Effect of dentinogenesis imperfecta and osteogensis imperfecta on the dental ultrastructures

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Abstract

Dentinogenesis imperfecta (DGI) is a disorder of tooth development mainly affecting the dentin. This condition causes the teeth to be discolored, weak, and prone to rapid wear. To date, the understanding of tooth ultrastructure affected with DGI is very limited. This study aims to investigate the mineral density and ultrastructure of the teeth from the patients affected with DGI and osteogenesis imperfecta (syndromic DGI). Teeth were collected from 2 patients diagnosed with syndromic DGI. Tooth color, surface roughness, microhardness, mineral composition, and microscopic morphology of the DGI teeth, compared with the similar tooth-type control teeth, were studied using the digital intraoral colorimeter, surface profilometer, Knoop microhardness, Energy-Dispersive X-ray (EDX), and Scanning Electron Microscopy (SEM). The syndromic DGI teeth were darker and more bluish and had higher surface roughness than the controls. The DGI dentin had lower mineral density than the controls. Correspondingly, the Knoop microhardness values of the DGI dentin and enamel were statistically significantly lower than those of the controls. The reduction in calcium, phosphorus, and oxygen levels and an increase in carbon content were observed in DGI teeth. The alteration in mineral contents and reduction in dentin density could lead to the reduced hardness of the DGI teeth, making them weak and prone to deteriorate. This study demonstrates that the ultrastructure of syndromic DGI teeth are diversely disturbed including tooth color, mineral density, microhardness, and mineral composition. These variations could affect the integrity of the teeth and the outcome of dental restoration.

Keywords**:** *Dentinogenesis imperfecta, Osteogenesis imperfecta, dentin, tooth ultrastructure*

1. Introduction

Dentinogenesis imperfecta (DGI) is an autosomal dominant genetic condition caused by abnormal dentin formation. Prevalence of DGI in the United stated is reported to be 1 in 6,000-8,000(Barron, McDonnell, Mackie, & Dixon, 2008) Both primary and permanent dentitions are affected. DGI is classified into 3 types based on phenotypic variability (Shields, Bixler, & El-Kafrawy, 1973). DGI Type I (syndromic DGI) is associated with bone disorder, osteogenesis imperfect (OI) (Leroi, Overshott, Byrne, Daniel, & Burns), mostly caused by mutations in *COL1A1* or *COL1A2* gene that encode type 1 collagen (Barron et al., 2008; Majorana et al., 2010). OI is an inherited brittle bone disease characterized by short stature and bone deformities. DGI Type II is non-syndromic and not associated with OI. Oral features of DGI type II are similar to type I. The DGI teeth are opalescent in color ranging from bluish-gray to yellow-brown. The teeth are weaker than normal making them prone to rapid wear and fracture (Kim & Simmer, 2007; Singhal, Arya, Vengal, & Bhalodia, 2015). Radiographically, the DGI teeth show bulbous crowns, cervical constrictions, various sizes of pulp cavities, and short and narrow roots. The pulp chambers and root canals may be abnormally wide and look like “shell teeth” which can be progressively obliterated due to dentin overproduction (Leal, Martins, Verli, de Souza, & Ramos-Jorge, 2010; Majorana et al., 2010; Sapir & Shapira, 2001). DGI type III or Brandywine type was found in the Brandywine triracial isolate in Southern Maryland. DGI type III shows variation in color and morphology of the teeth. Multiple pulp exposures and “shell teeth” are major characteristics of type III (Seow, 2014). Histologically, the dentinal tubules of the circumferential dentin in DGI teeth are coarse, branched, irregular, and reduced in number. A tubular area

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in the dentin with reduced mineralization and reduced number of odontoblasts is commonly present. The enamel is normal but can be crack easily due to abnormal dentin and dentinoenamel junction (DEJ). The fractured enamel results in rapid wearing and loss of interdental contact making the DGI teeth more susceptible to caries. DGI type II and III have been associated with mutations in the dentin sialophosphoprotein (*DSPP*) gene locating on chromosome 4q21. The *DSPP* encodes major non-collagenous proteins of the dentin including dentin phosphoprotein (DPP) and dentin sialoprotein (DSP). The *DSPP* is expressed in a number of tissues including bone, kidney, salivary gland, and lung but its expression in dentin is much higher than that in other tissues (Singhal et al., 2015). These three traditional categories of DGI were proposed based on clinical and radiographic manifestations. The recent genetic study attributes that DGI type II and type III could be the same disease with phenotypic variability (Barron et al., 2008).

Porntaveetus et al. (2017) reported that the primary teeth affected with non-syndromic DGI showed opalescent color, amorphous dentin, irregular and reduced dentinal tubules, and reduced mineral density compared to the controls. Kerbel et al. (1981) reported that DGI type II dentin showed increased calcium/phosphate ratio, reduced of calcium and phosphate, and low number of Magnesium. Wieczorek et al. (2012, 2013) showed that the enamel of DGI type II had decreased calcium and magnesium and increased phosphorus composition compared to the controls. Reductions in calcium-phosphorus ratio, modulus of elasticity, and hardness were reported in DGI type II teeth. The Vickers microhardness value of the DGI type II enamel was significantly reduced, compared with the normal enamel. To date, the studies of ultrastructure of syndromic DGI are limited.

2. Objectives

To study the mineral density and ultrastructure of the teeth affected by syndromic DGI. Tooth color, surface roughness, microhardness, mineral composition, and microscopic morphology of the affected teeth were studied using the digital intraoral colorimeter, surface profilometer, Knoop microhardness, Energy-Dispersive X-ray (EDX), and Scanning Electron Microscopy (SEM). This study aims to understand dental alterations caused by syndromic DGI and to provide appropriate dental management to the patients.

3. Materials and Methods

Subject enrollment

The teeth that were exfoliated or extracted according to dental treatment plan were subject for investigation. The primary maxillary right first molar was obtained from a 7-year-old Thai boy (OIDI1) and the primary mandibular right second molar from a 13-year-old Thai boy (OIDI2). Both patients were diagnosed with OI and DGI (DGI type I or syndromic DGI). Each sample was investigated and compared with two control teeth (similar tooth type) collected from age- and sex-matched healthy individuals. Patients' informed consents were obtained. The project was approved by the research ethics committee at the Faculty of Dentistry, Chulalongkorn, Thailand University Study code: HREC-DCU 2018-092).

Color measurement

Tooth shade was measured with a digital intraoral colorimeter (VITA Easyshade® V, VITA Zahnfabrik; H. Rauter GmbH & Co, KG, Germany) according to the manufacturer's recommendations. The measurements were acquired from each specimen by contacting the device tip on the smooth buccal and lingual surfaces of the enamel, repeated three times. The color was recorded in L*a*b values defined by the International Commission on Illumination (Wieczorek, Loster, Ryniewicz, & Ryniewicz). The L* displays the lightness alterations in the black and white scale, a* is the saturation in the red and green scale, and b* is the saturation in the blue and yellow scale. The color difference (ΔE*) was calculated using the equation.

$$\Delta E = \sqrt{(L_2 - L_1)^2 + (a_2 - a_1)^2 + (b_2 - b_1)^2}$$

Figure 1 The equation of color difference (ΔE*)

Micro CT analysis

The teeth scanned with specimen µCT 35 (SCANCO Medical, Brüttisellen, Switzerland) at the Mineralized Tissue Research Unit at the Faculty of Dentistry, Chulalongkorn University. All images were processed with the Image Processing Language (IPL, Scanco Medical AG). The data were available for three-dimensional (3D) reconstructions and cross-section.

Surface roughness measurement

Surface roughness was measured by a surface profilometer (model Talyscan 150, Taylor Hobson Ltd). Each specimen was measured 10 times randomly every 600 micrometers on Y-axis on the enamel at the buccal and lingual surface. Tracing area was at 1 mm x 1 mm; stylus speed at 1000 micrometer/second; and cut-off length at 0. 25 mm. The calculation of the surface topography parameters was carried out by the TalyMap Universal program

Microhardness measurement

Samples were embedded in an acrylic block and divided in Bucco-lingual axis to expose dentin surface using the low-speed precision saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) with a diamond disc at a speed of 450 rpm under constant water cooling. Then, a cut surface was grinded with Grit#1200 Silicon-carbide paper under water irrigation until the surface was flat and parallel. The alumina powder was used to polish the samples on the polishing pad (10-inch MICROPAD, Pace technologies). Once the samples prepared, the micrographs were acquired by stereo-microscopy (Olympus SZ61, Tokyo, Japan). The Knoop microhardness (HK) was measured using microhardness tester (FM700e Type D, FUTURE TECH; Japan). All specimens were impressed with a load of 100 gF for 15 seconds. Five indentations were performed at each surface. Values of Knoop hardness were calculated for enamel and dentine according to formula, HK value, Knoop hardness number; P value, test load (N); L value, indentation diagonal length(µm).

$$HK = 14229\frac{P}{L^2}$$

Figure 2 The equation of Knoop hardness values.

Gold coating and Energy-Dispersive X-ray (EDX) and Scanning Electron Microscopy (SEM)

The specimens were fixed in 2.5% glutaraldehyde and washed in phosphate buffered saline 3-5 minutes for 2 times. Dehydration with ethanol series (30%, 50%, 70%, 90%, and 100% ethanol) was performed at 10 minutes for each step. The specimens were bathed in 100% ethanol and placed in a critical point dryer (Emitech K850, Emitech Ltd, Kent, England). Dried specimens were placed on aluminum stubs and covered with golden powder in a media of argon-cathode atomization with fine coater (JFC 1200, Tokyo, Japan) for 10 seconds. EDX (ISIS 300 EDX-system; Oxford Instruments, UK) and SEM (Jeol, JSM 5410 LV Scanning electron microscope, Japan) were used to determine the elemental levels (%) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca).

Statistical Analysis

ANOVA and post hoc comparison for Tukey's test at P-value<0.05 were performed.

4. Results and Discussion

Color measurement

OIDI1 and OIDI2 samples had lower L* and b* values than those of the controls. The color different (ΔE) of OIDI1 and OIDI2 were 44.75 and 42.88, respectively (Figure 3 and Table 1). These suggest that the teeth affected with syndromic DGI were darker and bluer than the control teeth and the differences in tooth color between DGI and normal teeth can be perceived by human eyes.

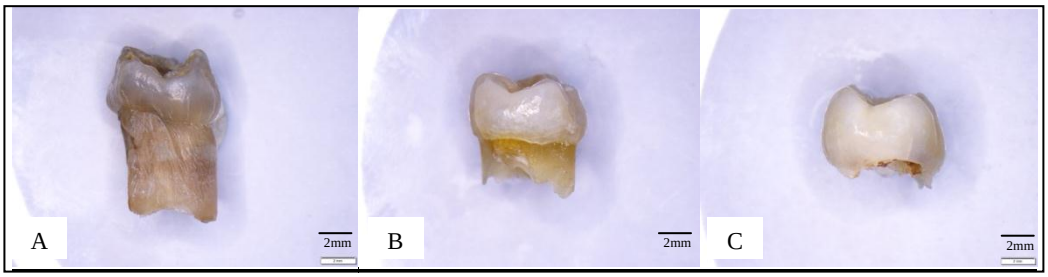

Figure 3 The teeth obtained from the patients affected with syndromic DGI. (A) OIDI1 (B) OIDI2 and (C) Control

Table 1 CIE L*a*b mean values and delta E values of the OIDI and control samples.

Tooth	L*	a*	b*	ΔE	Mean ΔE
OIDI1	38.00	3.15	26.15		
ControlOIDI1-1	90.35	3.35	43.45	45.72	44.75
ControlOIDI1-2	83.40	4.25	43.70	43.78	
OIDI2	62.00	2.20	21.55		42.88
ControlOIDI2-1	93.10	0.45	35.67	43.07	
ControlOIDI2-2	89.00	0.90	38.15	42.68	

Micro CT analysis

The vertical cross-sectional of teeth showed that the enamel thickness of OIDI samples was similar to that of the controls. The dentin thickness of OIDI1 was thinner while that of OIDI2 was thicker than that of the controls. The pulp cavity of OIDI2 was not seen by micro-CT (Figure 4). For OIDI1, the mineral density of enamel was 2162.48 mg/cm^3 (controls 2152.76 and 2155.24 mg/cm^3) and of dentin was 1190.13 mg/cm^3 (controls 1353.53 and 1227.06 mg/cm^3). For OIDI2, the mineral density of enamel was 2091.64 (controls 2191.61 and 2168.65 mg/cm^3) and of dentin was 1187.69 mg/cm^3 (control 1233.30 and 1207.95 mg/cm^3) (Figure 5).

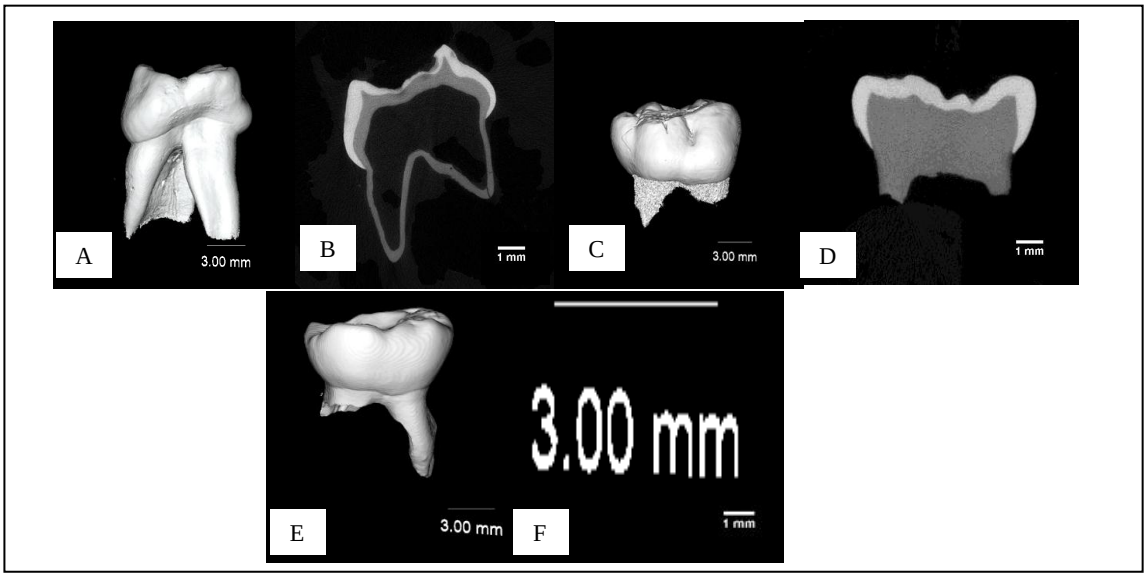

Figure 4 Micro-CT imaging showed three-dimensional (3D) reconstructions and vertical cross-section of teeth. In cross sections, the white area is the enamel and the grey area is the dentin. OIDI1(A,B), OIDI2(C,D) and control(E,F), respectively.

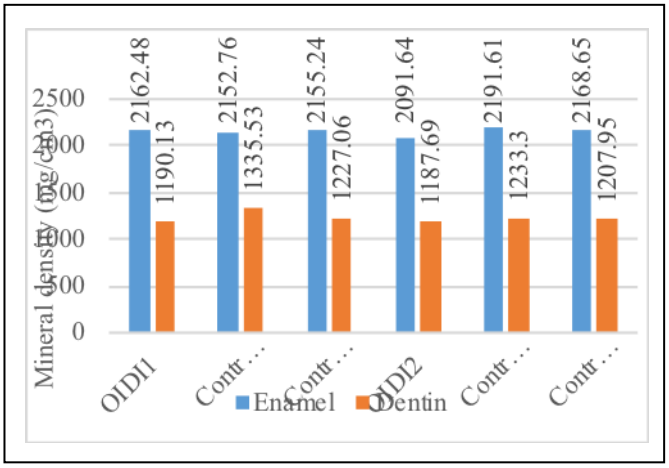

Figure 5 The mineral densities of enamel and dentin of the OIDI and control samples.

Surface roughness measurement

The mean surface roughness of OIDI1 was 0.49±0.10 μm (control 0.27±0.06 and 0.31±0.08 μm) and that of OIDI2 was 0.42±0.07 μm (control 0.28±0.03 and 0.30±0.04 μm). The statistically significant differences of surface roughness were observed between OIDI1 and controls and between OIDI2 and controls (Table2).

Table 2 Surface roughness of the enamel on buccal and lingual surfaces of OIDI samples and controls shown in mean ± standard deviation (SD). *P<0.05, N.S., not significant.

Surface roughness (μm)	Buccal enamel	Sig (P < 0.05)	Lingual enamel	Sig (P < 0.05)	Mean values
OIDI1	0.54±0.11	N.S.	0.43±006	N.S.	0.49±0.10
OIDI2	0.45±0.06		0.39±0.07		0.42±0.07
ControlOIDI1-1	0.29±0.03	*	0.25±0.06	*	0.27±0.06
ControlOIDI1-2	0.35±0.09	N.S.	0.27±0.04	N.S.	0.31±0.08
ControlOIDI2-1	0.28±0.03	*	0.27±0.02	*	0.28±0.03
ControlOIDI2-2	0.29±0.04		0.31±0.03		0.30±0.04

Microhardness measurement

For OIDI1, the mean Knoop microhardness of the enamel was 225.10±3.93 GPa (control 253.86±6.73 and 241.80±9.15 GPa) and of the dentin was 24.99±0.86 (controls 62.14±3.76 and 59.90±1.80 GPa). For OIDI2, the microhardness of the enamel was 266.86±6.78 GPa (control 247.83±5.78 and 251.29±8.21 GPa) and of the dentin was 34.35±1.83 GPa (control 62.24±1.13 and 63.21±1.04 GPa). In the enamel and dentin, the statistical differences were found between OIDI1-controls, OIDI2-controls, and OIDI1-OIDI2 (Figure 6 and Table 3).

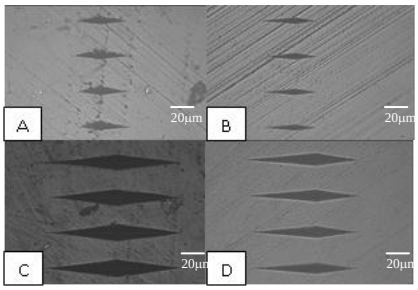

Figure 6 Stereomicroscope at 20x magnification showed Knoop microhardness indentation of OIDI enamel(A) and dentin(C) compared with control enamel(B) and control dentin(D).

Table 3 Knoop microhardness of the enamel and dentin of OIDI samples and controls shown in mean ± standard deviation (SD). *P<0.05, N.S., not significant.

Microhardness (GPa)	Enamel	Sig (P < 0.05)	Dentin	Sig (P < 0.05)
OIDI1	225.10±3.93	*	24.99±0.86	*
OIDI2	266.86±6.78		34.35±1.83	
Control OIDI1-1	253.86±6.73	*	62.14±3.76	*
Control OIDI1-2	241.80±9.15	*	59.90±1.80	*
Control OIDI2-1	247.83±5.78	N.S.	62.24±1.13	N.S.
Control OIDI2-2	251.29±8.21		63.21±1.04	

Gold coating and Energy-Dispersive X-ray (EDX)

The amount of carbon in OIDI1 and OIDI2 was higher than that in the controls whereas the levels O, P and Ca were less than those of the controls (Table 4).

Table 4 Element analysis (% weight) of carbon (C), oxygen (O), phosphorus (P), calcium (Ca), and Ca/P ratio of OIDI and control samples.

Elemental analysis (% weight)	Enamel					Dentin				
	C	O	P	Ca	Ca/P	C	O	P	Ca	Ca/P
OIDI1	32.26	35.29	11.04	21.43	1.94	26.44	36.82	12.39	24.37	1.97
OIDI2	79.69	11.75	2.53	6.03	2.38	72.61	10.54	3.77	13.09	3.48
Control OIDI1-1	9.58	40.18	16.35	33.9	2.07	14.51	40.23	14.84	30.43	2.05
Control OIDI1-2	8.94	36.98	16.89	37.2	2.2	15.745	37.37	14.91	32	2.15
Control OIDI2-1	13.58	40.33	15.42	30.68	1.99	18.23	40.79	14.12	26.87	1.9
Control OIDI2-2	5.83	36.81	17.78	39.585	2.23	14.27	37.16	15.48	33.1	2.14

Discussion

Our study identified that the teeth of the patients affected with syndromic DGI were different from the normal teeth in many aspects. The DGI teeth were shown to be opalescent in color ranging from bluish-gray to yellow-brown (Kim & Simmer, 2007; Shields et al., 1973; Singhal et al., 2015). (Johnston & Kao, 1989) reported human eyes can perceive the different color when $\Delta E > 3.7$. Consistently, this study observed that the color of DGI teeth was very obviously different from the control. They were darker and more bluish than normal teeth.

It was shown that the DGI teeth had a bulbous crown, cervical constriction, and variation in a size of pulp cavity. In this study, the enamel of DGI teeth was normal in thickness. However, thin dentin and large pulp chamber were radiologically found in OIDI1 while the thick dentin and no pulp chamber were

found in OIDI2. These suggest the diverse radiographic features of the dentin and pulp cavity associated with syndromic DGI. It is possible that the absence or obliteration of dental pulp in DGI teeth could be due to overproduction of the dentin (Sapir & Shapira, 2001; Singhal et al., 2015). The difference of tooth shade and illumination between OIDI and control teeth could be caused by alterations of dentinal tubules and pulp cavities in OIDI teeth.

We observed that the mineral density of the dentin and the microhardness of the teeth affected with syndromic DGI were reduced. The teeth affected with non-syndromic DGI showed a decrease in calcium and magnesium levels and an increase in phosphorus composition compared with the controls (Wieczorek & Loster, 2012; Wieczorek, Loster, Ryniewicz, & Ryniewicz, 2013b). Our EDX results showed that the syndromic DGI teeth had lower oxygen, phosphorus, and calcium levels, but higher carbon content than the controls. These imply that the teeth affected with both syndromic and non-syndromic DGI had a reduction in calcium composition. The decrease in mineral density and calcium could lead to reduced microhardness of the DGI teeth making them prone to rapid wear and fracture. Consistently, it was suggested that the reduction in hardness of DGI dentin was associated with the decrease of mineral content and the density of dentinal tubules (Gutiérrez-Salazar & Reyes-Gasga, 2003).

Due to the limitation of samples available in this study, the collection of more tooth samples obtained from the patients with syndromic DGI would validate the findings of our study. In addition, non-syndromic DGI should be included in the future study to expand the understanding of tooth anomalies associated with DGI.

5. Conclusion

We show that the ultrastructure of the teeth affected with syndromic DGI have diverse alterations. The color of the DGI teeth is noticeably dark by human eyes. The mineral density, microhardness, and levels of calcium, phosphorus, and oxygen of syndromic DGI teeth are reduced. These affect the integrity of the teeth and dental treatment tailored for the DGI patients. Our study provides an understanding of the effect of DGI and OI on dental ultrastructure.

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