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Dentinogenesis imperfecta in children with osteogenesis imperfecta: a clinical and ultrastructural study

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Aim. The aim of this study was to assess the correlation between osteogenesis imperfecta (OI) and dentinogenesis imperfecta (DI) from both a clinical and histological point of view, particularly clarifying the structural and ultrastructural dentine changes.

Design. Sixteen children (6–12 years aged) with diagnosis of OI were examined for dental alterations referable to DI. For each patient, the OI type (I, III, or IV) was recorded. Extracted or normally exfoliated primary teeth were subjected to a histological examination (to both optical microscopy and confocal laser-scanning microscopy).

Results. A total of ten patients had abnormal discolourations referable to DI: four patients were affected by OI type I, three patients by OI type III, and three patients by OI type IV. The discolourations, yellow/brown or opalescent grey, could not be related to the different types of OI. Histological exam of primary teeth showed severe pathological change in the dentin, structured into four different layers. A collagen defect due to odontoblast dysfunction was theorized to be on the base of the histological changes.

Conclusions. There is no correlation between the type of OI and the type of discolouration. The underlying dentinal defect seems to be related to an odontoblast dysfunction.

Introduction

Osteogenesis imperfecta (OI) or 'bone fragility disease' is a heterogeneous group of heritable connective tissue disorders, caused by a quantity and/or qualitative defect in type 1 collagen synthesis. The condition results from mutations in the genes (*COL1A1* and *COL1A2*) that encode for either chain of type 1 collagen. The most prominent clinical feature of the disease is fragile bone that leads to recurrent fractures (even in consequence of very mild traumas) and to skeletal deformities. All tissue rich in type 1 collagen can be affected. Patients may have blue sclera, hearing loss, dentinogenesis imperfecta (DI), growth deficiency, joint laxity, or any combination of these characteristics. OI was classified in 1979 by Sillence *et al.*[1] into four types, as shown in Table 1. On the basis of clinical, radiographical, and genetical findings, four main phenotypes of OI are recognized: a mild form (type I); a perinatally lethal syndrome (type II); a progressively deforming form (type III); and a moderately severe form (type IV) (Ref. 1, reviewed in Mini-Mendelian Inheritance in Man 166200, OMIM, 2000). A reduction in the quantity of collagen results in OI type I, whereas OI types II, III, and IV depend on both qualitative and quantitative alterations in collagen synthesis. According to the presence or absence of DI, Types I and IV are further divided into subgroups A (without DI) and B (with DI) whereas, in type III, DI is included in diagnostic criteria. No biochemical classification is currently available[1–4].

Dentinogenesis imperfecta is a hereditary disorder in dentin formation that comprises a group of autosomal dominant genetic conditions characterized by abnormal dentine structure affecting either the primary or both the primary and secondary dentitions[5]. DI may present as single trait disorders or

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Table 1. Classification of osteogenesis imperfecta (OI) modified from Byers (1993), Sillence *et al.* (1979)and Levin *et al.* (1998).

OI type	DI	Clinical features	Inheritance
IA	–	Normal or mild short stature	AD
IB	+	Little or no deformity Blue sclera Hearing loss common	
II	?	Extremely severe osseous fragility, still birth or death in the newborn period and beaded ribs	AD AR (rare)
III	+/–	Very short stature Progressively deforming bones, usually with moderate deformity at birth Scleral hue varies, often lightening with age Hearing loss less common than in type I	AD AR (uncommon)
IVA	–	Variably short stature	AD
IVB	+	Mild to moderate bone deformity Normal sclera Hearing loss less common than in type I	

DI, dentinogenesis imperfecta; AD, autosomal dominant; AR, autosomal recessive; ?, unknown.

associate with OI[6]. Clinically, the teeth are characterized by a discolouration that ranges from grey/brown to opalescent blue. Radiographically, they show short roots, bulbous crown with marked cervical constriction, and pulpal obliterations. The primary teeth are more severely affected than the permanent. Enamel is normal in thickness and radiodensity but the underlying dentinal defect of mineralization often induces the enamel detachment (leaving exposed weakened dentine that is prone to wear)[7,8]. The most familiar classification system is that formulated by Shields *et al.*[9] in 1973 recognizing three types of DI (types I, II, and III). DI type 1 is that associated with OI. The teeth of both dentitions are typically amber and translucent and show significant attrition. Radiographically, they have short, constricted roots and dentine hypertrophy leading to pulpal obliteration either before or just after eruption. Expressivity is variable even within an individual, with some teeth showing total pulpal obliteration, whereas in others the dentine appears normal. The dental features of DI type 2 are similar to those of DI type 1 but OI is not associated. Bulbous crowns with marked cervical constriction are a typical feature. Normal teeth are never found in DI type 2. DI type 3 is in a tri-racial population from Maryland and Washington, DC, known as the Brandywine isolate[10]. The clinical features are variable and resemble those seen in DI types 1 and 2 but the primary teeth show multiple pulp exposures and, radiographically, they often manifest 'shell' teeth, i.e., teeth that appear hollow due to hypotrophy of the dentine.[5,11]

Dentinogenesis imperfecta type I is inherited with OI and recent genetic studies have shown that mutations in the genes encoding collagen type 1, *COL1A1* and *COL1A2*, underlie this condition. All other forms of DI appear to result from mutations in the gene encoding dentine sialophosphoprotein (*DSPP*), suggesting that these conditions are allelic[12]. The Shields' system is increasingly out of date as it does not account for the genetic aetiologies of the hereditary dentine defects[7,8]. Unfortunately, the genetic defects that have been discovered to date are insufficient to allow the construction of a comprehensive classification based on the knowledge of the underlying mutations.

Histologically, the dentin is similarly affected in the three types of DI. A layer of normal mantle dentin with an irregular texture of dentinal matrix and an abnormal number and structure of dentine tubules is reported. Consistently there are atubular areas of dentin. [13–16] The normal scalloping of the dentin–enamel junction is present in teeth with DI. Loss of enamel is not a result of abnormal dentin–enamel junction but is rather due to a weakness within the dentin itself. Differential diagnoses include hypocalcified forms of amelogenesis imperfecta, congenital erythropoietic porphyria, conditions leading to early tooth loss (Kostmann's disease, cyclic neutropenia, Chediak–Hegashi syndrome, histiocytosis X, Papillon–Lefevre syndrome), permanent teeth discolourations due to tetracyclines, and Vitamin D-dependent and vitamin D-resistant rickets[5]. The aim of the study was to investigate the correlations between the different types of OI and DI, both from a clinical and histological point of view, particularly clarifying the structural

Table 2. Number of children with dentinogenesis imperfecta (only in primary teeth or only in permanent teeth or both in primary and in permanent teeth) for each type of osteogenesis imperfecta.

		Dentinogenesis imperfecta			
OI type	**Patients (*n*)**	**Primary**	**Permanent**	**Both**	**Total**
I	9	1	2	1	4
III	3	0	1	2	3
IV	4	1	0	2	3
	16				10

and ultrastructural dentine changes in children with OI.

Materials and methods

Sixteen children, six females and ten males, age range 6–12 years (mean age 9.6 ± 1.7), with diagnosis of OI were investigated. Twelve were recruited from the Department of Pediatric Dentistry of Brescia and four from the Department of Pediatric Dentistry of Bari.

Nine patients had OI type I, three with OI type III, and four with OI type IV.

All the patients were subjected to a careful examination of the oral cavity, in order to assess the presence or absence of dental alterations referable to DI, taking into account of clinical and radiographical findings.

Discolourations (from yellow–brown to blue–grey, with opalescent aspect), abnormalities in crown shape ('tulip' or 'bell' shape with cervical constriction), and dental attrition (graded according Eccles' criteria[17,18] based on clinical severity) were recorded. Panoramic radiographs were obtained to detect abnormally large or obliterated pulp chambers and short or thin roots with typical periapical radiotransparence due to fibrotic tissue. As most of the patients were in mixed dentition, the presence of DI only on primary teeth or only on permanent teeth or in both cases was separately recorded. The dental defects were in some cases documented photographically.

During the period of observation, the primary teeth in exfoliative phase were extracted and underwent histological examination, to both optical microscopy (OM) and confocal laser-scanning microscopy (CLSM). The primary teeth were decalcified with MIELODEC™ (BIO OPTICA, Milan, Italy), an appropriate fixing and decalcifying solution containing formalin and mercuric chloride as a fixative and EDTA as a decalcifying agent. Then the teeth were placed in paraffin, cut by microtome into sections with a thickness of 5 μm, and stained with Haematoxylin/Eosin and Masson Tricromic (Dako Italia s.p.a. Milan Italy).

Results

The prevalence and features of DI in our population is shown in Tables 2 and 3. A total of 10/16 patients (62.5%) had abnormal discolouration referable to DI, in particular four of nine patients were affected by OI type I, three of three patients were affected by OI type III, and three of four patients by OI type IV. Five patients (one with OI type I, two with OI type III, two with OI type IV) showed DI features both on primary and permanent teeth, whereas three patients had DI defects only on permanent teeth (Table 2).

The teeth discolouration in DI varied within each dentition, but overall it could be classified as either yellow/brown or opalescent grey. The yellow/brown discoloration occurred more frequently (80%) than the grey discolouration (Fig. 1). The discolourations could not be related to the different types of OI. Severe attrition or enamel fractures were observed on the primary teeth in six of ten patients with DI; in particular, out of six patients, two patients had attrition class II (localized lesions, <1/3 of surface involving dentin) and four patients had attrition class III (generalized lesions, >1/3 of surface involving dentin). Attrition was more prominent in those primary teeth that displayed the yellow/brown involvement. Permanent teeth did not exhibit excessive attrition or enamel fracture.

During the period of observation, seven primary teeth were extracted (three in patients with OI of type I, two in patients with OI type III, and two in patients with OI type IV) and underwent histological examination, to both OM and CLSM.

Table 3. Teeth discolourations (yellow/brown or grey) and attrition or enamel fractures in children with dentinogenesis imperfecta (DI) for each type of osteogenesis imperfecta (OI).

	OI type I (*n* = 4)		OI type III (*n* = 3)		OI type IV (*n* = 3)	
	Primary	Permanent	Primary	Permanent	Primary	Permanent
DI	22.2 (2)	33.3 (3)	66.6 (2)	100 (3)	75 (3)	50 (2)
Yellow/brown	100 (2)	66.6 (2)	100 (2)	66.6 (2)	100 (3)	50 (1)
Grey	0 (0)	33.3 (1)	0 (0)	33.3 (1)	0 (0)	50 (1)
Attrition or enamel fractures	100 (2)	0 (0)	100 (2)	0 (0)	66.6 (2)	0 (0)

Values are given as percentages (*n*).

Histological examination confirmed the diagnosis of DI. No histological differences were noted among the three types of OI. The enamel was essentially normal with a regular prismatic structure, and wide and long lamella extended from amelodentinal junction to enamel surface itself. The amelodentinal junction (DEJ) appeared to have normal scalloping. The dentin showed significant abnormalities. A normal appearing mantle dentin layer (10/15 μm wide) was continuous with a narrow (30/50 μm wide) normal appearing submantle band of tubules. This apparently normal zone ended in a large wavy laminated area (approximately 100 μm wide) parallel to DEJ; although appearing atubular in longitudinal section, in cross-section this area showed some occluded tubules with randomly oriented crystals (Fig. 2). Below and indenting this laminar zone, some widen structures similar to canals (5/10 μm diameter), cylindrical in shape, were present. These were wrapped in 'flow lines' of mineralized matrix and frequently demonstrated one or more dilated and back-curved extended processes in the middle of the channel. Numerous dilated, retrocurved (or 'U-turn') dilates structures (possibly odontoblast process) were seen in the laminar layer. Although, inside the channel, the preferred orientation of the collagen fibrils was parallel to the long axis of the channel itself, away from the channel the collagen fibrils were randomly oriented (Figs 3 and 4). Moreover, the dentin demonstrated a poor mineralization (Fig. 5) partially justified by an immunoreactivity to type III collagen (normally present only in reactive dentine) that could represent an obstacle to mineralization.

Discussions

Osteogenesis imperfecta is a rare hereditary disease, characterized by a defective formation of the collagen, involving bone fragility, consequent multiple bone fractures, and

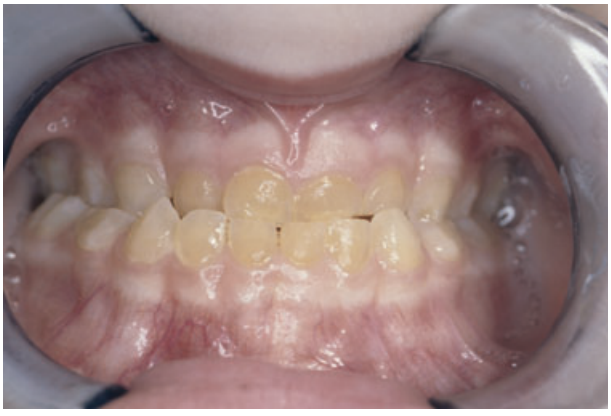

Fig. 1. Dentinogenesis imperfecta on primary teeth.

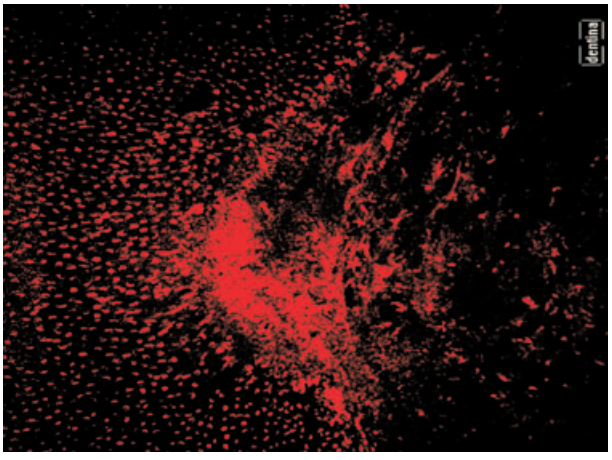

Fig. 2. Dentinogenesis imperfecta to CLSM (monochromatic): normal appearing band of dentinal tubules near area with occluded tubules.

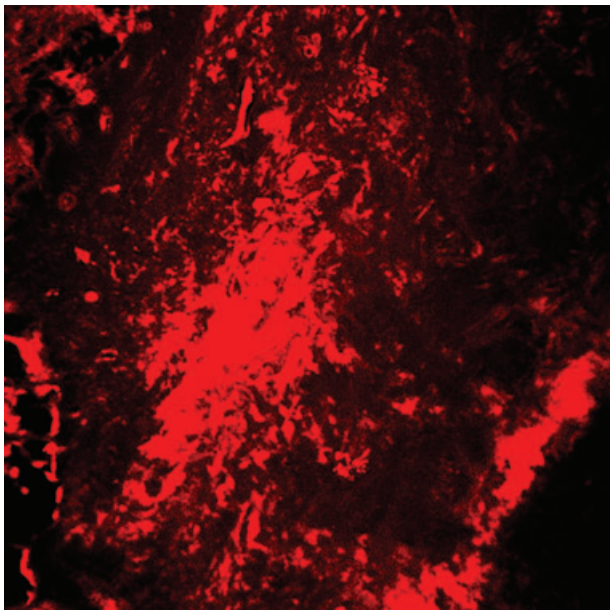

Fig. 3. Dentinogenesis imperfecta to CLSM (monochromatic): aberrant dentin with randomly orientated collagen fibrils.

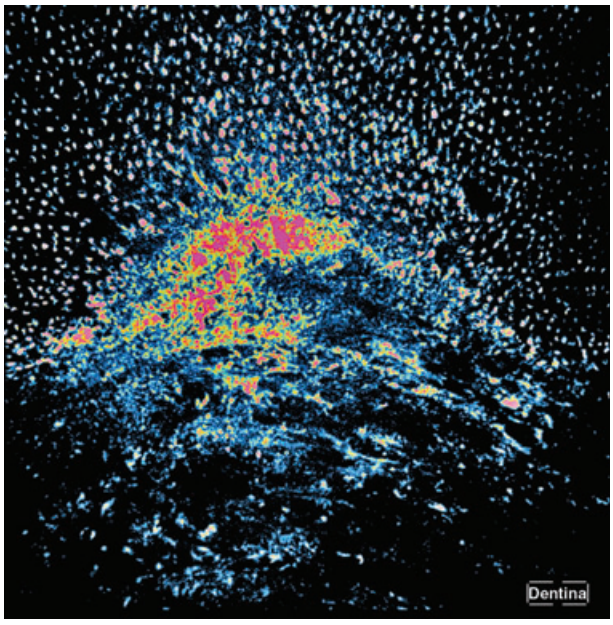

Fig. 4. Dentinogenesis imperfecta to CLSM (dual stain): randomly orientated collagen fibrils.

skeletal deformities. The gravity and the extension of this disease are more variable and generally referable to four clinical types.

Dental aberration is common and may be of diagnostic importance when the diagnosis is uncertain[1–8].

Out of 16 patients affected by OI, ten (62.5%) showed dental alterations due to DI.

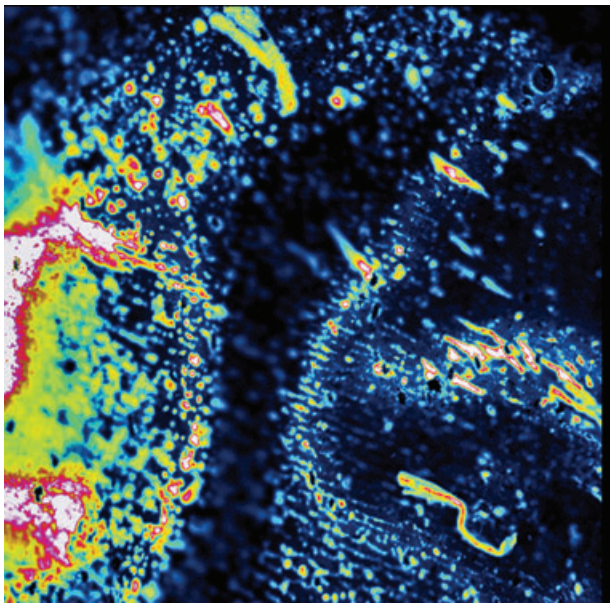

Fig. 5. Dentinogenesis imperfecta to CLSM (dual stain): poorly mineralized dentin with randomly orientated chanel-like structures.

The prevalence of DI in patients with OI reported in previous studies varies considerably, ranging from 21% to 73%[19,20]. Several factors may contribute to this discrepancy, for example the inclusion of more than one member from each family or of a certain number of OI type III cases or the possibility to perform radiographical examination. Yet, the strong association between OI and DI had induced various authors, including Levin *et al.*[4], to propose an opposite classification by making diagnosis of OI, depending on the presence of DI.

Clinical dental features were similar to those previously reported. Yet, our report shows that DI on the primary teeth presents more frequently with the yellow-brown discolouration, which seems to be associated with increased attrition or enamel fractures. This occurs regardless of the type of OI. For a minority of patients, although the primary teeth were affected, DI was not clinically present on the permanent teeth, but it was not possible to predict in which patients this would occur.

Teeth affected by DI showed radiographic evidence of DI to varying degrees, pulpal obliteration being the most frequent feature.

The histological examination (to both OM and CLSM) of extracted or normally

exfoliated primary teeth demonstrated a consistently abnormal appearance of the dentin in all specimens without difference and allowed to postulate reasons for the structural changes found, explaining them in terms of odontoblast dysfunction. These findings are in agreement with those described by Hall *et al.*[14], using light and polarized-light microscopy, scanning and transmission electron microscopy (SEM, TEM), selected area diffraction, and X-ray spectroscopy (EDX), which supported their well-explained theory about the ultrastructural dentin changes in the teeth of children with OI and its origin, linked to the odontoblast dysfunction[14]. The normal appearing mantle layer and the adjacent tubular zone suggest an initial normal function of the odontoblast. This normal layer then changes into a laminated area, atubular in longitudinal section but characterized by dilated channel-like structures in cross one. This area can be interpreted as the mineralization of an abnormal secretion of the alterated collagen fibrils and other matrix components.

A possible mechanism to explain the histological findings is based on the known effects of collagen mutation on the intracellular process of fibroblast[21,22]. The dysfunctional odontoblast may dilate, due to the intracellular accumulation of abnormal procollagen, and slow down until arresting. The secreted abnormal gel-like matrix mineralizes eventually enveloping the dilated odontoblast and its process and preventing further collagen secretion. The odontoblast process is forced to curve back on itself as it meets the viscous mineralizing front and this may explain the 'U-turn' tubules. Concerning the origin of the channel-like structures, their parallel appearance support the theory that they are 'fossilized' dilated odontoblast cells, processes, and tubule spaces. Despite the normal appearance of the mantle layer, odontoblast may be dysfunctional from the outset as odontoblast differentiation is controlled by gene expression[23,24]. Some reports showed that the mutant collagen can be expressed differently in bone and in skin[25,26]. The incorporation of the mutant collagen secreted by the odontoblast-like cells into the matrix may explain the different pathology of bone, skin, and possibly dentin in patients affected.

Analysis by CLSM allowed to examine the aberrations of collagen (just by means of collagen self-fluorescence at laser) otherwise impossible to notice under polarizing light OM and to clarify the structural and ultrastructural dentin alterations with time and cost considerably reduced compared with SEM and TEM analyses. Therefore, it could be interesting in future studies, planned on a larger group of patients, to examine histological changes of the dentin for all types of OI both in primary and permanent teeth.

What this paper adds

- Clinical associations between OI and DI, regarding for the first time both primary and permanent teeth.
- Detailed histological analysis of dentin alterations in patients with OI.
- Hypothesis about the ultrastructural changes found, regarding the odontoblast dysfunction in DI.

Why this paper is important to paediatric dentistry

- It contains useful information about dental alterations in a rare disease, OI.

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