Ultrastructures of Enamel, Dentin, and Cementum associated with Hypocalcified Amelogenesis Imperfecta

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Abstract

Objectives: Amelogenesis imperfecta (AI) is a group of hereditary disorder of tooth development that affects the structure and appearance of tooth enamel. AI has been categorized based on clinical and radiographic appearances. However, understandings of the defects in ultrastructure and mineral composition defects of AI are very limited. This study aimed to analyze the ultrastructure, mineral density, and chemical compositions of enamel, dentin, and cementum in two teeth affected by autosomal dominant hypocalcified type of AI (ADHCAI). This study will expand knowledge of abnormalities in dental hard tissues associated with ADHCAI.

Methods: Two Thai patients from different families affected with ADHCAI participated in this study. The history, clinical, and radiographic examinations were performed. An extracted tooth obtained from each patient was subjected for studies. The mineral density was examined by micro-CT, mineral composition by dispersive x-ray spectrometry (EDX), and ultrastructure by scanning electron microscopy (SEM).

Results: The first patient exhibited yellowish brown discoloration with pitted enamel. The second patient showed brown-black colored crown with smooth surface. In both patients, the radiopacity of enamel was reduced. The disease was inherited through families suggesting autosomal dominant mode of inheritance. These suggest ADHCAI in both patients. SEM revealed that AI enamel showed atypical enamel rods containing several porosities and irregular dentinoenamel junction. Dentin exhibited partially obliterated dentinal tubules while cementum appeared unremarkable. The mineral density of AI enamel was decreased. The levels of calcium and phosphorus in affected enamel, dentin, and cementum were significantly reduced compared to the controls. These show alterations in the ultrastructure, mineral density, and compositions of the enamel, dentin, and cementum associated with ADHCAI.

Conclusions: Our study demonstrates that ADHCAI could influence clinical and radiographic characteristics, ultrastructure, mineral density, and inorganic elements in the enamel, dentin, and cementum.

Key words: Amelogenesis Imperfecta, ultrastructure, teeth, EDX, SEM

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**Introduction**

Amelogenesis imperfecta (AI) is a hereditary disorder causing defects in dental enamel. It affects both primary and permanent dentitions. The prevalence of AI varies from 1:14,000 to 1:700 depending on population studied.\(^1,^2\) AI has been divided into four types including hypoplastic (type I), hypomaturation (type II), hypocalcification (type III) and hypomaturation-hypoplastic with taurodontism (type IV)\(^3\) depending on the stage of enamel formation affected by genetic defects.

Hypoplastic type is caused by the reduced secretion of extracellular matrix. The quantity of enamel is decreased, which may be expressed clinically as thin or missing enamel such as pits and grooves. Hypomaturation type caused by anomalies at the maturation stage of enamel formation, presents clinically as opaque, mottled, discolored, and porous enamel but normal thickness. Hypocalcified type is due to defects in mineralization, characterized by discolored, rough, and soft enamel which is rapidly lost by attrition. Hypomaturation-hypoplastic with taurodontism is characterized by thin, mottled or spotted, and discolored and taurodont molars.\(^4,^5\) Radiographic features of AI reveal a variety of defects, depending on type of AI. Hypoplastic type shows reduced thickness of enamel and normal to slightly reduced radiopacity. The enamel contrast of hypomaturation type is similar to or higher than that of the dentin while that of hypocalcified type is similar to or lower than dentin. The hypomaturation-hypoplastic with taurodontism is characterized by altered enamel contrast and large pulp chambers. The genetic inheritance patterns of AI can be autosomal dominant, autosomal recessive, or X-linked trait. The autosomal dominant hypocalcified type of AI (ADHCAI) is one of the most common forms of AI.

The development of dental hard tissues including enamel, dentin, and cementum is a complex process controlled by reciprocal interactions of genes and signaling. The abnormalities of enamel could affect the formation of dentin which influences that of the cementum. Majorities of previous ADHCAI studies have reported the structural defects of tooth enamel but showed highly variable data. These compromise the understandings of not only enamel anomalies but changes in dentin and cementum associated with ADHCAI. This study, identified two Thai patients from different families having different phenotypes of ADHCAI, therefore aimed to investigate ultrastructure, mineral density, and chemical compositions of two affected teeth using micro-computerized tomography, scanning electron microscopy, and energy dispersive x-ray spectrometry. The findings would expand the ultrastructural knowledge of affected dental hard tissues including enamel, dentin, and cementum and benefit treatment approach of ADHCAI in the future.

**Materials and Methods**

**Subjects**

Two Thai patients from different families were recruited for the study. They were diagnosed with ADHCAI according to the criteria by Witkop\(^3\) at the Faculty of Dentistry, Chulalongkorn University based on clinical and radiographic examinations. The written informed consents were obtained from all individuals who participated in the study. The mandibular right first premolar was obtained from the first patient (called AI1 sample in this study) and the maxillary right lateral incisor was from the second patient (called AI2 sample) which were extracted according to their orthodontic treatment plans and poor prognoses, respectively. The research protocol was approved by the research ethics committee at Faculty of Dentistry, Chulalongkorn University. The patients’ teeth were subjected for ultrastructural analyses compared with two age- and tooth type-matched sound teeth obtained from healthy individuals.
Micro-Computerized Tomography (µCT) analysis

The teeth were rinsed with phosphate-buffered saline and stored in 10% formalin solution. The samples were scanned with specimen µCT 35 (SCANCO Medical, Brüttisellen, Switzerland) and processed using the Image Processing Language (IPL, Scanco Medical AG). Thirty spots in the enamel and thirty spots in the dentin of the patients’ teeth were selected to evaluate its mineral density compared with same areas in the controls via cross sections of micro CT. Subject to the availability of remaining tooth structure, the enamel and dentin were examined in A11.

Energy-Dispersive X-ray (EDX) evaluation

The teeth were mounted on acrylic resin blocks with sticky wax and cut longitudinally along buccal-lingual direction using the slow-speed precision saw (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA) with diamond disc at a speed of 450 rpm under constant water. They were then grinded with Grit#1200 silicon-carbide paper and polished with alumina powder on polishing pad (10-inch MICROPAD, Pace technologies). The samples were dehydrated with ethanol series and exposed to a critical point drying process using critical point dryer (Emitech K850, Emitech Ltd, Kent, England). Dried samples were coated with gold powder in argon-cathode atomization with fine coater media (JFC 1200, Tokyo, Japan) for 10 seconds. Three spots in the enamel, dentin, and cementum between A1 and control teeth were selected to calculate the elemental levels (%) of carbon (C), oxygen (O), phosphorus (P), and calcium (Ca) under the EDX (Jeol, JSM-6610 LV Scanning Electron Microscopy, Japan) by a single operator who was not aware of the samples’ preferences.

Scanning Electron Microscopy (SEM) evaluation

The specimens were etched with 37 percent phosphoric acid, rinsed with water, dehydrated, dried, and covered with gold powder for 110 seconds. For qualitative evaluation of the hard tissue surface, scanning electron microscopy (Quanta Feg 250, FEI Company, Oregon, USA) was performed on the affected samples and compared with the controls.

Statistical analysis

The data were analyzed by GraphPad Prism 5 software package (GraphPad Prism Software Inc., San Diego, CA, USA). Independent T-test was performed to determine significant differences of percent weight of each element between affected and control group (p < 0.05).

Results

Manifestation of AI

The first patient (A11) is a 13 year-old Thai boy who presented for dental treatment at the Faculty of Dentistry, Chulalongkorn University with the complaint of yellowish teeth. Oral examination exhibited yellowish to brownish discolored, rough, and soft enamel with severe wear. Dental caries was found on his upper first molars. Generalized heavy plaque deposition and gingival inflammation were presented (Fig. 1A-B). Periapical radiograph revealed minimal enamel thickness with radiopacity slightly brighter than that of dentin. The unerupted third molar showed normal enamel thickness (Fig. 1C). Extractions of mandibular first premolars were performed based on orthodontic treatment plan. The mandibular premolar was collected for further evaluation (Fig 1D). Enamel defects were also observed in his mother, younger sister, and other mother’s relatives.
The second patient (AI2) is a 42 year-old Thai male who presented for dental treatment at the Faculty of Dentistry, Chulalongkorn University due to his discolored and worn teeth. Oral examination revealed smooth, shiny, brownish-black teeth with severe attrition and caries leaving multiple retained roots. Several teeth were clinically absent (Fig. 2A). Radiologically, the enamel and dentin showed similar radiopacity. Pulp obliteration was presented. Multiple teeth had pulp necrosis with asymptomatic apical periodontitis (Fig. 2B) which were later extracted (Fig. 2C). His daughter was also affected with enamel abnormalities.

Fig. 1  Oro-dental manifestations of AI1 patient. A. The teeth were yellowish, rough, crowded, and deposited with heavy plaque and calculus. B. Oral photograph of maxillary right teeth showed loss of enamel and proximal contact. Large cavity was observed on maxillary right first molar. C. Dental radiograph showed irregular thin enamel and loss of tooth contact. D. Photograph of extracted mandibular right first premolar showed yellowish brown enamel with multiple pits.

Fig. 2  Oro-dental manifestations of AI2 patient. A. Oral photograph of the teeth showed brownish-black, smooth, shiny teeth with severe attrition and caries leaving multiple retained roots. B. Panoramic radiograph showed similar radiopacity between enamel and dentin. Multiple teeth were broken and developed periapical lesions. C. Photograph of the extracted root of the maxillary right lateral incisor showed shiny white to yellowish color and irregular surface.
Micro CT analysis

In AI1, the mineral density of the enamel was 1,581.891 mgHA/cm², compared to the controls (2,137.418 and 2,091.817 mgHA/cm²) while that of the dentin was 1,133.752 mgHA/cm², compared to the controls (1,234.914 and 1,173.842 mgHA/cm²). These show that the AI tooth had substantially reduced mineral density in the enamel while slightly reduced density was observed in the dentin (Fig. 3).

Energy-Dispersive X-ray (EDX) analysis

Elemental analyses of the enamel revealed that AI1 had significantly lower levels of calcium (Ca, 31.73%) compared to the controls (33.57% and 33.06%). Its phosphate (P, 16.51%) and oxygen (O, 41.54%) contents were also significantly decreased (control; P, 17.63% and 17.57%; O, 42.25% and 42.81%) while the carbon (C, 10.21%) content and the mean C/O ratio (0.25) were significantly higher compared to the controls (C, 6.56%; C/O, 0.15). The calcium/phosphate (Ca/P) ratio in AI1 enamel (ratio 1.92) was significantly higher than controlAI1-2 (1.88) (p=0.0132) but not significantly different to controlAI1-1 (1.91) (Fig. 4A-B).

Fig. 3  Mineral density of enamel and dentin in AI1 sample and the controls analyzed by micro-CT. The mineral density of the AI1 enamel was apparently decreased while that of the dentin was similar to the controls.

Fig. 4  Graphs of elemental quantities and ratios in the enamel. A. The AI1 enamel showed significantly higher C content but lower O, P, and Ca levels than the controls (p<0.05). B. The C/O and Ca/P ratios AI1 was significantly increased compared to the controls (p<0.05).
In the dentin, elemental analysis of AI1 exhibited that the C content and C/O ratio were statistically significantly higher in the AI1 dentin compared to the controls. On the other hand, the P and Ca contents were statistically significantly lower in the AI1 dentin compared to the controls. The O content in AI1 dentin was statistically significantly lower in the AI1 compared to the control AI1-2. The Ca/P ratio in AI1 dentin was statistically significantly higher in the AI1 compared to the control AI1-2 but was not statistically significantly different when compared with control AI1-1 (Fig. 5A-B).

![Dentin Graphs](image)

**Fig. 5** Graphs of elemental quantities and ratios in the dentin. A. The Ca, O, and P contents of AI1 dentin were significantly lower than the controls. In contrast, the C content of AI1 was significantly increased \((p<0.05)\). B. The C/O and Ca/P ratios of AI1’ dentin was significantly increased compared to the controls \((p<0.05)\).

In the cementum, AI2 showed significantly increased C and O contents and C/O and Ca/P ratios but decreased P and Ca levels, compared to the controls (Fig. 6A-B).

![Cementum Graphs](image)

**Fig. 6** Graphs of elemental quantities and ratios in the cementum. A. AI2 showed significant decreased Ca and P contents but increased C and O contents compared to the controls \((p<0.05)\). B. AI2 cementum showed increased Ca/P and C/O ratios \((p<0.05)\).
**Scanning Electron Microscopy (SEM) Analysis**

The AI1 enamel revealed porosities and disorganized and collapsed enamel rods. The interspaces between affected enamel rods were wider compared to those of the controls (Fig. 7A, B). The dentinoenamel junction of AI tooth was irregular (Fig. 7C, D). The dentinal tubules were present in AI dentin but partially obliterated (Fig. 7E, F). No obvious difference was observed between the cementum of AI2 and the controls (Fig. 7G, H).

![SEM images](image-url)
Discussion

The ADHCAI is characterized by hypocalcified soft enamel with yellowish-brown in color affecting the whole crown. The disease is inherited as an autosomal dominance. In this study, we identified two Thai AI patients from different families exhibiting diverse dental manifestations. The features of the AI1 were consistent with ADHCAI categorized by Witkop and other previous reports. The AI1 exhibited rough and soft enamel with yellowish to brownish discoloration. Radiographically, the enamel thickness of erupted teeth was thin while that of unerupted teeth was normal. This phenotype corresponds to the developmental defects associated with ADHCAI. It was shown that the ameloblasts secreted normal quantities of enamel matrix proteins resulting in normal enamel thickness in ADHCAI, but the mineralization process was disturbed. Correspondingly, our patient’s teeth were weak, subject to wear and fracture soon after eruption and exposure to mastication forces. In AI1 family, his mother, younger sister, and other mother’s relatives also presented enamel anomalies, suggesting that ADHCAI is inherited in this family.

In AI2 family, the patient and his daughter were affected with AI, indicating autosomal dominant mode of inheritance. Interestingly, the phenotype of AI2 was dissimilar to that of AI1 and to the typical rough enamel of ADHCAI. The clinical appearance of AI2 enamel was a brown-black discoloration with smooth and shiny surfaces. Consistently, Song et al. also observed smooth and shiny enamel surface related to ADHCAI. These show that the ADHCAI patients could present a wide range of enamel features from yellow, brown, and black smooth to rough surface. These could be due to different ages of patients and/or genetic backgrounds.

In this study, only enamel and dentin of AI1 sample were available for analyses because its root was discarded during the operation. For AI2 sample, only the root portion was obtained. The formation of root dentin has unique control mechanisms involving Hertwig’s epithelial root sheath and integration of the root with alveolar bone, blood supply, and nerve innervations which are different from that of crown dentin. The root dentin was thus not included in this study.

The ultrastructure of AI1 enamel was porous containing irregular enamel rods and multiple crack lines. These were consistent with previous studies showing non-crystalline amorphous materials, crack lines, crevices, and porosity in the enamel of ADHCAI. The enamel rods were irregularly associated with inappropriate retention of organic matrix. These indicate that ADHCAI has enamel malformations. Quevado et al. (2004) showed that the dentin in ADHCAI had partial obliteration of the dentinal tubules. Moreover, Zhang et al. reported that ADHCAI had abnormal dentinal tubules with irregular, narrow, or partially obliterated lumens. These are consistent with partially obstructed dentinal tubules observed in our AI teeth. To our knowledge, there are no previous studies on ultrastructure of the cementum in ADHCAI. Kammoun et al. in 2017 reported the cementum ultrastructure of hypoplastic AI. The thickening of cellular cementum was observed, but was not consistent in their study. In addition, the pathogenesis of hypoplastic AI is different from that of ADHCAI. The comparison between AI2 findings and previous studies cannot therefore be concluded. We show here that the structure of enamel is severely affected, the dentin is mildly altered, and the cementum is not likely to be influenced by ADHCAI. Wright et al. found that the mineral density of the enamel in ADHCAI was decreased (45 - 80 % mineral per volume) compared to the normal range (70 - 98 %). This indicates that the mineral content of the enamel affected with ADHCAI could be widely varied from
A forty percent reduction to nearly normal level. Consistently, we observed that the mineral density of AI enamel was approximately twenty-five percent reduced compared to the controls.

Calcium and phosphorus are the main inorganic compounds involved in crystal formation and influence the physical and chemical properties of the enamel. The EDX analyses revealed that the Ca and P values in the enamel, dentin, and cementum of AI teeth were significantly decreased compared to those of the controls. It was shown the ADHCAI had marked reduction in the inorganic component of enamel, compared to other AI types including hypoplastic and hypomaturation. These imply that the teeth affected with ADHCAI had reduced inorganic, but increased organic compositions causing fragile enamel. El-Sayed et al. (2010) reported that the Ca/P ratio in the ADHCAI enamel was not significantly different from that of the controls. However, we found the inconsistent difference in Ca/P ratio between our AI sample and each control. More samples should be investigated to clarify this aspect in future studies. Interestingly, the C content and C/O ratio in the enamel, dentin, and cementum of AI teeth were significantly increased. Similarly, it was reported that the enamel in ADHCAI had higher C value and C/O ratio. These demonstrate a wide range of alterations in inorganic contents of dental hard tissues in ADHCAI. The etiology and mechanisms of the abnormal AI features still require further studies.

Our study observed deviations in clinical and radiographic manifestations, ultrastructure, mineral density, and inorganic components of dental hard tissues in ADHCAI. These changes not only affect the appearance but also physical and mechanical properties of AI teeth. The teeth are prone to deteriorate upon mastication. The applications of dental adhesives, tooth-colored filling materials, and adhesive resins on AI teeth could be jeopardized. Knowledge about AI tooth defects would therefore assist dental practitioners to provide the suitable treatment for AI patients improving their quality of life.

Conclusions

Our study demonstrated that ADHCAI could present a wide clinical spectrum ranging from pitted, rough to smooth discolored enamel. The ultrastructural changes in enamel prisms and crystallite organization, and the reduction in mineral density and composition in enamel were the main ADHCAI features. The DEJ, dentin, and cementum in ADHCAI were also altered. Therefore, the weakness of AI teeth might not effect only enamel anomalies, but changes in the structure and compositions of other parts of the teeth as well. Our study demonstrated comprehensive characteristics of ADHCAI in Thai patients. These findings lead to a better understanding and proper management of AI in the future.

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