

Chemical Changes of Enamel Occlusal Surfaces Affected by Incipient Dental Caries: an EDX Study

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The aim of this study is to assess the quantitative ions changes in enamel occlusal surfaces affected by white-spot and brown-spot dental caries. Energy dispersive X-rays analysis (EDX) has been used for qualitative and quantitative chemical analysis of 30 enamel samples (10 sound enamel samples, 10 white-spot enamel samples, 10 brown-spot enamel samples). For samples with white-spot and brown-spot carious lesions, the mean percentages of calcium and phosphorus ions were considered. The statistical study used test t for independent samples. P-values less than 0.05 were considered to be statistically significant. It was recorded a significant decrease of calcium and phosphorus ions levels for enamel surfaces associated with white-spot and brown-spot lesions, comparing with sound enamel ($p < 0.05$). The highest decrease of calcium ions mean values was recorded for white-spot enamel surfaces (from 51.21 wt% in sound enamel samples to 28.37 wt% in white-spot enamel samples). The same decreasing trend was recorded for phosphorus ions between sound enamel samples and brown-spot and white-spot enamel samples (from 20.36 wt% in sound enamel surfaces to 17.88 wt% for white-spot enamel surfaces and 18.60 wt% for brown-spot enamel surfaces). The study proved the existance of significant mineral loss in white-spot carious lesions and brown-spot carious lesions comparing with sound enamel. White-spot carious lesions also present significant Ca, P ions loss comparing with brown-spot carious lesions.

Keywords: EDX, white-spot, brown-spot, calcium ions, phosphorus ions

The use of the systemic and local fluoridation methods on large scale produced a significant change of clinical aspect in the case of occlusal dental caries and made more difficult the clinical detection for early enamel caries as well as for hidden dentinal caries [1-6]. Other factors related to insufficient detection include complex anatomy of pits and fissures, superposition of structures in the radiographic evaluation, lack of accurate diagnostic devices [7, 8]. In this context an accurate diagnostic must rely both on clinical examen and paraclinical methods that assess the enamel mineralisation or demineralisation degree. Early diagnosis is requested to establish a proper plan of therapeutical-preventive measures and minimal invasive operative treatment [9].

The incipient occlusal carious lesion is initiated at the level of fissure opening, following the metabolic activity of bacterial biofilm [10]. Regarding histological and morphological aspects, occlusal early dental caries are characterized by specific features [11]. In the first stages early dental caries appear in SEM images as diffuse demineralizations of intraprismatic and interprismatic enamel with different degrees of peripheral dissolution of enamel crystals. Accordingly to morphopatological features and carious activity level, early dental caries are classified in two categories, white spot and brown spot carious lesions. White-spot enamel lesions are a stage of the caries process, prior to cavitation, where mineral has been lost from the enamel subsurface, with an intact surface layer overlying the mineral-poor region [12]. The white spot carious lesions on dried occlusal surfaces appear as white, opaque areas and are considered active dental caries. In this category of dental caries the presence of bacteria determined a high level of demineralization. The precipitation of mineral ions, following remineralization

processes as well as the adsorption of organic colorants conduct to the change of white spot in brown spot carious lesions. Lesions can be considered active if are associated with whitish/yellowish opaque surface with loss of luster and rough appearance [13].

There is a renew interest for treatment of early dental caries using preventive-therapeutical approaches like remineralisation or infiltration with low-viscosity resins [14, 15]. However a low number of studies focus on the chemical changes of white-spot and brown-spot dental caries [16-19]. In this context, are requested studies that highlight the pattern of demineralization from surface to depth layers and measure the mineral loss in diverse types of incipient dental caries.

The objective of this study was to test the hypothesis that occlusal white-spot and brown-spot dental caries induce a significant decrease of calcium and phosphorus ions content in the enamel tissue.

Experimental part

30 human molars teeth were extracted for diverse reasons (orthodontic, periodontal, malpositioned). Immediately after extraction, the teeth were imersed in 10% neutral phosphate-buffered formalin acetate solution for minimum 2 weeks. The calculus, soft tissues or alveolar bone were removed using ultrasound device. The teeth were divided in three study groups as follows: Group A (control)- 10 specimens of sound enamel surfaces; Group B- 10 samples of enamel surfaces with white-spot occlusal carious lesions; Group C- 10 samples of enamel surfaces with brown-spot carious lesions.

The clinical criteria for the carious lesion activity were as follows:

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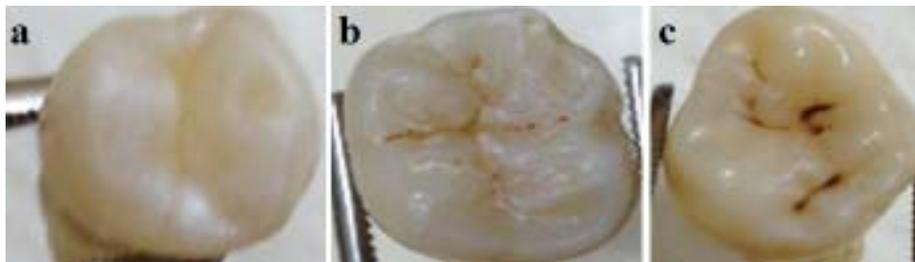


Fig. 1. Images of theets: a- Occlusal sound enamel (tooth 2.5.), b - Occlusal white-spot carious lesion (tooth 4.8.), c - Occlusal brown-spot carious lesion (tooth 3.8.)

-sound enamel surfaces- the absence of colour changes or substance loss;

-white-spot carious lesions- presence of whitish/yellowish, opaque areas with rough appearance along occlusal fissures and pits;

-brown-spot carious lesions- presence of brown or dark areas along occlusal fissures and pits.

To select only teeth with incipient dental caries limited to enamel surfaces, we also used laserfluorescence method (DiagnoDent, KaVo). The principle of laserfluorescence method consists in the measurement of fluorescence produced by bacterial products present in demineralization areas [20]. The criteria were as follows:

-sound enamel surfaces - range values 1-9;

-enamel dental caries (white-spot, brown-spot) - range values 10-25

The enamel surfaces were sectioned from teeth in buccal-oral direction using active water-cooled diamond discs (Gebr. Brasseler GmbH&Co, Germania). Blocks of approximate 8 x 3 x 4 mm were obtained. These blocks were mounted vertically with wax. It was avoided any area of cavitation. The examen was performed on single section including the demineralised enamel surface. Energy dispersive X-rays analysis (EDX) has been used for elemental analysis for all enamel samples. For white-spot and brown-spot carious lesions, the concentrations of calcium and phosphorus ions were measured at three areas

(external, medium, internal third of each carious lesions). The mean value of these three measurements was recorded. The enamel samples were analysed using a EDX detector QUANTAX QX2 (Bruker/Roentec, Germania) with active area of 10 mm² and resolution under 1,33 eV (MnK, 1000 cps). A spectrum of the energy versus relative counts of the detected x-rays is obtained and evaluated for qualitative and quantitative determinations of the chemical elements present in the specimen using a computer based program.

The concentrations of calcium and phosphorus ions were expressed as weight proportions (wt%). The statistical study used test *t* for independent samples. The null hypothesis for the independent *t* test is that there are no differences between the variables (calcium, phosphorus ions in sound enamel, white-spot, brown-spot). If the calculated probability is low ($P < 0.05$) the null-hypothesis is rejected and it can be concluded that variables are significantly different from each other. P-values less than 0.05 were considered to be statistically significant.

Results and discussions

The EDX method of sound enamel samples detected ions calcium and phosphorus as principal chemical elements (figs. 1, 2 and 3). Using EDX analysis we assessed quantitatively the percentage of each element for sound

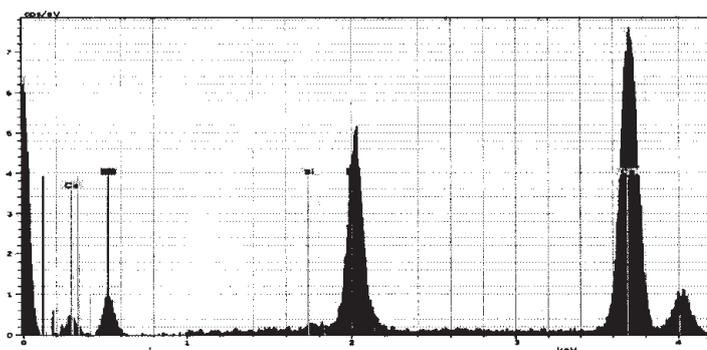


Fig. 2. The EDX spectrum and composition for a sound enamel sample

Element	Weight [%]	Atoms [%]	Error %
Calcium	51.38	34.84	1.57
Phosphorus	20.56	18.04	0.88
Oxygen	28.05	47.11	3.86
	100	100	

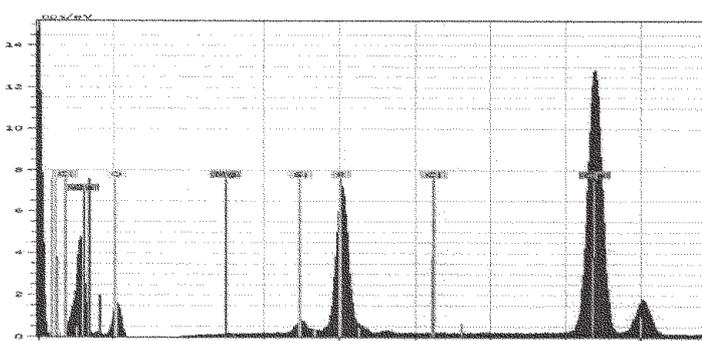


Fig. 3. The EDX spectrum and composition for a white-spot carious lesion sample

Element	Weight [%]	Atoms [%]	Error %
Calcium	29.09	17.25	0.88
Phosphorus	18.17	15.25	0.51
Oxygen	62.73	67.49	3.43
	100	100	

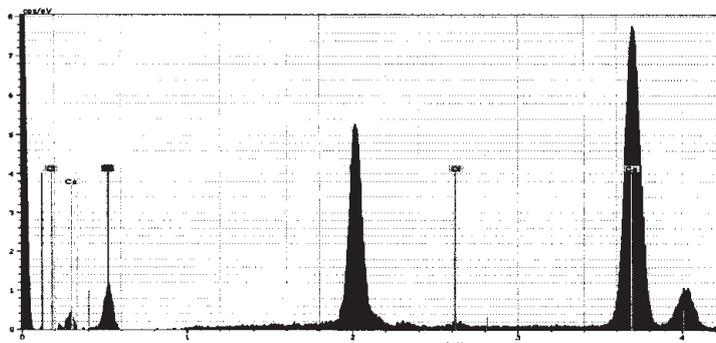


Fig. 4. The EDX spectrum and composition for a brown-spot carious lesion sample

Element	Weight [%]	Atoms [%]	Error %
Calcium	35.16	23.12	1.19
Phosphorus	17.99	13.59	0.75
Oxygen	46.84	63.28	3.43
	100	100	

enamel surfaces, white-spot carious lesions and brown-spot carious lesions.

Figure 2 presents qualitative analysis of sound enamel sample and mean values of mineral ions in the sound enamel sample with 51.38 wt% calcium ions and 20.56 wt% phosphorus ions.

Figure 3 presents qualitative analysis of white-spot enamel sample and mean values of mineral ions in the white-spot enamel sample with 29.09 wt% calcium ions and 18.17 wt% phosphorus ions.

Figure 4 presents qualitative analysis of brown-spot enamel sample and mean values of mineral ions in the brown-spot enamel sample with 35.16 wt% calcium ions and 17.99 wt% phosphorus ions.

The mineral content of sound enamel, expressed as weight percentage, had a mean value of 51.21 (ranged between 50.79 and 51.69) for calcium and 20.36 (ranged between 19.97 and 20.79) for phosphorus. The mineral content of enamel surfaces affected by white-spot carious lesions had a mean value of 28.37 (ranged between 25.59 and 29.26) for calcium and 17.88 (ranged between 17.41 and 18.22) for phosphorus. The mineral content of enamel surfaces affected by brown-spot carious lesions had a mean value of 34.11 (ranged between 25.59 and 29.26) for calcium and 18.6 (ranged between 17.99 and 19.01) for phosphorus.

Enamel surfaces	Mean values of Ca, P (wt%)	
	Calcium ions	Phosphorus ions
Sound	51.21	20.36
White-spot	28.37	17.88
Brown-spot	34.11	18.60

It was recorded a significant decrease of mean values for calcium and phosphorus ions for enamel surfaces associated with white-spot and brown-spot lesions, comparing with sound enamel (table 1). The highest decrease of calcium ions mean values was recorded for white-spot enamel surfaces (from 51.21 wt% in sound enamel samples to 28.37 wt% in white-spot enamel samples). Regarding phosphorus ions mean values, the same decreasing trend was recorded between sound enamel samples and brown-spot and white-spot enamel samples (from 20.36 wt% in sound enamel surfaces to 18.6 wt% for brown-spot enamel surfaces and 17.88 wt% for white-spot enamel surfaces).

In order to determine whether these differences are statistically significant, the recorded data were analyzed using test t for independent samples. P-values less than 0.05 were considered to be statistically significant.

Significant statistical results were also obtained when compared mean values of calcium ions as well as between sound enamel and white-spot carious lesions samples (significance level $p = 0.000 < 0.05$, t value 59.012, freedom degrees 10.085) (table 2). The test t results proved significant statistical results when compared mean values of calcium ions between sound enamel and brown-spot carious lesions samples (significance level $p = 0.000 < 0.05$, t value 47.716, freedom degree 10.33). Significant

Table 1
MEAN VALUES OF CALCIUM AND PHOSPHORUS IONS LEVELS FOR SOUND ENAMEL, WHITE-SPOT AND BROWN-SPOT CARIOUS LESIONS

Table 2

RESULTS OF THE STATISTICAL TEST t APPLIED IN ORDER TO ESTIMATE THE DIFFERENCES FOR CALCIUM BETWEEN: A - SOUND ENAMEL AND WHITE-SPOT, B - SOUND ENAMEL AND BROWN-SPOT, AND RESPECTIVELY C - BETWEEN WHITE-SPOT AND BROWN-SPOT CARIOUS LESIONS SAMPLES

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
A	Equal variance assumed	8.496	.009	59.012	18.000	.000	23.29300	.39472	22.46373	24.12227
	Equal variance not assumed			59.012	10.085	.000	23.29300	.39472	22.41452	24.17148
B	Equal variance assumed	13.868	.002	47.716	18.000	.000	7.10400	.35846	6.35091	7.85709
	Equal variance not assumed			47.716	10.330	.000	7.10400	.35846	6.30876	7.89924
C	Equal variance assumed	.005	.942	11.988	18.000	.000	6.18900	.51625	5.10439	7.27361
	Equal variance not assumed			11.988	17.813	.000	6.18900	.51625	5.10358	7.27442

Table 3

RESULTS OF THE STATISTICAL TEST *t* APPLIED IN ORDER TO ESTIMATE THE DIFFERENCES FOR PHOSPHORUS BETWEEN: A - SOUND ENAMEL AND WHITE-SPOT, B - SOUND ENAMEL AND BROWN-SPOT, AND RESPECTIVELY C - BETWEEN WHITE-SPOT AND BROWN-SPOT CARIOUS LESIONS SAMPLES

		Levene's Test for Equality of Variances		t-test for Equality of Means						
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
									Lower	Upper
A	Equal variance assumed	.725	.406	19.945	18.000	.000	2.48100	.12439	2.21967	2.74233
	Equal variance not assumed			19.945	17.500	.000	2.48100	.12439	2.21913	2.74287
B	Equal variance assumed	.002	.968	12.577	18.000	.000	1.76700	.14049	1.47183	2.06217
	Equal variance not assumed			12.577	17.875	.000	1.76700	.14049	1.47169	2.06231
C	Equal variance assumed	.435	.518	5.456	18.000	.000	.71400	.13086	.43908	.98892
	Equal variance not assumed			5.456	16.948	.000	.71400	.13086	.43785	.99015

statistical results were also obtained when compared mean values of calcium ions between white-spot and brown-spot carious lesions samples (significance level $p = 0.000 < 0.05$, *t* value 11.988, freedom degrees 18).

The test *t* proved significant statistically results when compared mean values of phosphorus ions between sound enamel and white-spot carious lesions samples (significance level $p = 0.000 < 0.05$, *t* value 19.945, freedom degrees 18) (table 3). The test *t* proved significant statistical results when compared mean values of phosphorus ions between sound enamel and brown-spot carious lesions samples (significance level $p = 0.000 < 0.05$, *t* value 12.577, freedom degree 18). Significant statistical results were recorded when compared mean values of phosphorus ions between white-spot and brown-spot carious lesions samples (significance level $p = 0.000 < 0.05$, *t* value 5.456, freedom degrees 18).

The study focused on the assessment of changes in mineral content regarding a white-spot and brown-spot carious lesions and compared the recorded data with sound enamel. Because the calcium and phosphorus ions were found as having greatest proportions, the study measured only the concentrations of these two ions for all the occlusal enamel samples.

The mineral content of sound enamel, expressed as weight percentage, had a mean value of 51.21 for calcium and 20.36 for phosphorus. These values recorded for control group are highest, comparing with values recorded in enamel surfaces affected by white-spot and brown-spot carious lesions. The results regarding study groups are recorded for active carious lesions as a mean value of measurements in external third, medium third and internal third of carious lesion. The active carious lesions exhibit a lower mineral content comparing with inactive carious lesions [16]. The average lesion mineral content in the enamel surfaces affected by white-spot dental caries is only 60% from mineral content of the area with lowest demineralization [17]. Other study based on x-ray microtomography, found in the white-spot caries, a mineral concentration in body lesion at lowest level, comparing with sound enamel, where calcium and phosphorus ions level can raise to over 50% [18]. The results of this study agree with our results regarding the difference between demineralised enamel areas and sound enamel. Our study found different mineral concentrations at different depth

of the same carious lesion. The white-spot carious lesions present highest mineral content at the level of the surface layer, approximately 90% of the mineral content of sound enamel, while other areas present lower minerals concentrations [19].

The importance of mineral content for early dental caries is related to the fact that active carious lesions are more likely to regress under saliva action or remineralization treatments, comparing with inactive carious lesions. There is a variance of calcium and phosphorus concentrations related to different depth of the same carious lesion. Further studies focusing on a more complex assessment of mineral content related to different layers of carious lesions are required.

Conclusions

The study proved the existence of significant mineral loss in white-spot carious lesions and brown-spot carious lesions comparing with sound enamel. White-spot carious lesions also present significant calcium and phosphorus ions loss comparing with brown-spot carious lesions.

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