 ±0.463	0.0947
O	45.228 ±10.646	41.852 ±0.444	0.6761

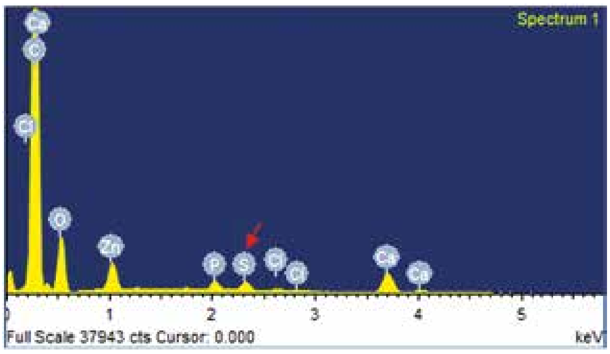

Fig. 4. Cementum spectrogram of tooth in patient after chemotherapy, indicating the prevalence of sulphur (S)

ed with chemotherapeutics than in macroscopically unchanged enamel after chemotherapy or in healthy enamel (Table 1).

The enamel mass content of Mg was also higher in patients after chemotherapy than in controls, while that of Cl was lower. Na levels were also assessed in both groups (Table 2).

Furthermore, the enamel of two teeth after chemotherapy tested positive for zinc (Zn) (Fig. 3), whereas healthy enamel did not.

Chlorine and a lower magnesium level were detected in the dentine of patients after chemotherapy when comparing to controls (Table 3).

Chlorine was detected in the cementum of patients after chemotherapy and sulphur was also detected in two teeth. Sulphur was not detected in any of the teeth in the controls (Table 4, Fig. 4).

The surface of the interior of the canal in the post chemotherapy group contained statistically insignificantly less calcium and phosphorus than the surface of the canal in controls (Table 5). Chlorine (Cl) was also detected in the inner wall of the canal in teeth after chemotherapy, but not in healthy teeth.

Discussion

The analysis of the chemical composition of various tooth areas in children and adolescents after antineoplastic treatment indicated a lower calcium level (Ca) and an increased calcium to phosphorus ratio in enamel, when compared to controls. The level of magnesium (Mg) decreased; chlorine (Cl) was detected in dentine; trace amounts of sulphur (S) and important amounts of chlorine (CL) were detected in the cementum of certain teeth, when compared to controls.

Table 5. Mass percent of elements on the interior surface of the canal

Chemical element	Patients after chemotherapy (group 1)	Controls (group 2)	*p*
Ca	26.870 ±9.839	33.420 ±2.789	0.4034
P	17.472 ±6.247	19.528 ±0.698	0.8345
Na	1.326 ±1.192	1.594 ±0.873	0.4034
Cl	0.214 ±0.265	-	0.1797
Mg	1.444 ±0.897	5.320 ±7.106	0.2492
O	46.106 ±8.916	42.236 ±0.755	0.8345

Hydroxyapatite, a crucial enamel component, is mainly composed of calcium and phosphorus. The normal Ca level in healthy enamel is between 36.5 and 40.0 mass percent, depending on age, and the normal phosphorus (P) level is between 17.25 and 18.25 mass percent [12]. A study reported the levels at 32.68 mass percent for Ca and 17.48 for P [12]. However, the Ca level increased with age [13].

The present analysis of enamel established a Ca level at 35 mass percent in children not treated with chemotherapeutics, and at 34.67 mass percent in children treated with chemotherapeutics; therefore considerably higher than in adult teeth. This did not confirm the generally suggested tendency when comparing the teeth of children/adolescents and young adults [13].

Enamel hardness depends on calcium and phosphorous levels, i.e. the calcium-to-phosphorus ratio. The higher the ratio, the lower the enamel mineralisation. Normal ratio should fluctuate between 1.8 and 2.3 [12]. The present results established the calcium to phosphorus ratio at 1.76 in healthy enamel and at 1.851 in carious enamel, both being within the normal limits. Studies varyingly described the calcium-to-phosphorus ratio in demineralised enamel; Piesiak-Pańczyszyn *et al.* reported it to be decreased in carious enamel vs. healthy enamel (1.5 vs. 1.9) [12]; so did Jalevik *et al.* (1.8 vs. 1.4) [9]; however Knychalska-Karwan (2.34 vs. 3.38) *et al.* and Fagrell *et al.* reported it to be increased, similarly to the present study [8, 14]. Divergences could result from unidentified defects during tooth mineralisation.

Furthermore, the present study detected such trace elements as chlorine, sodium, and magnesium in the enamel of both groups, i.e. in patients after chemotherapy and in controls, with slightly higher results in the former. These elements could promote caries [10, 11, 15, 16]. Magnesium (Mg) is known to increase the susceptibility of mineralised dental tissues to acids and therefore also to caries. In the present study, the level of Mg in dental enamel was higher in the group after chemotherapy than in controls, which could be responsible for more severe caries in that group. Amr *et al.* determined that the level of magnesium in carious enamel was higher than in healthy enamel of teeth removed due to orthodontic treatment [17]. The level of Mg in enamel could also be related to environmental factors because, in industrialised regions, levels of magnesium contained in animal and plant-based foods have been decreasing. Opalko *et al.* observed a lower Mg level in the enamel of children living in the region of the city of Szczecin than in that of children living in the region of the city of Bialystok [18]. At the meantime, it resulted in less severe caries in children from the Szczecin region than in those from the Bialystok region [18]. Jalevik *et al.* also confirmed higher levels of Mg in demineralised enamel than in the healthy one [9]. The correlation between Cl prevalence in enamel and enamel mineralisation remained unclear. Jalevik *et al.* did not confirm that the prevalence of Cl affected enamel demineralisation and promoted caries [9]. In the present study, the amount of Cl in the enamel of patients after chemotherapy was lower than the amount of Cl in controls, although the difference was not statistically significant. Furthermore, Zn was detected in one tooth from group 1 and was not detected in any of the teeth from group 2. Mazurek-Machol *et al.* and Gomes *et al.* established that Zn was necessary for proper enamel formation and its deficiency meant teeth were more prone to developing caries [15, 19]. They showed that zinc helped strengthen enamel and prevented caries. Therefore, the prevalence of Zn detected in the enamel of patients after chemotherapy and not detected in controls remained unclear.

Many studies have emphasised that even a low level of trace elements could impact the size of the formed enamel prisms, determining their hardness, and therefore their resistance to acids [10, 11]. Such trace elements as lead (Pb), titanium (Ti), manganese (Mn), selenium (Se), chromium (Cr), and nickel (Ni) affected the crystal structure of hydroxyapatites [10].

Such trace elements as fluorine (F), aluminium (Al), iron (Fe), selenium (Se), and strontium (Sr) were detected in teeth at a low risk of caries; manganese (Mn), copper (Cu), and cadmium (Cd) were detected in teeth at a high risk of caries. In the mean time, the levels of fluorine (F), strontium (Sr), potassium (K), and aluminium (Al) were higher in healthy than in carious enamel, and the level of silicon (Si) was higher in carious enamel [11]. In the present study, fluorine was detected in one patient after chemotherapy and in one control, and silicon only in one patient after chemotherapy. No other trace elements were detected in the enamel in both groups.

When considering the impact of trace elements on enamel mineralisation, it is important to note that because of fluctuations in the levels of other elements, which ranged from very low to very high, averaging the results could lead to interpretation errors [20, 12]. Divergences between the chemical composition of carious and healthy teeth could also result from environmental factors (diet and pollution), the impact of other diseases, different patient ages in subsequent studies, and the use of different measurement tools. The main dentine components – calcium, inorganic phosphorous, and fluorine – play a key role in tooth demineralisation and remineralisation [21, 22].

Pawlicki *et al.* assessed the chemical composition of dentine [13] and found lower mass percentage of calcium (21.76–25.82 depending on age group) and phosphorous (7.91–15.20) than in the present study. The present calcium and phosphorous mass percentages in outer dentine was higher than in the interior of the canal. These results differed from those of Magnus *et al.* [22], who compared Ca and P mass percentages in both outer dentine and in the interior of the canal. Chlorine was detected in the dentine of teeth after chemotherapy but not in that of controls. However, according to Jalevik *et al*., chlorine did not affect tooth mineralisation [9].

Brodzikowska assessed the mass percentage of these elements in healthy and carious cementum (23). She established that the calcium mass percentage (38.1) was higher than in the present study (23). Furthermore, carious cementum contained less Ca (33.1) than healthy cementum. Brodzikowska also showed that the phosphorous mass percentage was higher in both healthy (18.08) and carious (17.02) cementum than in the present group 1 (14.47), but higher than in the present group 2 (19.48). In the present study, the calcium to phosphorus ratio in the cementum of children after chemotherapy was higher (2.92) than Brodzikowska's ratio in healthy cementum (2.12). For controls, the calcium to phosphorus ratio (1.76) was lower than Brodzikowska's. It is difficult to compare the present study to the existing ones because there are no publications reporting on the element composition in teeth extracted after antineoplastic treatment. Höltta and Macleod analysed teeth extracted from patients after chemotherapy with a microscope, however they only determined the morphology of mineralised tissues [5, 24].

The present X-ray analysis of the chemical composition of mineralised dental tissues showed that multidrug antineoplastic treatments in childhood, together with the complications they cause, including malnutrition, malabsorption, and metabolic disorders, could considerably impact developmental anomalies, caries, and their severity. It could be caused by abnormal calcium and phosphorus levels and the prevalence of trace elements in these tissues. Since antineoplastic treatments are complex and may impact oral health in numerous ways, studies on this impact should be continued.

In conclusion, antineoplastic treatment, and the complications it causes, in childhood may lead to a decrease in the calcium-to-phosphorus ratio and also modify the levels of these elements in mineralised teeth. Antineoplastic treatments and concurrent disorders could also have an impact on the levels of trace elements. A higher predisposition to caries in children after oncological treatment could result from modifications in the chemical composition of teeth.

The authors declare no conflict of interest.

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