Case report of a molar-root incisor malformation in a patient with an autoimmune lymphoproliferative syndrome

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Abstract

Background: Molar-root incisor malformation (MRIM) is a novel dental phenotype likely related to a patient’s past medical history. This case aimed to confirm MRIM by histological and scanning electron microscopy (SEM) examination for the first time in a patient diagnosed with autoimmune lymphoproliferative syndrome (ALPS) and to propose a possible link between ALPS and MRIM that could be attributable to abnormally proliferated bone marrow.

Case presentation: A 12.5-year-old boy with an extensive medical history, including diagnosis of ALPS, was examined clinically and radiologically to elucidate the reason for pain primarily originating from the area of the lower left permanent first molar tooth (PFM; tooth 36). Dental examination and radiographic survey revealed abnormal pulp cavity morphology of all four PFMs, and these were extracted, resolving the dental pain in the patient. The extracted PFMs were subjected to light microscopy, SEM evaluation and mineral density and elemental composition analyses. Histology of two PFMs revealed the presence of dentin-, bone- and cartilage-like tissues with abundant blood vessels occupying the majority of the pulp chamber. The root canals were obliterated with mineralized structures resembling pulp stones. Two different, highly mineralized abnormal tissues filling the majority of the pulp chamber revealed by SEM and confirming the diagnosis of MRIM displayed a mineral density and elemental composition similar to those of enamel and dentin, respectively.

Conclusions: It appears likely that in addition to the complex medical history during early childhood in the present case, extensive lymphoid infiltrates that are possible in ALPS patients can be regarded as a cofactor in the development of MRIM by exerting considerable pressure on the developing tooth bud and providing cells capable of differentiating into diverse cell types.

Keywords: Molar-root incisor malformation, Tooth development, Pulp biology, Histology, Scanning electron microscopy

Background

In 2014, a new type of dental malformation was described by Witt et al. [1], who found that root malformation of the permanent first molars (PFMs) was associated with a distinct structure: the ectopic mineralized plate or the cervical mineralized diaphragm (CMD). A comparable condition was described by Lee et al. [2], who termed this molar-incisor malformation (MIM) and described it as affecting PFMs and, in some cases, maxillary incisors. Another 30 similar cases were later described, and the condition was named molar root-incisor malformation (MRIM) [3]. An overview of previously published findings in teeth of patients with MRIM is presented in Table 1.

MRIM is characterized by underdeveloped aberrant roots of the PFMs with the crowns of these teeth having normal contour and surface strength [2]. Typically, the roots of all of the PFMs, especially those on the mandible, are affected [4]. The pulp chamber of the PFM is abnormal, being constricted into a narrow straight form in the crown [1]. In addition to the PFMs, the permanent maxillary incisors and/or primary second molars [2] or canines and mandibular incisors can be affected [3] (Table 1). Teeth with MRIM show diverse clinical
| Author(s) | Patient(s) | Medical history | Dental features |
|----------|------------|-----------------|----------------|
| Witt et al., 2014 [1] | 8-year-old boy | At the age of 9 months, osteomyelitis of the left femur was successfully treated with antibiotics (cephalosporine, penicillin, lincosamide and glycopeptide). The girl had frequent middle ear infections from 2 years of age, which were treated with amoxicillin and clavulanic acid. | In both cases, the roots of all four PFMs were malformed, with barely visible or very short roots and narrow appearance of the pulp cavities. All PFMs extracted. |
| Lee et al., 2014 [2] | N = 12 Male: 6 Female: 6 Aged 4–13 years | Ten of the patients had at age 1 to 2 years meningitis (3), brain injury by dystocia (1), hydrocephaulus (1), spina bifida (1), cerebral cyst (1), cephalohematoma (1), or seizure (2). | All PFMs affected. Mandibular deciduous second molars: 5 patients. Maxillary incisors (wedge-shaped defect at the cervical portion): 7 patients. Additional pathology reported: impaction of the PFMs, space loss due to early exfoliation of the deciduous second molar, impaction, hypo-occlusion, dental caries, adjacent tooth eruption disorder, periodontitis, spontaneous pain. |
| Lee et al., 2015 [5] | 6-year-old girl | Premature birth (28th week, birth weight of 1.1 kg) was reported; at 8 weeks, she was diagnosed with perinatal asphyxia. At the age of 17 months, she was diagnosed with frontal intracerebral hemorrhage and a zygomaticomaxillary fracture. | All PFMs had barely developed roots, partly obliterated pulp cavities, constriction in the crown area, thickened pulpal floor, convex appearance of the furcation floor and normal tooth crown contour. All PFMs extracted at the age of 9 during orthodontic treatment. |
| Wright et al., 2016 [3] | N = 30 Male: 18 Female: 12 | During the neonatal period, patients had meningomyelocele or sacral dimple (7), meningitis (6), preterm birth (4), or chronic renal disease (4). In individual patients, the following were reported: meconium aspiration, urinary tract infections, hemiplegia (stroke), cerebral thrombosis, possible cerebrovascular accident, or cerebral palsy with placenta previa. No major problems were reported in 4 patients. | In all patients, all four PFMs were affected; dysplastic root formation and diminished pulp chamber of the PFMs were observed. Deciduous second molars: - all four (14 patients) - both mandibular (one patient) Maxillary central incisor (12 patients) Maxillary and mandibular canine (5 patients) One patient had all PFMs, all permanent incisors and canines, and two premolars affected. |
| McCreedy et al., 2016 [6] | 8-year-old boy | Sacrococcygeal teratoma was diagnosed prenatally and excised the second day after birth. | In both patients, all PFMs were present with abnormal morphology of the roots (hypoplastic and malformed) and narrow pulp canals but normal contour of the crowns. Ectopic eruption of the mandibular PFMs. Ectopic eruption of the permanent maxillary second molars (one patient). |
problems [2, 3, 5–7], and endodontic treatment of such teeth is typically complicated [4, 6]. Hence, knowledge of pulp space morphology is essential for the development of a rational treatment plan [8]. Recommendations for diagnosis/treatment planning of MRIM were recently made by Brusevold et al. [7].

Tooth root malformations can occur as a result of various genetic and environmental factors (reviewed in [9]). Premature termination of root development can be due to infection, trauma, chemotherapy or radiation therapy [1]. Disruption in the development of many roots can be associated with dentine dysplasia type I and regional odontodysplasia [5]. In these cases, the root dysplasia is generalized or affects certain sections of a dental arch [1]. Molar root hypoplasia is observed in patients with Schimke immunooosseus dysplasia; its likelihood increases with the severity of the disease [10]. However, this autosomal-recessive disorder is also characterized by other dental anomalies (microdontia, hypodontia), dimorphic features (facial dimorphism, a short neck, hyperpigmented macules, protuberant trunk, short limbs), renal dysfunction, and T-cell immunodeficiency [10].

The etiology of MRIM remains unclear. A common feature reported in the majority of patients with MRIM is a serious medical condition and treatments (especially antibiotic) during the first 2 years of life [1–7]. No particular disease has been identified as a causal factor in MRIM [11] (Table 1). It appears that severe systemic conditions likely provoke a secondary effect that is expressed locally and affects the development of the tooth bud, resulting in the malformation of PFMs. Different environmental stressors occurring during early childhood have been associated with the abnormal formation of the MRIM tooth roots [3].

Autoimmune lymphoproliferative syndrome (ALPS) is an extremely rare disorder [12] characterized by the increased size of selected organs (mainly lymphadenopathy, hepatomegaly and splenomegaly) resulting from an abnormally large number of lymphocytes accumulating...
in the tissues as well as autoimmune destruction of blood cells (hemolytic anemia, thrombocytopenia and neutropenia) [13]. The bone marrow of ALPS patients is also commonly affected by lymphocytosis, with the possibility that extensive infiltrates can replace normal bone marrow elements [12]. In two-thirds of patients, a mutation in the FAS gene has been confirmed; in many cases, the etiology remains undefined [14].

Data on the oral health of ALPS patients are scarce. To the best of our knowledge, only one study, involving seven patients, has investigated the oral pathologies of patients with ALPS [15]. The authors of that study concluded that ALPS patients exhibit a wide spectrum of signs and symptoms affecting oral soft tissues (e.g., recurrent mucosal ulcers, a smooth tongue, gingivitis). Although caries was reported, no other dental diseases were mentioned, and no X-rays have been presented to date.

The aim of the present case report was to confirm MRIM in a patient diagnosed with ALPS by histological and scanning electron microscopy (SEM) examination and to determine the mineral density and elemental composition of the affected PFMs. To the best of the authors’ knowledge, no such case has been reported previously. Based on the findings, the authors propose a possible link between ALPS and MRIM that could be attributable to abnormally proliferated bone marrow.

**Case presentation**

The medical history of a 12.5-year-old boy, referred due to pain in the area of the lower left PFM (tooth 36), reported serious health conditions since the first year of life (Fig. 1). At the age of 3.5 years, he was diagnosed with ALPS; although all of the findings were indicative of ALPS, there was no history of ALPS in the family and no mutation of the most commonly involved genes (FAS, FASLG) [13], as confirmed by genetic analysis. His dental history reported fillings on all second primary molars; however, no inflammatory complications were reported. A dental panoramic tomogram (DPT), obtained when the patient was 6 years old, is presented in Fig. 2a.

At the age of 12.5 years, a dental clinical examination revealed complete permanent dentition, and both upper
central incisors were built up (Fig. 2b). Reportedly, this treatment was performed by a general dentist as soon as the incisors erupted as they had hypoplastic incisal thirds. Otherwise, the crown morphology was normal. On the cervical halves of the PFMs, poor mineralization of the enamel was identified, which most likely occurred during enamel formation. The remaining tooth crowns appeared intact. Except in the area of the right mandibular PFM, the oral mucosa was of normal coral pink color, size and resilience and showed no inflammatory or other pathologic signs. Swelling was observed buccally in the area of the right PFM (tooth 46), whereas the patient reported constantly present spontaneous pain related to the left PFM (tooth 36). Both mandibular PFMs were sensitive to percussion and did not respond to cold or an electric pulp test. The right one was pathologically mobile. Diagnostic evaluation findings are presented in Fig. 1. DPT and periapical radiographs revealed profoundly malformed pulp cavities and tooth roots of all four PFMs (Figs. 2c, d). There was an appreciable peri- and para-apical radiolucency related to tooth 46. In addition, DPT showed the presence of both maxillary third molars but no mandibular third molars.

Based on clinical and radiographic findings, a diagnosis of symptomatic apical periodontitis associated with the necrotic mandibular left PFM and of pulpal necrosis with acute apical abscess for the mandibular right PFM was reached. Due to the very poor prognosis for the endodontic treatment of mandibular PFMs with aberrant root canal morphology, the recommendation of the interdisciplinary team (endodontist, orthodontist and
pediatric dentist) was extraction of all four abnormally formed PFMs. This was performed followed by an orthodontic space closure. Prior to any procedures, written informed consent was obtained from the patient and his parents. Extraction resulted in an immediate post-operative resolution of pain, and uneventful healing was observed at the 2-week clinical recheck. No further oral/dental problems have been reported.

**Histological analysis**

Teeth 16 and 36 (Figs. 3a, b) were fixed with 10% neutral buffered formalin and demineralized with the Shandon TBD-2 Decalcifier (Thermo Fisher Scientific Inc., Waltham, MA, USA) and the mild bone-decalcification solution Osteosoft® (Merck KGaA, Darmstadt, Germany), respectively [16, 17]. Progression of the demineralization was monitored by dental radiographs (Figs. 3c, d).

Both decalcified specimens were dehydrated and embedded in paraffin to prepare a series of 5-μm histological sections cut in a mesiodistal direction at 50-μm intervals using a Leica SM 2000R microtome (Leica Biosystems, Nussloch, Germany). The specimens were stained with hematoxylin and eosin (HE), a Masson-Goldner kit (Merck KGaA, Darmstadt, Germany), toluidine blue (pH 7.2) and alcian blue (pH 2.5; Merck KGaA, Darmstadt, Germany). A Nikon Microphot-FXA microscope equipped with a DS-Fi1 camera and NIS-Elements imaging software (NIS Elements D.32; Nikon Instruments Europe B.V., Badhoevedorp, the Netherlands) were used for histological examination. Representative tissue sections were presented using Adobe Creative Cloud.

Examination by light microscopy revealed profoundly folded dentin (especially in the root portion) as well as an unusual tissue filling in the majority of the pulp chamber, comparable to CMD [1]. Only minor areas of preserved normal pulp were evident (tooth 16; Fig. 4a, b; tooth 36; Fig. 5a, b). The course of the dentinal tubules appeared normal only in the occlusal third of the crown (Figs. 4a, b). The CMD contained connective tissue canals with blood vessels, many of which resembled osteons (Figs. 4c, f and 5e, f). The surrounding tissue consisted mainly of globules and interglobular matrix that contained only scarce collagen fibers (Figs. 4d and 5g). At the border between the CMD and the occlusal dentin, amorphous tissue resembling tertiary dentin was present (Figs. 4c and 5c). In areas where the dentinal wall was thinner, there were chondrocyte-like cells (Figs. 4d, e). The cervical area (where the floor of the dental pulp chamber and furcation should have been) contained cellular cementum and some periodontal ligament tissue. The root canals were obliterated with mineralized structures resembling pulp stones (Figs. 4g-k) with remnants of normal pulp tissue (Figs. 4a, b, i, j). Sequential histological sections cut in a mesiodistal direction at 50 μm displayed similar findings.

**Scanning electron microscopy**

Tooth 46 was fixed in 10% neutral buffered formalin, rinsed, bisected bucco-palatally and embedded in epoxy resin (Araldite, Ciba-Geigy, East Lansing, MI, USA) with the cut side exposed. After polymerization, the exposed axial cross-section was polished, etched with 37% orthophosphoric acid for 30 s, rinsed with distilled water spray for 30 s, dried with compressed air, dehydrated with 70% ethanol, dried again and sputter-coated with carbon (Vacuum Evaporator, Type JEE-SS; Japan Electron Optics, Tokyo, Japan). Subsequently, the sample was subjected to ultrastructural analysis via SEM (JEOL JSM-5610, JEOL, Tokyo, Japan) performed in the secondary electron mode.

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**Fig. 3** Photographs of the left mandibular first molar (36) taken before the demineralization procedure and progression of the demineralization process as monitored by dental radiographs. Tooth 36 from the (a) buccal and (b) mesial surface. Note the aberrant root morphology and a ribbon of hypomineralized enamel extending circularly around the tooth crown and toward the cervical area. (c) Radiograph of tooth 36 taken 14 weeks after demineralization with the mild bone-decalcification solution Osteosoft®. Note incomplete decalcification with unusual mineralized tissue in the area of the pulp chamber (asterisk). (d) The process was successfully completed after additional overnight decalcification with a 1:1 mixture of Osteosoft® and Osteomoll® solution.
electron imaging (SEI) and backscattered electron (BSE) modes. Micrographs were recorded at 15 kV and a working distance of 20 mm.

The SEM images revealed a normal structure and thickness of the enamel, areas with normal and abnormal dentin, and an almost completely mineralized pulp chamber (i.e., a CMD). Higher-magnification micrographs marked with rectangles show the course of the dentinal tubules in the coronal dentin interspersed between the CMD, amorphous tissue at the border between the CMD and dentin (yellow double-sided arrow) and the connective tissue canal containing blood vessels (yellow arrow). This connective tissue exhibits pale staining with toluidine blue, whereas the surrounding tissue consists of globules and a toluidine blue-positive interglobular matrix. CMD consisting of globules and interglobular matrix. At the border with dentin, there are enlarged chondrocyte-like cells residing in the lacunae and surrounded by the alcian blue-positive matrix, suggestive of cartilage proteoglycans.

Mineral density and elemental composition analyses
Mineral densities and the elemental composition of four areas were compared: 1) brighter tissue and 2) darker tissue of the abnormal hard tissue located at the site of the pulp chamber (CMD), 3) dentin and 4) enamel.

For mineral density analysis, 10 BSE images of each selected tissue were taken at a magnification of 1500x, 15 kV and a working distance of 20 mm. For enamel, images were obtained from normal dentin located on
occlusal side between the enamel and pulp chamber area, approximately in the middle of its thickness. For brighter tissue and darker tissue of the CMD, we randomly took images in the area of the CMD in regions where we found typical appearance of brighter and darker tissue. Using the OpenCV library in Python (Windows, Microsoft), for each pixel in an image, the grayscale value in the range of 0 to 255 (black to white) was determined, and the average grayscale value was then computed for each image.

For elemental analysis, five representative images were taken of each of the four tissues, selected in the same manner as described above. Elemental analysis was performed with energy dispersive X-ray spectroscopy-EDXS (500 Digital Processing; IXRF Systems, Houston, TX, USA) at a working distance of 20 mm, an acceleration voltage of 15 kV and a counting time of 80 s. This approach was conducted on the entire surface area of the SEI images under 1500x magnification. For each of the four tissue groups, five images were analyzed, and the values obtained were expressed as the mean ± SD. The results of the EDXS spectra were further evaluated in relation to the carbon-oxygen ratio (C:O) and calcium-phosphorous ratio (Ca:P) as described previously [18].

The statistical significance of differences between analyzed tissues was determined by one-way analysis of variance (ANOVA) followed by Tukey’s post hoc test using SPSS 20.0 for Windows (SPSS Inc., Chicago, Ill, USA). A *P*-value ≤ 0.05 was considered statistically significant.

Mineral density and elemental composition analyses confirmed the similarities between the brighter tissue and enamel, and between the darker tissue and dentin. Figure 7 (a–d) shows representative SEM images of each of the tissues from the crown of the tooth. The mineral density of the darker tissue was comparable to that of the dentin (29.16 ± 0.37 and 29.24 ± 0.16, respectively; *P* = 0.458), whereas the mineral density of the brighter tissue was lower than that of the enamel (39.64 ± 0.88 and 42.63 ± 0.49, respectively; *P* = 0.05). Data on the elemental composition are summarized in Table 2. The darker tissue and brighter tissue displayed elemental compositions that resembled dentin and enamel, respectively. The C:O ratio differed significantly between the pairs of groups (enamel/
brighter tissue vs. dentin/darker tissue), whereas the Ca:P ratio was only reduced in the darker tissue (Table 2).

**Discussion and conclusions**
This report describes MRIM in a young boy affected by ALPS, with clinical and radiographic findings in agreement with those of previous reports [1–3, 5–7]. Mandibular PFMs with MRIM caused spontaneous pain, and an apical abscess in the absence of dental caries was observed. Abscesses in these teeth are thought to be caused by pulp necrosis induced by pulp chamber obliteration or by pulp exposure induced by dentinal defects.
Endodontic treatment of the MRIM tooth with a profoundly calcified pulpal floor and malformed root is very difficult or unfeasible [4], which is why the treatment option to extract these teeth was chosen. The histological findings were largely consistent with those previously reported for MRIM-affected teeth [1, 3, 5]. Obscure mineralized content filling in the majority of the pulp chamber (i.e., CMD) consisted of several different tissues, including enamel-like tissue as determined by mineral density and elemental composition analyses. Specifically, when mineral density and elemental composition were analyzed, the darker tissue of the CMD resembled dentin, whereas the brighter tissue of the CMD was more enamel-like. Witt and coworkers [1] reported similar mineral densities of the “interglobular part of CMD” and dentin and a much higher mean density value of the “globular part of CMD”, which approached the mean value of the enamel. Three distinct layers of tissues reportedly visualized by microcomputed tomography in the pulpal floor of MRIM-affected teeth [5] were not distinguished in our case. The root canal was almost completely filled in by structures resembling pulp stones, similar to previous reports [1, 3]. In MRIM, the exact cause of

![Fig. 7 Mineral density of different dental tissues. Representative SEM-BSE micrographs of (a) “brighter tissue” and (b) “darker tissue” of the CMD, (c) dentin and (d) enamel (× 1500, BSE) (Table 2)]](image)

| Parameter       | Darker area (S) | Brighter area (S) | Dentin (S)    | Enamel (S)    |
|-----------------|----------------|------------------|---------------|---------------|
| Carbon (%)      | 78.81 ± 3.10^{a,b,d} | 29.60 ± 1.39^{a,b,c,d} | 79.31 ± 1.76^{a,b,d} | 14.21 ± 0.95^{a,b,c} |
| Oxygen (%)      | 10.94 ± 2.42^{a,b,d} | 16.75 ± 0.26^{a,c} | 10.14 ± 0.61^{b,d} | 18.75 ± 0.95^{a,b,c} |
| Phosphorus (%)  | 3.77 ± 1.01^{a,b,d} | 18.98 ± 0.30^{a,c,d} | 4.32 ± 0.88^{b,d} | 23.77 ± 0.47^{a,b,c} |
| Calcium (%)     | 5.25 ± 1.60^{a,b,d} | 33.36 ± 1.39^{a,c,d} | 6.49 ± 1.69^{a,b,d} | 42.56 ± 0.84^{a,b,c} |
| C:O ratio (%)   | 7.49 ± 1.61^{a,b,d} | 1.77 ± 0.10^{a,c} | 7.74 ± 0.32^{b,d} | 0.76 ± 0.06^{a,b,c} |
| Ca:P ratio      | 1.42 ± 0.32^{a,b,d} | 1.76 ± 0.08^{a} | 1.49 ± 0.14 | 1.79 ± 0.04^{a} |

*significantly different from the darker area
^significantly different from the brighter area
\$significantly different from the dentin
"significantly different from the enamel
Differences were considered significant at $P \leq 0.05$
pulp stone formation is not known but may be related to systemic disease [19].

The formation of MRIM has been proposed to result from damage to the vascular plexus at the base of the dental papilla during crown development, giving rise to the precipitation of calcified globules, with the interglobular components of the CMD deriving from the dental follicle [1]. Alternatively, the middle layer of the CMD may originate primarily from the apical pulp and partially from the dental follicle, with the lower layer formed by the dental follicle [5]. However, these hypotheses cannot explain the origin of the enamel-like tissue in the mineralized content of the pulp chamber. Its presence may be the result of Hertwig’s epithelial root sheath (HERS) cell differentiation into ameloblasts [20] or differentiation of other pluripotent stem cells into ameloblast-like cells in the presence of HERS cells [21]. Alternatively, bone marrow-derived cells, which appear to have the greatest capability to differentiate into diverse cell types [22, 23], including ameloblast-like cells [24], could contribute to the source of cells forming the CMD. Moreover, highly active bone marrow may represent a locally expressed factor as a secondary effect of a severe systemic condition, resulting in MRIM. This could also explain why PFMs (especially mandibular PFMs) are consistently affected by MRIM, whereas the occurrence of MRIM in other teeth varies. In a healthy newborn and in infants up to 3 years of age, hematopoietic marrow is distributed in both jaws, particularly in the mandible [25]. Before the age of one year, 100, 67 and 50% of subjects have hematopoietic marrow throughout the condyle, ramus and angle of the mandible, respectively [25]. With increasing age, hematopoietic marrow is gradually replaced with fatty marrow; in the most distal part of the mandible, conversion occurs by the age of 3 years [25]. If there was profoundly abnormal growth of bone marrow in the jaw, it would most likely be present in the area of developing mandibular PFMs. Accordingly, aberrant roots (divergent, twined, hypoplastic or undeveloped) and pulp chambers (constricted into a narrow straight form) of the PFMs are pathognomonic for MRIM. As extensive lymphoid infiltrates in the bone marrow are possible in ALPS patients [12], hypothetically, such proliferated bone marrow could exert pressure and present a mechanical obstacle that interferes with the developing tooth during the first year and thus plays a role in the etiology of MRIM. In support of this view, i) the aberrant pulp chamber and the abnormal course of the dentinal tubules indicate possible mechanical obstruction to the developing tooth bud, and ii) bone marrow-derived cells have the greatest capability to give rise to the diverse types of tissue found in the mineralized content of the pulp chamber area. Interestingly, the hearing loss observed in a murine model of ALPS (i.e., MRL/lpr mice) also results from defects in the bone marrow [26].

In summary, based on the rarity of ALPS and the lack of reports on oral/dental health in ALPS patients, it cannot be concluded that ALPS causes MRIM. At this point, the presented case may represent a unique, singular case, and one can only speculate about the MRIM incidence in ALPS patients. In addition, the environmental factors, medical conditions and medications to which the patient was subjected to during his first 2 years of life cannot be ruled out as possible cofactors in the development of MRIM in the presented case, but lymphoid infiltrates in the bone marrow, which interfere with the development of the tooth bud, should be considered as another possible (co)factor. Further investigations into the bone marrow abnormalities that serve as a link between ALPS and MRIM are warranted.

**Abbreviations**

ALPS: Autoimmune lymphoproliferative syndrome; C: Carbon; Ca: Calcium; CMD: Cervical mineralized diaphragm; DPT: Dental panoramic tomogram; HERS: Hertwig’s epithelial root sheath; MIM: Molar-incisor malformation; MRIM: Molar-root incisor malformation; O: Oxygen; P: Phosphorus; PFM: Permanent first molar tooth; SEM: Scanning electron microscopy

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**Availability of data and materials**

The datasets, used and/or analyzed in this paper, are available from the corresponding author on request.

**Authors’ contributions**

AP was a primary clinician on the case and designed the further analysis as described in the paper, collected and interpreted the data and drafted the manuscript. MV performed histological examinations of the teeth, interpreted the data, and was a major contributor in writing the manuscript. JJ interpreted the data and critically revised the manuscript. MB performed SEM, mineral density and elemental composition analyses and statistical analyses. AN interpreted the data and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Prior to extraction of the teeth with very poor prognosis, we obtained written informed consent from the patient and his parents. For further analysis of the extracted teeth, we obtained a consent of the Slovenian National Committee for Medical Ethics (No. 0120–648/2016).

**Consent for publication**

All authors of this manuscript clearly state that consent was obtained from the patient and his parents to publish this case report and all accompanying images.

**Competing interests**

The authors declare that they have no competing interests.
References

1. Witt CV, Hirt T, Rutz G, Luder HU. Root malformation associated with a cervical mineralized diaphragm-a distinct form of tooth abnormality? Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;117:e311–9.

2. Lee HS, Kim SH, Kim SO, Lee JH, Choi HJ, Jung HS, Song JS. A new type of dental anomaly: molar-incisor malformation (MIM). Oral Surg Oral Med Oral Pathol Oral Radiol. 2014;118:1019–93.

3. Wright JT, Cuman A, Kim KJ, Yang YM, Nam SH, Shin TJ, Hyun HK, Kim YJ, Lee SH, Kim JW. Molar root incisor malformation: considerations of diverse developmental and etiologic factors. Oral Surg Oral Med Oral Pathol Oral Radiol. 2016;121:164–72.

4. Yue W, Kim E. Nonsurgical endodontic management of a molar-incisor malformation-affected mandibular first molar: a case report. J Endod. 2016;42:664–8.

5. Lee HS, Kim SH, Kim SO, Choi BJ, Cho SW, Park W, Song JS. Microscopic analysis of molar-incisor malformation. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;119:544–52.

6. McCready C, Robbins H, Newell A, Mallya SM. Molar-incisor malformation: two cases of a newly described dental anomaly. J Dent Child (Chic). 2016;83:33–7.

7. Brusevold Ul, Bie T, Baumgartner CS, Das R, Espelid I. Molar incisor malformation in six cases: description and diagnostic protocol. Oral Surg Oral Med Oral Pathol Oral Radiol. 2017;124:52–61.

8. de Pablo OV, Estevez R, Peix Sanchez M, Heilborn C, Cohenca N. Root anatomy and canal configuration of the permanent mandibular first molar: a systematic review. J Endod. 2010;36:1919–31.

9. Luder HU. Malformations of the root tooth in humans. Front Physiol. 2015;6:307.

10. Morimoto M, Kerouedan O, Gendronneau M, Shuen C, Baradaran-Heravi A, Asakura Y, Basiratnia M, Bogdanovic R, Bonneau D, Buck A, et al. Dental abnormalities in Schimke immuno-osseous dysplasia. J Dent Res. 2012;91:29S–37S.

11. Choi S, Lee J, Song J. Molar-incisor malformation: three cases of a newly identified dental anomaly. J Korean Acad Pediatr Dent. 2017;44:370–7.

12. Xie Y, Pittaluga S, Price S, Raffeld M, Hahn J, Jaffe ES, Rao VK, Maic I. Bone marrow findings in autoimmune lymphoproliferative syndrome with germline FAS mutation. Haematologica. 2017;102:364–72.

13. Li P, Huang P, Yang Y, Hao M, Peng H, Li F. Updated understanding of autoimmune lymphoproliferative syndrome, consisting of autosomal-recessive cone-rod dystrophy and amelogenesis imperfecta. Am J Hum Genet. 2009;84:266–73.

14. Talla HV, Kommineni NK, Yalamanchi S, Avula JS, Chilakur A. A study on pulp stones in a group of the population in Andhra Pradesh, India an institutional study. J Conserv Dent. 2014;17:111–4.

15. Shimura Y, Tsuchiya S, Hata K, Honda MJ. Quiescent epithelial cell rests of Malassez can differentiate into ameloblast-like cells. J Cell Physiol. 2008;217:728–38.

16. Yoshiida K, Sato J, Takai R, Uehara O, Kurashige Y, Nishimura M, Chiba I, Saitho M, Abiko Y. Differentiation of mouse iPSC cells into ameloblast-like cells in cultures using medium conditioned by epithelial cell rests of Malassez and gelatin-coated dishes. Med Mol Morphol. 2015;48:138–45.

17. Pierdomenico L, Bonisi L, Calvitti M, Rondelli D, Arpinati M, Chirumbolo G, Becchetti E, Marchionni C, Alviano F, Fossati V, et al. Multipotent mesenchymal stem cells with immunosuppressive activity can be easily isolated from dental pulp. Transplantation. 2005;80:836–42.

18. Yen AH, Sharpe PT. Stem cells and tooth tissue engineering. Cell Tissue Res. 2008;331:359–72.

19. Hu B, Linda F, Bopp-Kuchler S, Jimenez L, Wang XJ, Haikel Y, Wang SL, Lesot H. Bone marrow cells can give rise to ameloblast-like cells. J Dent Res. 2006;85:416–21.

20. Yamada M, Matsuoka T, Uetani M, Hayashi K, Tsuji Y, Nakamura T. Normal age-related conversion of bone marrow in the mandible: MR imaging findings. AJR Am J Roentgenol. 1995;165:1223–8.

21. Ivai H, Lee S, Baba S, Tomoda K, Inaba M, Ikehara S, Yamashita T. Bone marrow cells as an origin of immune-mediated hearing loss. Acta Otolaryngol. 2004;124:8–12.