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Aspects on dental hard tissues in primary teeth from patients with Ehlers–Danlos syndrome

GUNILLA KLINGBERG[1,2,3], CATHARINA HAGBERG[1,4], JÖRGEN G. NORÉN[2] & SANDOR NIETZSCHE[5]

[1]Mun-H-Center, National Orofacial Resource Centre for Rare Disorders, Göteborg, Sweden, [2]Department of Pedodontics, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, [3]Department of Pediatric Dentistry, Faculty of Odontology, Malmö University, Malmö, Sweden, [4]Department of Orthodontics, Institute of Odontology, Sahlgrenska Academy, University of Gothenburg, Göteborg, Sweden, [5]Centre of Electron Microscopy, Friedrich-Schiller-University Jena, Germany

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Background. Ehlers–Danlos syndrome (EDS) is a rare hereditary condition affecting connective tissues and dental hard tissues.

Hypotheses. Primary enamel and dentine from EDS patients were expected to differ from those of healthy subjects regarding morphology and chemical composition.

Design. Forty-seven exfoliated primary teeth from 25 patients with EDS were investigated. Morphology was studied using a polarized light microscope, scanning electron microscope, and X-ray microanalysis. Comparisons were made with 36 primary teeth from 36 healthy patients.

Results. Morphological analysis of enamel in EDS teeth showed a high frequency of postnatally hypomineralized enamel and postnatally located incremental lines, whereas dentine was normal in all patients. Chemical analysis could not reveal any differences between EDS and control patients except for lower content of C and a higher Ca/P ratio in the enamel in the EDS teeth, indicating porous enamel. Regarding dentine, EDS teeth had a lower content of C, and a higher content of Ca, P, and O. Ratios for Ca/C and Ca/O were also higher compared with controls.

Conclusions. There are several aberrations of booth enamel and dentine in primary teeth from patients with EDS. These could explain the occurrence of both more dental caries and tooth fractures in patients with EDS.

Introduction

Ehlers–Danlos syndrome (EDS) is a rare hereditary disorder affecting the connective tissues[1]. Prevalence figures vary between 1 in 5000 to 1 in 10 000[2]. The disorder has a wide-ranging expressivity depending on which types of collagen that are affected. Some of the main findings in EDS are soft, thin, fragile and hyperelastic skin, hypermobility of joints, dystrophic scars, and excessive bleeding[1–4]. Many different phenotypes have been described; however, in 1997, a new classification based on the cause of the disorder was agreed upon describing six types of EDS[4]. The hypermobility type is the most common, followed by the classical type, and together they account for approximately 90% of the cases. In Sweden, the Villefranche nosology is used for diagnosis and classification[4]. EDS is a clinical diagnosis based on symptoms and pedigree. However, for vascular or kyphoscoliosis types, biochemical and genetic testing are possible. In the vascular type, skin biopsies may be used for biochemical analyses of collagen III, followed by mutation analyses of the *COL3A1* gene. For the kyphoscoliosis type, molecular genetic testing of the *PLOD* gene is available.

Oral manifestations include fragile and sensitive mucous membranes, aggressive periodontitis with extensive bone loss, temporomandibular joint problems, pain from masticatory muscles, dental caries problems, high vaulted palate, a very flexible tongue, and reports of spontaneous

Correspondence to:
Gunilla Klingberg, Mun-H-Center, National Orofacial Resource Centre, Odontologen Göteborg, Medicinaregatan 12 A, SE 413 90 Göteborg, Sweden.
E-mail: gunilla.klingberg@vgregion.se

fractures of teeth[5–8]. Because collagen is the major constituent of dentine, some dental aberrations have been reported in connection with EDS, such as pulp stones, short and deformed roots, and affected dental hard tissues[5,9–11]. In 1969, Barabas[9] described a large number of histological abnormalities in 13 teeth from six patients. However, the classification of EDS was different and the diagnoses of the patients were not described. Apart from that study, most reports on histological findings are case reports. There are no studies describing the histology and chemical composition of enamel and dentine in larger series of primary teeth from patients with EDS.

The aims of this study were to examine and describe the histomorphology of enamel and dentine in primary teeth from patients diagnosed with EDS, using polarized light microscopy and scanning electron microscopy, and to analyse C, O, P, and Ca in these tissues using X-ray microanalysis (XRMA) and to compare with findings in enamel and dentine from unaffected patients. It was hypothesized that primary enamel and dentine from EDS patients would exhibit more abnormalities regarding morphology, as well as their chemical composition, than dental hard tissues from healthy controls.

Materials and methods

Subjects and tooth material

Forty-seven exfoliated primary teeth and one extracted tooth because of dental trauma (16 incisors, 10 canines, 12 molars) were collected from 25 patients (14 girls, 11 boys) with EDS. The diagnoses of the patients (as provided by patients/parents) are given in Table 1. The teeth were collected in collaboration with the Swedish patient organization, which has over 350 members. The patients were repeatedly asked to send in exfoliated or extracted teeth over a 3-year period.

After 24 h in 70% ethanol, the teeth were embedded in an epoxy resin (Epofix, Electron Microscopy Sciences, Fort Washington, PA, USA), and sagittal longitudinal sections, with a thickness of approximately 100 μm, were prepared in a Leitz Low Speed Saw Microtome (Leitz, Wetzlar, Germany). Central sections were used for histological examination in a polarized light microscope (POLMI). The teeth were broken into two parts or more. One part was used for scanning electron microscope (SEM) analysis and XRMA. One tooth (patient with hypermobile type) was destroyed during preparation, wherefore 47 teeth were available for further analyses.

For the XRMA analyses, 36 primary teeth from 36 healthy patients, serving as controls, were prepared according to what is described earlier. The Mann–Whitney *U*-test revealed that there were no statistically significant differences between different tooth types.

POLMI examination

All sections, from EDS patients and controls, were examined in polarized light, both dry in air and after water imbibition, in an polarizing microscope employing strain-free objectives (Olympus Corporation, Tokyo, Japan).

SEM/XRMA

Fifteen teeth, representing 15 EDS patients (three hypermobility, six classical, six unspecified) and 36 teeth from healthy control patients were, after the POLMI analysis, mounted on sample holders for SEM analysis and XRMA with carbon tape. The sections for SEM were etched for 45 s with 30% phosphoric acid, carefully rinsed with deionized water, and

Table 1. Numbers of patients and teeth by classification of Ehlers–Danlos syndrome.

	Classical	Hypermobility	Unspecified	Total
Patients (*N*)	4	6	15	25
Gender (female/male)	2/2	3/3	9/6	14/11
Teeth (*N*)	22	9	17	48
Tooth type (I/C/M)	10/4/8	5/2/2	6/4/7	21/10/17

Tooth type: I, incisor; C, canine; M, molar.

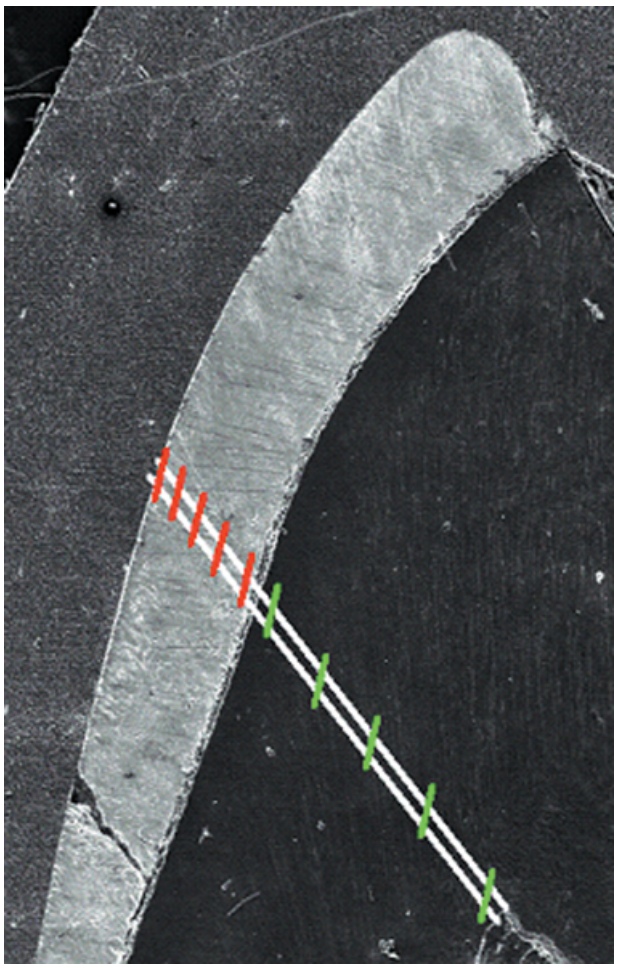

Fig. 1. Overview taken in scanning electron microscope analysis of an undecalcified section coated with carbon for X-ray microanalyses showing the principle locations of measurements in enamel and dentine.

sputter coated with gold by vapour deposition. The sections used for XRMA were sputter coated with carbon by vapour deposition. The SEM and XRMA were performed in a Philips SEM 515 (Philips, Eindhoven, The Netherlands). The sections were analysed in the enamel and the dentine with XRMA for C, Ca, P, and O in a Philips SEM 515 at 12 kV; EDAX DX4, ECON-detector (Philips).

Two lines of measurements were performed in each of the 15 EDS teeth and 36 control teeth. Five locations for measurements were identified along each line in the enamel and dentine, respectively (Fig. 1). The first location being located 10 μm under the enamel surface and the following three located at 1/4, 1/2, and 3/4 of the enamel thickness. The fifth location was located 10 μm above the enamel–dentine junction (EDJ). The locations in the dentine were 10 μm under the EDJ followed by three locations at 1/4, 1/2, and 3/4 of the dentine thickness and the fifth being 10 μm above the dentine–pulp border. Thus, in every specimen, ten measurements were made in each tissue. For each individual, the mean value for the corresponding measurements was calculated and then used for the statistical analyses.

All measurements were carried out on the buccal side of the tooth, always aiming at morphologically analysing the same area. For all measurements, the emitted X-rays were detected during continuously fast-scanning small window of 6.1 μm × 4.3 μm at a magnification of 650×. The relative amounts of C, Ca, P, and O were obtained by a semi-quantitative analysis using the EDAX DX-4 software. All values were considered semi-quantitative.

The Mann–Whitney *U*-test was used for statistical analyses to analyse differences between EDS and the control teeth regarding the amount of C, O, P, and Ca, and the ratios Ca/P, Ca/C, and Ca/O registered in the XRMA.

Results

POLMI examination

The morphological findings from the POLMI analysis and data of the EDS patients are given in Table 2. The presence of a neonatal line (NNL) was found in all but one section, which made it possible to discriminate prenatal enamel from postnatally formed enamel. When the sections were examined in dry air, the NNL appeared positively birefringent, extending from the EDJ coronally (Fig. 2a). Water imbibition did not change the positive birefringence of the NNL.

The prenatal enamel appeared positively birefringent in all teeth examined dry in air, seen as an inner microporous zone (Fig. 2b). After water imbibition, the microporous zone decreased in its extension but remained positively birefringent, indicating a degree of porosity more than 5%. Thus, the prenatal enamel appeared less mineralized compared with the postnatal enamel.

Table 2. Number of primary teeth by classification of Ehlers–Danlos syndrome, type of tooth, and histological findings.

		Prenatal enamel		Postnatal enamel				
	Neonatal line	HM	IL	HM	HP	IL	SSL	Dentine
Classical type (*N* = 22)	22	19	1	15	1	16	14	1
Incisors (*N* = 10)	10	8	1	4	1	5	7	0
Canines (*N* = 4)	4	3	0	3	0	4	3	1
Molars (*N* = 8)	8	8	0	8	0	7	4	0
Hypermobile type (*N* = 8*)	8	5	0	4	0	4	6	0
Incisors (*N* = 4*)	4	2	0	1	0	1	4	0
Canines (*N* = 2)	2	2	0	2	0	2	2	0
Molars (*N* = 2)	2	1	0	1	0	1	0	0
Unspecified type (*N* = 17)	16	8	0	11	0	10	6	0
Incisors (*N* = 6)	5	1	0	3	0	2	0	0
Canines (*N* = 4)	4	0	0	1	0	1	2	0
Molars (*N* = 7)	7	7	0	7	0	7	4	0
Total (*N* = 47*)	46	32	1	30	1	30	26	1
Incisors (*N* = 20*)	19	11	1	8	1	8	11	0
Canines (*N* = 10)	10	5	0	6	0	7	7	1
Molars (*N* = 17)	17	16	0	16	0	15	8	0

*One tooth lost during preparation why eight of originally nine teeth from hypermobile subgroup were used; in total 47 of 48 teeth were analysed.
HM, hypomineralization; HP, hypoplasia; IL, incremental line; SSL, subsurface lesion.

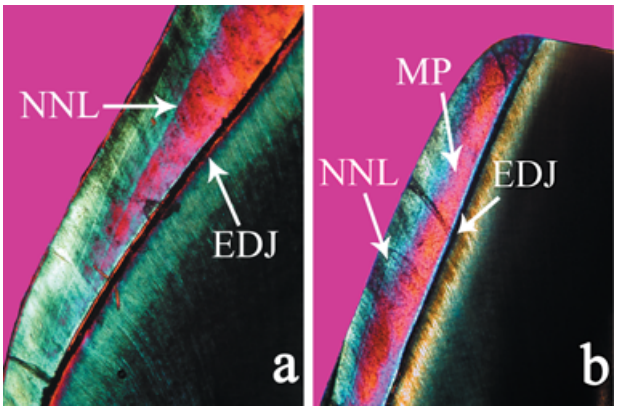

Fig. 2. (a) Undecalcified section of a primary incisor from an Ehlers–Danlos syndrome (EDS) patient seen dry in air in polarized light with a neonatal line (NNL). Magnification ×40. (EDJ, enamel–dentine junction). (b) Undecalcified section of a primary incisor from an EDS patient seen dry in air in polarized light with a NNL and an inner microporous prenatal zone in the enamel. Magnification ×40. (MP, prenatal microporous zone).

When the postnatal enamel was examined dry in air, it appeared negatively birefringent in 15 teeth. In one tooth, it was not possible to examine the postnatal enamel. The remaining 31 teeth had a positive birefringence of varying extension in the enamel.

If the positively birefringent enamel changed to negative birefringence after water imbibition, it was regarded as being of a normal degree of mineralization. Enamel remaining positively birefringent after water imbibition indicated a degree of porosity of more than 5%. The extension of postnatal enamel with a pore volume distribution of more than 5% was divided into two groups: group 1, extending over less than 1/2 of the postnatal enamel and group 2, extending over more than 1/2 of the postnatal enamel.

Seventeen teeth were regarded as having a normal degree of mineralization (7 of 22 in the classical group, 4 of 9 in the hypermobility group, 6 of 17 in the unspecific group).

Four teeth were classified into group 1 (2 of 22 in the classical group, 0 of 9 in the hypermobility group, 2 of 17 in the unspecific group).

Twenty-six teeth were classified into group 2 (13 of 22 in the classical group, 4 of 9 in the hypermobility group, 9 of 17 in the unspecific group) having a positive birefringence extending over more than 1/2 of the postnatal enamel (Fig. 3a).

One or more incremental lines in the postnatal enamel were found in 30 teeth (Fig. 3b). In the prenatal enamel, incremental lines were found only in one tooth. All incremental lines

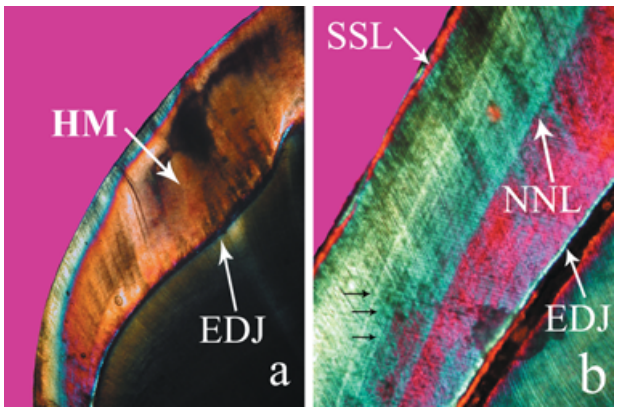

Fig. 3. (a) Undecalcified section of a primary molar from an Ehlers–Danlos syndrome (EDS) patient seen dry in air in polarized light with hypomineralized postnatal enamel. Magnification ×40. (EDJ, enamel–dentine junction; HM, hypomineralized postnatal enamel). (b) Undecalcified section of a primary incisor from an EDS patient seen dry in air in polarized light with a neonatal line (NNL) and an inner prenatal microporous zone and subsurface lesions located close to the surface of the postnatal enamel. Magnification ×40. (SSL, subsurface lesion; black arrows indicate incremental lines).

appeared positively birefringent both dry in air and after water imbibition. No correlation between microporosities and recorded incremental lines could be found.

Subsurface lesions were found mainly in the buccal enamel in 26 teeth (Fig. 3b). The lesion was always located in the postnatal enamel and was seen as a thin microporous zone under a well-mineralized surface. No connection or relation with other structural changes in the enamel could be found.

Enamel hypoplasia, seen as a macroscopic defect with rounded cervical borders, was found in one tooth, and was located at the NNL. No carious lesions were found in the examined sections.

The dentine appeared normal in all sections. In one section, interglobular dentine was found in the coronal part of the dentine crown. Reparative dentine in the coronal pulp areas was found in all teeth which were abraded.

In all teeth studied, the normal resorption process had reached the tooth crown, whereby no normal pulp tissue could be found.

SEM/XRMA

The SEM analyses showed normal enamel structure (Fig. 4a). In areas with hypomineralized enamel, the prism structure appeared less distinct compared with normal enamel (Fig. 4b). The dentine showed normal structure in all parts with normal dentinal tubules (Fig. 4c). In areas of reparative dentine in the pulpal areas, the dentine showed a more irregular appearance (Fig. 4d).

There were no statistically significant differences between incisors and molars concerning the XRMA in either of the groups (controls or EDS teeth).

Enamel

The results of the XRMA measurements are given in charts based on the median values for each location in the enamel and dentine, respectively. The curves for the different elements and ratios had, in principle, an identical outline from the enamel surface to the EDJ (Fig. 5a–c) irrespective of being normal enamel or enamel from EDS teeth. No statistical significant differences were seen except for C and the ratio Ca/C. Carbon had lower median values in all locations in the enamel in teeth from EDS patients compared with normal enamel (P values < 0.01 and < 0.05) (Fig. 5a). The ratio Ca/C was significantly higher ($P < 0.05$) in EDS enamel compared with normal enamel except for location 2 (Fig. 5c). The ratio Ca/P did not differ between enamel from EDS or normal primary teeth (Fig. 5c).

Dentine

As for the enamel, all dentine gradients had the same appearance from the EDJ to the pulp, irrespective if the teeth were from EDS or normal patients. However, the differences between normal dentine from primary teeth and dentine in teeth from EDS patients showed statistically significant differences for all elements and ratios with the exception for Ca/P ratio (Fig. 6a–c). In the EDS teeth, C had lower values in all locations ($P < 0.001$) (Fig. 6a). The oxygen values were statistically significantly higher (P values < 0.01 and

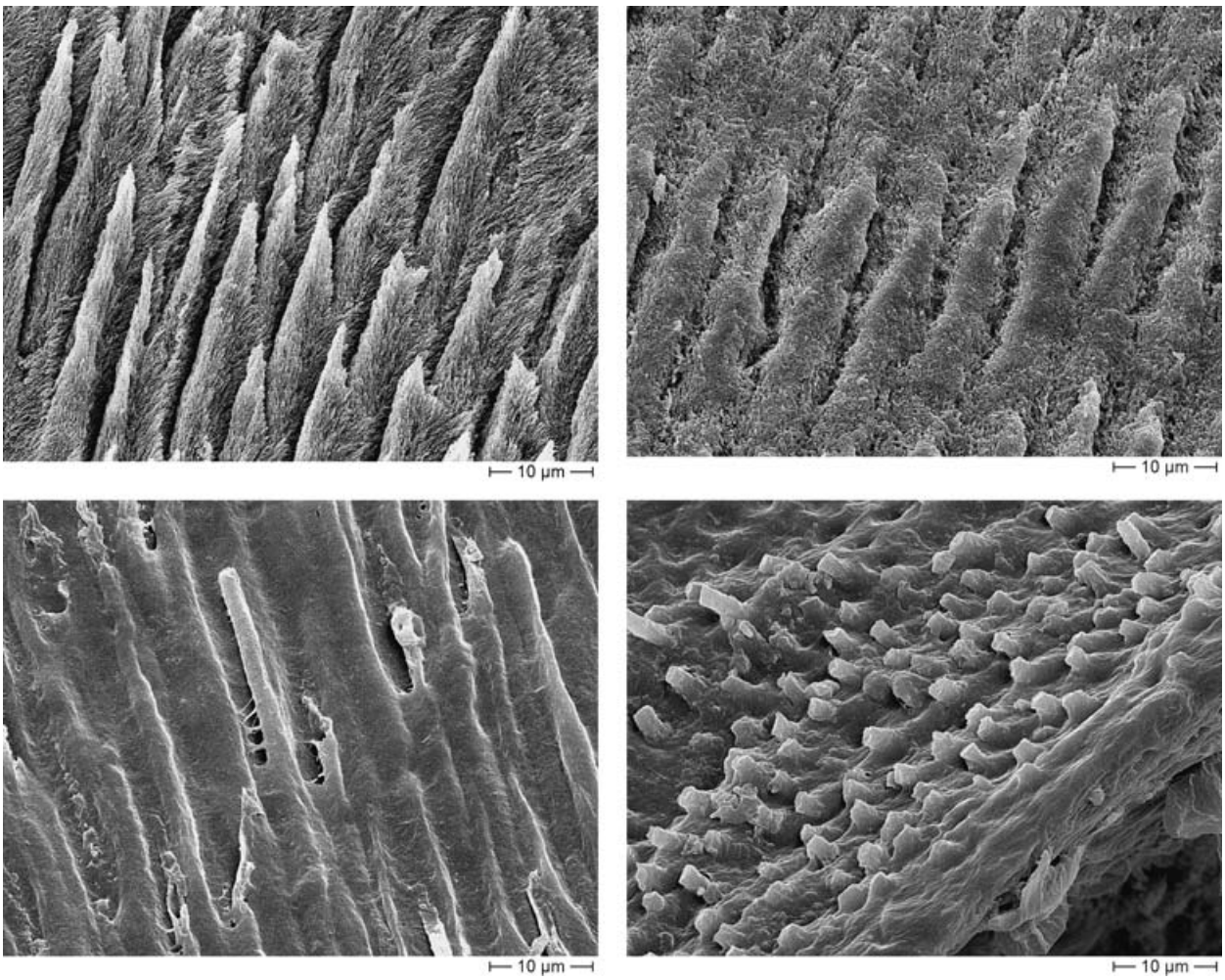

Fig. 4. (a) (top left) Scanning electron microscope (SEM) image of normal enamel in a primary incisor from an Ehlers–Danlos syndrome (EDS) patient (magnification 2000×). (b) (top right) SEM image of hypomineralized enamel in a primary incisor from an EDS patient (magnification 2000×). (c) (bottom left) SEM image of dentine in a primary incisor from an EDS patient (magnification ×2000). (d) (bottom right) SEM image of reparative dentine in a primary incisor from an EDS patient (magnification ×2000).

< 0.05) in the EDS dentine compared with normal dentine, with the exception for location 2 (Fig. 6a). P and Ca had significantly higher values in all locations (*P* values < 0.001, < 0.01, and < 0.05) (Fig. 6b). All ratios, except for Ca/P, differed significantly between normal dentine and dentine in the EDS teeth. For Ca/C, the dentine of EDS teeth had higher values in all locations ($P < 0.001$) (Fig. 6c). In the EDS dentine, Ca/O had significantly higher values compared with normal dentine (*P* values < 0.01 and < 0.05) except for location 5 (Fig. 6c).

As the number of individuals and teeth in each of the different types of EDS (classical, hypermobility, and unspecified) was low, no comparisons were made between the different types, or between the types and healthy controls.

Discussion

This study has shown that the enamel in primary teeth from patients diagnosed with EDS exhibits a high frequency of postnatally hypomineralized enamel and postnatally located incremental lines. Further, the elemental analyses revealed statistically significant differences between dentine in primary teeth from patients diagnosed with EDS and dentine from healthy children. In the enamel, the chemical differences were mainly found for carbon.

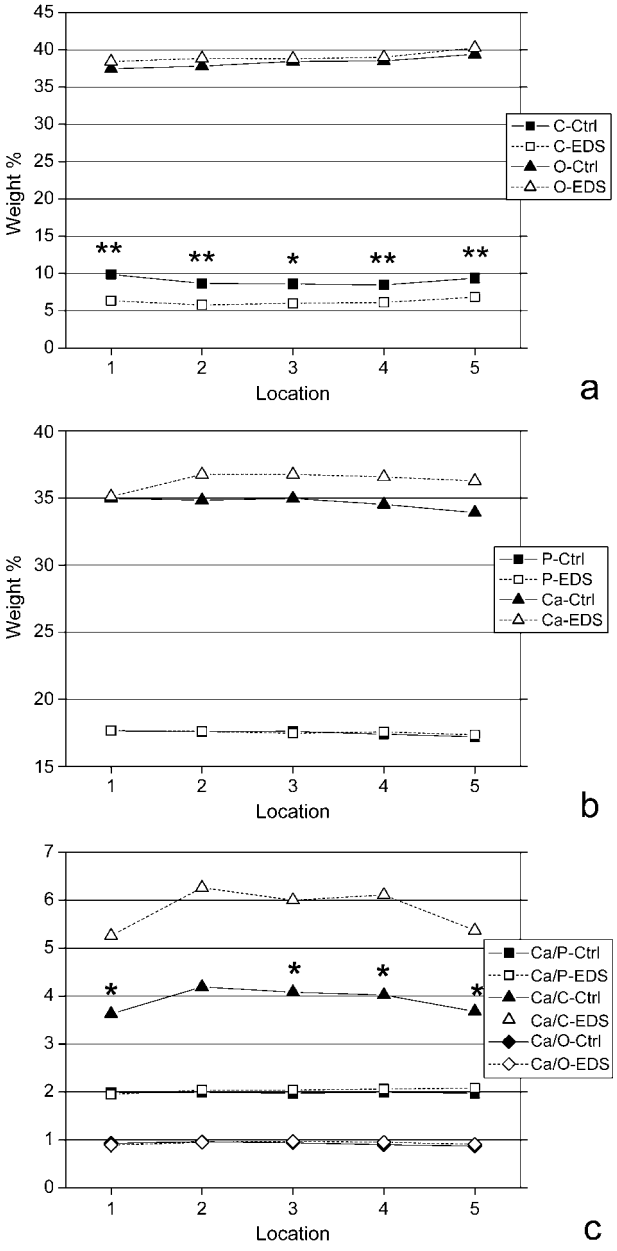

Fig. 5. (a) Chart of median values form the X-ray microanalysis (XRMA) measurement of C and O in five locations in the enamel of primary teeth from Ehlers–Danlos syndrome (EDS) patients and controls (weight %). Mann–Whitney *U*-test (**P* < 0.05; ***P* < 0.01). (b) Chart of median values form the XRMA measurement of P and Ca in five locations in the enamel of primary teeth from EDS patients and controls (weight %). Mann–Whitney *U*-test. (c) Chart of median values form the XRMA measurement of the ratios Ca/P, Ca/C, and Ca/O in five locations in the enamel of primary teeth from EDS patients and controls. Mann–Whitney *U*-test (**P* < 0.05).

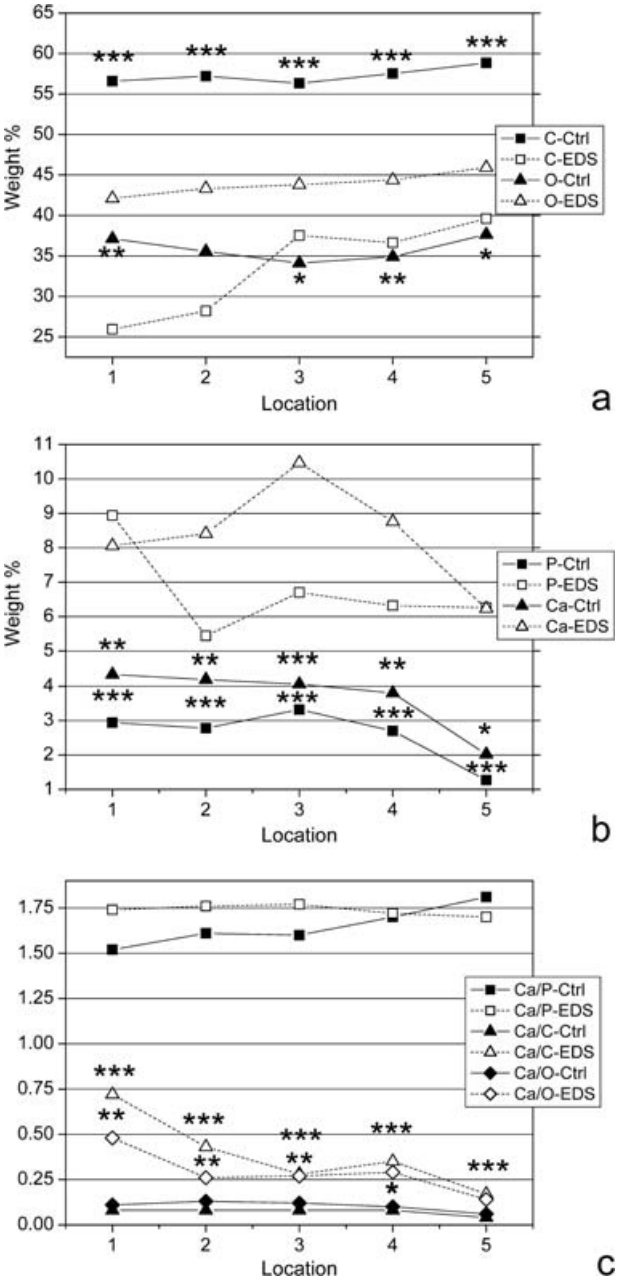

Fig. 6. (a) Chart of median values form the X-ray microanalysis (XRMA) measurement of C and O in five locations in the dentine of primary teeth from Ehlers–Danlos syndrome (EDS) patients and controls (weight %). Mann–Whitney *U*-test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). (b) Chart of median values form the XRMA measurement of P and Ca in five locations in the dentine of primary teeth from EDS patients and controls (weight %). Mann–Whitney *U*-test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001). (c) Chart of median values form the XRMA measurement of the ratios Ca/P, Ca/C, and Ca/O in five locations in the dentine of primary teeth from EDS patients and controls. Mann–Whitney *U*-test (**P* < 0.05; ***P* < 0.01; ****P* < 0.001).

This study included teeth from 25 patients. This might not seem to be a high number. It should be pointed out, however, that EDS is regarded to be a rare disorder, and this study is in fact one of very few studies including specimens from more than just one patient. Further, comparisons were made with a material of normal teeth from healthy individuals. A majority of the patients had EDS of unspecified type followed by classical and hypermobility types. This is not what might have been expected as the hypermobility type is the most common subtype. This discrepancy is probably owing to the fact that the material was collected based on voluntary sending of teeth for examination, and for the high number of unspecified cases. Having an unspecified type of EDS is possible according to the Villefranche nosolgy[4], and probably more frequent in young individuals where all symptoms and clinical problems are not always apparent. In the clinical situation, several patients presented clear symptoms of EDS, but do not fit into the lists of symptoms of the specific subtypes found in the Villefranche nosology[4]. These are today labelled as EDS unspecified subtype.

Morphologically, few aberrations were found in the dentine of primary teeth from patients with EDS but were found in high frequency in the postnatal enamel. The microporous prenatal enamel coincides with what has been described earlier in primary teeth from healthy individuals[12]. The anatomy of the examined teeth showed no abnormal morphology which previously has been reported[9–11]. The histomorphological appearance of enamel has previously been reported to show hypoplastic areas in the enamel and some structural changes of the EDJ[9]; this was not revealed in this study. The high frequency of postnatally hypomineralized enamel and postnatally located incremental lines in this study is a new finding not reported before. Whether the hypomineralization of the postnatal enamel may be directly attributed to EDS or not is not possible to elucidate; however, because EDS is a condition of connective tissue disorder with impaired extracellular structure matrix affecting the blood vessels, it is not unlikely that the ameloblasts would be affected as well. This may be supported by the fact that the vascularization of the enamel organ occurs when the tooth germ is fully developed[13].

Because the primary teeth examined in this study were exfoliated and thus physiologically resorbed, no root dentine or cementum could be seen. But, in the remaining coronal dentine, no aberrations were discerned which is in concordance with what has earlier been found[9,11].

The chemical analysis revealed a lower carbon content in primary enamel in teeth from EDS patients. Because the Ca/P ratio did not differ between the groups, the basic mineralization of the hydroxyl apatite may be regarded as normal. Further, the crystallites of the enamel may be smaller than normal, and the interprismatic organic content is lower than in normal enamel.

The ratio Ca/P did not differ between normal and EDS dentine which indicates that the apatite of the dental hard tissues was normal and in concordance with previous studies[14]. The same pattern for Ca/P in enamel from EDS patients and controls indicates a normal degree of mineralization. The higher values for Ca and P, and the higher Ca/O ratio and lower *C* values in the dentine from the EDS teeth indicate that the dentine is more mineralized compared with normal dentine. From a chemical point of view, it is obvious that the dentine in primary teeth from patients with EDS, even though it does not reveal any morphological changes, differs considerably from that of dentine from normal patients.

The deviations in primary enamel could lead to vulnerability for dental caries. When the demineralization process in a caries lesion has started, the enamel in patients with EDS has a structure that could make the progress faster than in healthy patients with normal enamel. The importance of a higher degree of mineralization in the dentine for the caries process is hard to evaluate. Still, the findings of aberrations in enamel support findings of more dental caries in patients with EDS than previously reported from clinical studies[5]. The study by De Coster *et al.*[5] also reported of poorer oral hygiene in patients with EDS, and a relationship between plaque and fragile oral mucosa. Problems with oral hygiene could probably further increase the risk of initiation of the demineralization process and should thus be

recognized as a risk factor in these patients. It should be pointed out that the present investigation was limited to primary teeth, and it therefore is important to look at histomorphology and chemical composition also in permanent teeth from patients with EDS in order to gain a more comprehensive understanding.

Another clinical implication is the importance of higher mineralization of the dentine in relation to tooth fracture. Dentists treating patients with EDS frequently meet patients describing spontaneous tooth fractures in non-carious teeth. This was also pinpointed in a recent study where 41% of a large study population of patients with EDS reported these problems[7]. It is plausible that altered mineralization of the dentine leads to changed elasticity of the dentine which increases the risk for fractures. Again, the results of this study are based on analyses of primary teeth, and thus, it would be valuable to also analyse the permanent successors.

In conclusion, this study has revealed a high frequency of aberrations in both enamel and dentine in primary teeth from patients with EDS. These could explain the occurrence of both more dental caries and tooth fractures in patients with EDS.

What this paper adds

- This paper adds new information about morphology and chemical composition of both enamel and dentine in primary teeth in EDS.
- Deviations in foremost enamel of primary teeth could explain the caries problems reported in patients with EDS.
- The chemical composition of dentine in primary teeth is different from that in normal teeth and could explain the increased occurrence of tooth fracture in EDS.

Why this paper is important to paediatric dentists

- Child patients with EDS often need specialist paediatric dental treatment, wherefore it is important that paediatric dentists know about the syndrome and its oral manifestations.

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