Hypophosphatasia: Evaluation of Size and Mineral Density of Exfoliated Teeth

Hayashi-Sakai S*/ Numa-Kinjoh N**/ Sakamoto M***/ Sakai J****/ Matsuyama J*****/ Mitomi T******/ Sano-Asahito T*******/ Kinoshita-Kawano S********

Objective: *Most cases of hypophosphatasia (HPP) exhibit early loss of primary teeth. Results of micro-computed tomography (micro-CT) analysis of teeth with HPP have not yet been reported. The purpose of the present study was to describe the size and mineral density distribution and mapping of exfoliated teeth with HPP using micro CT.* ***Study design:*** *Seven exfoliated teeth were obtained from a patient with HPP. Exfoliated teeth sizes were measured on micro CT images and mineral densities of the mandibular primary central incisors were determined.* ***Results:*** *Partial dentures were fabricated for the patient to replace the eight primary teeth which had exfoliated. Most primary teeth sizes were within the normal range. The mean values of enamel and dentin mineral densities in teeth with HPP were 1.35 and 0.88 g/cm*^{3}*, respectively, in the mandibular primary central incisors.* ***Conclusion:*** *Mineral density distribution and mapping revealed that the values in teeth with HPP were lower than the homonymous teeth controls in all regions from the crown to apex. Furthermore, it was demonstrated that the differences between HPP and controls were larger on the crown side and the differences tended to converge on the apex side. These results suggested that the present patient showed mild hypomineralization in the primary dentition.*

Key words: *hypophosphatasia, , mineral density, micro-CT.*

*Sachiko Hayashi-Sakai, DDS, PhD, Assistant Professor, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.
**Natsuko Numa-Kinjoh, DDS, PhD, Researcher, Division of Oral Biochemistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.
***Makoto Sakamoto, PhD, Professor, Department of Health Sciences, Faculty of Medicine, Niigata University, Japan.
****Jun Sakai, PhD, Lecturer, Department of System and Automotive Engineering, Niigata College of Technology, Japan.
*****Junko Matsuyama, DDS, PhD, Assistant Professor, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.
******Tomoe Mitomi, DDS, PhD, Assistant Professor, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.
*******Tomiko Sano-Asahito, DDS, PhD, Assistant Professor, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.
********Shoko Kinoshita-Kawano, DDS, PhD, Assistant Professor, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, Japan.

Send all correspondence to:
Sachiko Hayashi-Sakai, Division of Pediatric Dentistry, Niigata University Graduate School of Medical and Dental Sciences, 2-5274 Gakkocho-Dori, Chuo-ku, Niigata 951-8514, Japan
Phone: +81 25 227 2912
E-mail: sachipro@dent.niigata-u.ac.jp

INTRODUCTION

Hypophosphatasia (HPP) is an in-born error of metabolism characterized by defective calcification of hard tissues such as bone and teeth and manifests with autosomal recessive or dominant inheritance. HPP is classified into six clinical forms according to the age at diagnosis: 1) perinatal, 2) infantile, 3) childhood, 4) adult, 5) odonto and 6) a rare benign perinatal form [1-5]. Symptoms vary widely from lethal skeletal hypomineralization in perinatal HPP to dental abnormalities without skeletal manifestation in odonto HPP [1, 2, 4]. The severity of the symptoms of HPP is inversely related to the patient's levels of serum alkaline phosphatase (ALP) activity, with the more severe symptoms associated with lower levels of serum ALP [1-4]. The reduction in the level of serum ALP is caused by various mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene [1-4]. As TNSALP maintains appropriate levels of inorganic pyrophosphate, an inhibitor of hydroxyapatite formation, by hydrolyzing it at the site of mineralization, loss of function of TNSALP leads to accumulation of pyrophosphate and hence to hypomineralization [1-3]. To date, a total of 287 mutations have been reported world wide (http://www.sesep.uvsq.fr/03_hypo_mutations.php).

Infantile and childhood HPP patients develop rachitic changes, but premature loss of dentition is a major feature, with incisors most commonly affected. Previous studies on HPP have revealed that hypoplasia of cementum tissue, is responsible for early loss of primary teeth in childhood HPP [1, 2, 6, 7]. However, there have been few reports on exfoliated teeth obtained from childhood patients with HPP.

Micro-computed tomography (micro-CT) is an imaging technique widely used for visualizing the internal structure of objects using the x-ray attenuation method. As it enables non-destructive, noninvasive evaluation and three-dimensional analysis, it is useful for various studies of human hard tissues [8]. Many recent studies have reported assessment of the mineral density of dental hard tissues using commercial micro-CT systems. However, the micro-CT analysis of exfoliated teeth with HPP has not been reported yet.

The purpose of the present study was to describe the size and mineral density distribution and mapping of exfoliated teeth from a patient with HPP using micro CT.

MATERIALS AND METHOD

A 1-year old Japanese boy was referred to the Pediatric and Special Needs Dental Clinic of Niigata University Medical and Dental Hospital from the pediatric clinic of a prefectural hospital for dental follow-up as the pediatrician was apprehensive about the early exfoliation of primary teeth.

The patient was a full-term infant delivered normally at 39 weeks of pregnancy. His birth weight (2,996 g) and height (49.0 cm) were within normal range. He was under observation in the neonatal intensive care unit as his mother had hypothyroidism. His condition was diagnosed as infantile HPP based on the following findings: serum alkaline phosphatase was under the reference interval upon blood test, crural bones showed slight curve on radiographic examination, calcium/creatinine rose and phosphoethanolamine exceeded the reference interval upon urine analysis. He was fed therapeutic special milk added to breast milk from 5 months of age. His mandibular primary central incisor started to erupt at 8 months of age.

At the first visit to our clinic, the patient's weight was 9,000 g and his height was 75.0 cm, which were within the normal range. Motor development was also normal. Intraoral examination revealed that the mandibular primary central incisors (71, 81) were present. These teeth exhibited physiological mobility, however, the cervical regions were exposed (Fig. 1). Eight primary teeth exfoliated spontaneously during dental follow-up (Fig. 2A, Table 1). At the age of 3 years 5 months, partial dentures were placed in the maxilla and mandible (Fig. 2B). His oral hygiene was good and he had no caries to date.

Radiographic examination revealed enlarged pulp chambers and abnormal alveolar bone resorption (Fig. 3). Permanent teeth germs showed normal development. Seven exfoliated teeth except the mandibular primary left lateral incisor (72) obtained from the patient were immersed in Teeth Keeper Neo® (Neo Dental Chemical Products Co., Ltd., Tokyo, Japan) at 4°C as the patient's parents wished to entrust them to our clinic (Table 1). All the teeth were used after obtaining informed consent from the donating patient's parents. The study was performed according to an approved protocol issued by the Institutional Review Board of the Faculty of Dentistry, Niigata University (approval number: 26-R30-10-09).

Experimental Micro-CT parameters

All measurements were performed using a micro-CT compact desktop system SkyScan-1174 (Bruker-micro CT Corp., Kontich, Belgium) with a camera resolution of 1280 ×1024 pixels. A 1.0-mm-thick aluminum filter was set in the beam path to remove low energy X-rays. A new flat field reference was acquired to reduce ring artifacts in every sample before scanning. This serves to correct for sensor non-uniformity and ensures the sensor is in optimal working condition. Samples were scanned with X-rays. Rotation step was set to 0.7°, resulting in 272 two-dimensional projections over a 180° rotation of the sample. To improve the signal-to-noise ratio, a 3-frame averaging mode, geometrical correction and flat field correction were applied. All images were scanned at a source voltage of 50 kV and 800 µA, and an image pixel size of 32 µm.

Cross-sections were reconstructed using software (NRecon, Version 1.6.6.0, Bruker-micro CT Corp., Kontich, Belgium) provided by the scanner manufacturer. Beam hardening artifacts were corrected by preselecting a correction level that minimized the artifact shown in the preview image in the NRecon software. Scanning slices were used to reconstruct images with a resolution of 1024 ×1024 pixels and with an isotropic size of 32 µm. Data were saved in 16 bit TIF formatted files.

Figure. 1 Clinical intraoral views at the age of 1 year 0 months.

Table 1. Age at which teeth were exfoliated in the present case. Seven exfoliated teeth, except the mandibular primary left lateral incisor (72), were used as samples.

		Left	Right
Maxillary			
Primary central incisor	(51, 61)	3y4m	3y1m
Mandibular			
Primary central incisor	(71, 81)	1y0m	1y0m
Primary lateral incisor	(72, 82)	(2y0m)	2y1m
Primary canine	(73, 83)	2y1m	3y2m

Figure. 2 Clinical intraoral views at 3 years 5 months with (A) 8 primary teeth missing and (B) with partial dentures.

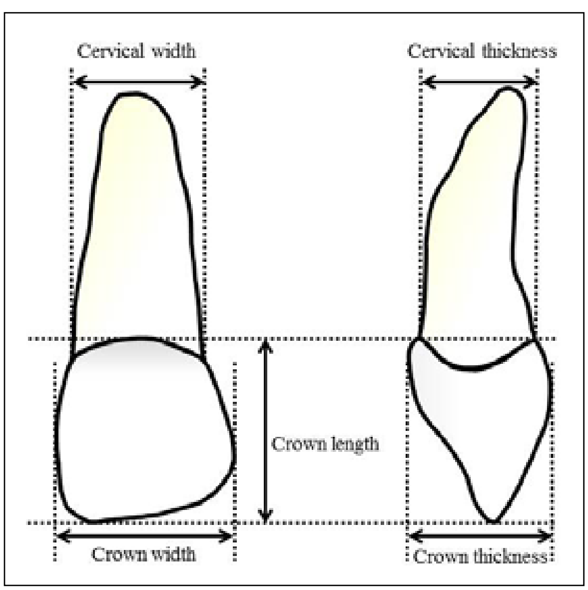
Figure. 3 Radiographic appearance at 4 years 0 months of age.

Size measurement of exfoliated teeth

Seven exfoliated primary teeth in the present case were measured from the reconstructed micro CT images according to Onda's method [9]. The software package CT Analyzer (Version 1.14.4.1, Bruker-micro CT Corp., Kontich, Belgium) was used for measurements. As some roots of the exfoliated teeth showed incomplete formation or abnormal external resorption, the following 5 items that could be measured were adopted (Fig. 4):

Crown length: the distance from the top of the cervical curve to edge of the labial surface. *Crown width*: the distance from the top of the mesial surface curve to the top of the distal surface curve. *Crown thickness*: the distance from the top of the labial surface curve to the top of the palatal surface curve. *Cervical width*: the distance from the mesial surface to the distal surface along the cervical line.

Figure. 4 Inspection items for size measurement of exfoliated teeth.



Cervical thickness: the distance from the labial surface to the palatal surface along the cervical line

The obtained data were compared with the standard mean value of normal Japanese children in each tooth type.

Mineral density analysis of exfoliated teeth

Mineral densities of the exfoliated left and right mandibular primary central incisors (n=2) were investigated. Exfoliated teeth with incomplete root formation could not be compared with data of teeth with complete root formation. For comparison of mineral densities, exfoliated and control teeth need to be in the same stage of root formation. Therefore, left and right mandibular primary central incisors (n=2) which had completely luxated due to trauma from a healthy boy aged 1 year were used as controls.

Data analyses were performed from edge to apex in each sample. For quantitative measurements of enamel and dentin mineral densities, the software package CT Analyzer was used. Two hydroxyapatite phantoms with different mineral densities (0.25 and 0.75 g/cm^3) were used as calibration standards. For each sample, regions of interests (ROIs) were drawn on each enamel and dentin, and then their data sets were extracted from the data obtained by scanning whole teeth, respectively. Grey values were detected and mineral densities could be calculated, then the mean values of enamel and dentin mineral densities were obtained. The obtained data for teeth with HPP were compared with control teeth.

Mineral density distribution was investigated. In the present study, the edge was defined as 0% and the dentinoenamel junction was defined as 100% of the distance of enamel, the dentinoenamel junction was defined as 0% and the apex was defined as 100% of the distance of dentin (Fig. 6A). Each mineral density on each scanned image was plotted to a scatter diagram. Mineral density distribution of enamel and dentin in teeth with HPP was compared with controls.

The reconstructed dataset was imported into DataViewer (Version 1.4.4, Bruker-micro CT Corp., Kontich, Belgium) and false colored for visualization. A mapping of the mineral density was also made and HPP and control mandibular primary left incisors were compared.

Figure. 5 Teeth size comparison between those with HPP and the standard mean value of normal Japanese children. (A) Maxillary primary central incisors, (B) mandibular primary central incisors, (C) mandibular primary lateral incisors, (D) mandibular primary canines. Bar top represent the maximum and bar bottom represent the minimum values in healthy teeth [9].

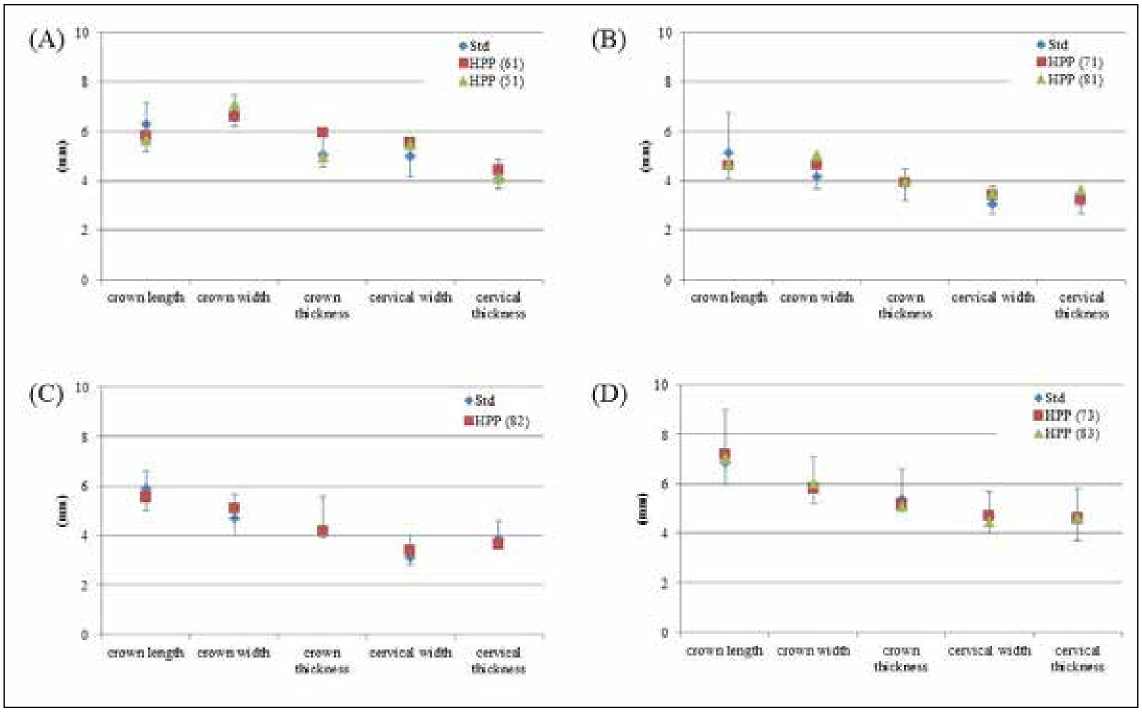


Figure. 6 Comparison of mineral density distribution between teeth with HPP and control teeth in mandibular primary central incisors. (A) Schematic representation of enamel and dentin distance. (B) Mineral density distribution in enamel and (C) dentin.

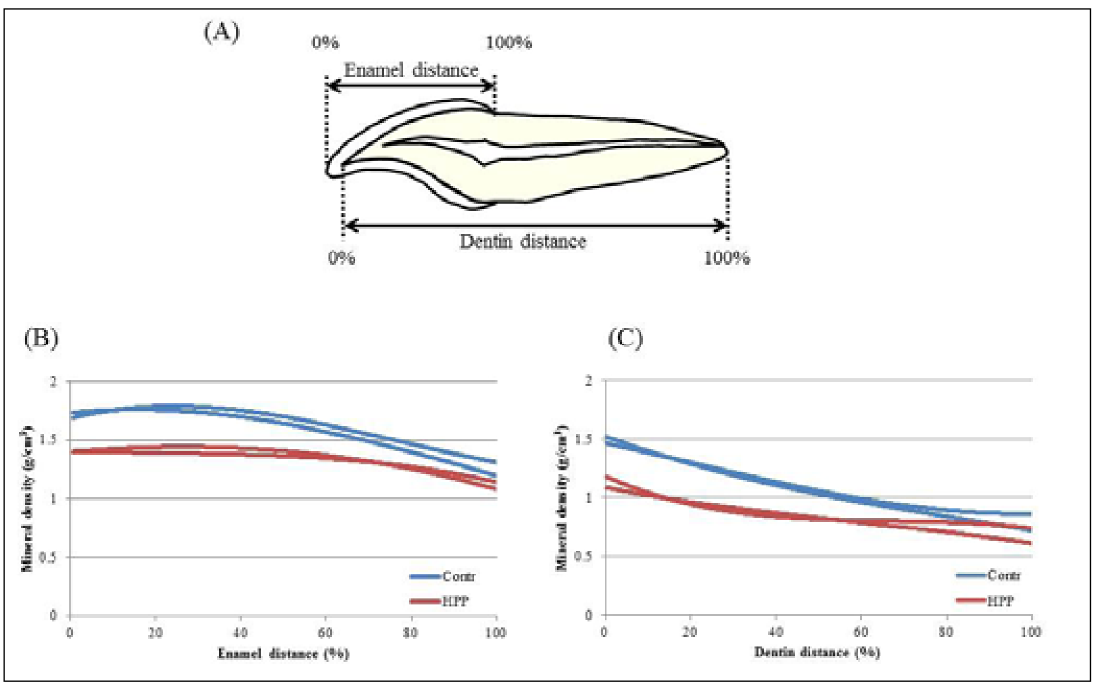
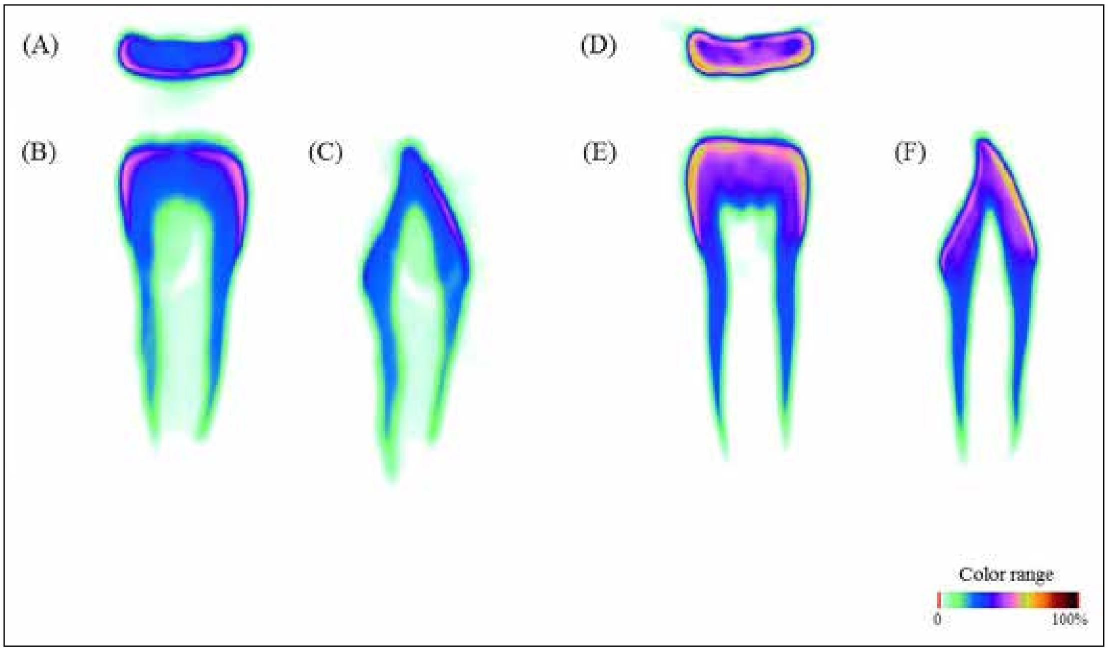


Figure. 7 Mineral density mapping of (A, D) incisal, (B, E) labial and (C, F) mesial view in (A, B, C) HPP and (D, E, F) control on mandibular primary left central incisor (71). Color range represents the relative values of attenuation coefficient. It means that 100% is equal to 3.0 g/cm^3.



RESULTS

The patient in this case was originally diagnosed with infantile HPP. He is a compound heterozygote carrying the genotype R119H/c.1559delT in the *ALPL* gene (MIM 171760) encoding TNSALP. The missense mutation R119H was transmitted from his father, while the c.1559delT frame-shift mutation was from his mother. R119H has an ALP activity of 35% of that of the wild type-TNSALP. The replacement of arginine with histidine maintains the positive nature of this mutation, giving rise to the moderate phenotype of this missense mutation [2, 10]. In contrast, c.1559delT frame-shift mutant protein having an 80 amino acid-long extension at the C-terminus, exhibits very low ALP activity and thus a severe allele [11]. The c.1559delT allele is the most frequent mutation among HPP patients in Japan [4].

Eight primary anterior teeth had exfoliated spontaneously between the age of 1 year 0 months (at first visit to our clinic) and 4 years 0 months (at present). The age of teeth exfoliation is shown in Table 1.

The sizes of exfoliated primary teeth with HPP are shown in Figure 5. Most of the primary teeth sizes were within the normal range of Japanese children. However, crown width of the mandibular right primary central incisor exceeded the maximum and crown thickness of both mandibular primary canines, and was below the minimum for healthy teeth.

Mineral density of primary teeth with HPP

The mean enamel mineral density of mandibular primary central incisors with HPP was 1.35 g/cm^3 and the corresponding value for controls was 1.66 g/cm^3. The mean dentin mineral density was 0.88 g/cm^3 in mandibular primary central incisors with HPP and 1.10 g/cm^3 in controls.

The mineral density distribution in enamel and dentin is shown in Figure 6B and C. All teeth tended to show a decrease in gradient toward 100% in enamel and dentin. Mineral densities in teeth with HPP were lower than the controls in all regions. These findings were also revealed in the visualized mapping of mineral densities (Fig. 7).

DISCUSSION

HPP is a rare inherited disorder. As we treated this patient with HPP, we were able to describe the clinical oral findings in terms of exfoliated teeth size and mineral density. Although the patient's parents entrusted us with the exfoliated teeth, they were not willing to subject them to destructive tests. Therefore, non-destructive tests using micro CT were adopted for the measurement and analysis of the exfoliated teeth in the present study.

Based on the general phase of root formation, it is considered that mandibular primary central incisors and canines exfoliated before root formation was complete, the primary lateral incisors exfoliated around the phase of root completion, and the maxillary central incisors exfoliated after root completion [12]. As the maxillary central incisors exhibited external root resorption, it is suggested that these teeth had developed and completed root formation, and then exfoliated with abnormal external root resorption.

Primary teeth size obtained was compared with the mean value of normal Japanese children standard in each tooth type. The findings in the present case did not correspond to the previous report that the HPP had large values of mesio-distal and buccal-lingual width because most primary teeth sizes were within the normal range [13].

Exfoliated teeth with incomplete root formation could not be compared with data of teeth with complete root formation in the mineral density analysis of exfoliated teeth because enamel development leads to post-eruptive maturation. Therefore, sound mandibular primary central incisors from children of the same age were used as controls for comparison. The mineral densities were measured in the homonymous teeth. Both enamel and dentin mineral densities in subjects with HPP proved to be lower than the controls in the present case, although there were no statistically significant differences between control and HPP in the mineral content of dentin [6]. In particular, mineral density distribution revealed that the values in teeth with HPP were lower than those of the controls in all regions from crown to apex. Furthermore, it was proved that the differences between HPP and controls were large on the crown side while the differences tended to converge on the apex side. In the mapping of mineral densities, the above findings were clearly demonstrated. These results suggested that the present patient showed mild hypomineralization in the primary dentition.

As HPP presents with various symptoms and hypomineralization levels, the results of the present study on primary teeth size and mineral densities may not be generalized to all patients with HPP. The general bone mineral density of the present patient has not been investigated as he is too young. We will also study the correlation between bone mineral density and teeth mineral density in the future. A positive correlation may suggest that the general bone mineral density can be predicted in cases of premature exfoliation of teeth. More case reports on HPP from various viewpoints would help dentists to perform clinical treatment more smoothly.

CONCLUSIONS

HPP is a rare inherited disorder and most cases are accompanied with the early loss of primary teeth. However, there is little useful information regarding the dental characteristics. Therefore, the clinical oral findings, treatment, exfoliated teeth sizes and mineral densities of teeth with HPP were described in this paper. Most exfoliated primary teeth with HPP sizes were within the normal range; however, mineral densities of enamel and dentin of exfoliated primary teeth with HPP were lower than homonymous control teeth of the same age. These results suggested that the present patient showed mild hypomineralization in the primary dentition.

ACKNOWLEDGEMENT

The authors would like to acknowledge the patient and his parents for their kind cooperation, and Prof. Kimimitsu Oda, Division of Oral Biochemistry, Niigata University Graduate School of Medical and Dental Sciences, for his kind and valuable suggestions. This study was supported in part by a Grant-in-Aid for Scientific Research (C) (No. 26462835) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

1. Whyte MP. The Metabolic and Molecular Bases of Inherited Diseases. 8th Ed. New York, McGraw-Hill; 5319-5329, 2001.
2. Mornet E. Hypophosphatasia. Orphanet J Rare Dis 2: 40, 2007. Available from: http://www.ojrd.com/content/pdf/1750-1172-2-40.pdf
3. Whyte MP. Physiological role of alkaline phosphatase explored in hypophosphatasia. Ann NY Acad Sci 1192: 190-200, 2010.
4. Mornet E. Genetics of Hypophosphatasia. Clinic Rev Miner Metab 11 (2): 71-77, 2013.
5. Oda K, Numa-Kinjoh N, Sohda M, Komaru K, Amizuka N. Tissue-nonspecific alkaline phosphatase and hypophosphatasia. Clin Calcium 24 (2): 233-239, 2014 (in Japanese).
6. Van den Bos T, Handoko G, Niehof A, Ryan LM, Coburn SP, Whyte MP, BeertsenW. Cementum and dentin in hypophosphatasia. J Dent Res 84 (11): 1021-1025, 2005.
7. Wei KW, Xuan K, Liu YL, Fang J, Ji K, Wang X, Jin Y, Watanabe S, Watanabe K, Ojihara T. Clinical, pathological and genetic evaluations of Chinese patients with autosomal-dominant hypophosphatasia. Arch Oral Biol 55 (12): 1017-1023, 2010.
8. Zou W, Hunter N, Swain MV. Application of polychromatic μCT for mineral density determination. J Dent Res 90 (1): 18-30, 2011.
9. Onda S. Anatomy of primary teeth, 2nd Ed. Tokyo, Oral health association of Japan; 3-5, 1995 (in Japanese).
10. Zurutuza L, Muller F, Gibrat JF, Taillandier A, Simon-Bouy B, Serre JL, Mornet E. Correlations of genotype and phenotype in hypophosphatasia. Hum Mol Genet 8 (6): 1039–1046, 1999.
11. Orimo H, Goseki-Sone M, Inoue M, Tsubakio Y, Sakiyama T, Shimada T. Importance of deletion of T at nucleotide 1559 in the tissue-nonspecific alkaline phosphatase gene in Japanese patients with hypophosphatasia. J Bone Miner Metab 20 (1): 28–33, 2002.
12. Schour I, Massler M. The development of the human dentition. J Am Dent Assoc 28: 1153-1160, 1941.
13. Inoue M, Suzuki S, Nakata M. Dental findings in a case of hypophosphatasia. Jpn J Ped Dent 15 (1): 62-70. 1977 (in Japanese).