Evaluation of Alveolar Bone Hypomineralization in Hypophosphatasia Patients Using Orthopantomography

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Abstract

Hypophosphatasia (HPP) is a metabolic disease characterized by impaired bone mineralization and early exfoliation of primary teeth. The purpose of this study was to develop a method for quantitatively evaluating alveolar bone hypomineralization using orthopantomographic images. Alveolar bone density was defined according to the pixel values and corrected by brightness shown by an indicator applied to the orthopantomographic device. Images of 200 systemically healthy subjects (aged 2–15 years) were classified into five age groups. The corrected pixel values were significantly lower in the younger group than in those aged 14–15 years (2–4, 5–7 and 8–10 years versus 14–15 years: $P < 0.0001$, 11–13 years versus 14–15 years: $P < 0.01$). Orthopantomographic images of 17 HPP patients were evaluated. The corrected pixel values of three-quarters of the odonto type HPP patients were lower than the mean values for the healthy group. One-third of patients treated with enzyme replacement therapy showed higher corrected pixel values than the healthy group. Our results suggest that odonto type HPP without skeletal problems is occasionally accompanied by hypomineralization of alveolar bone, and that alveolar bone hypomineralization in severe HPP patients is possibly improved by enzyme replacement therapy.

Introduction

Hypophosphatasia (HPP) is an inherited metabolic disease caused by mutations in the *ALPL* gene encoding tissue-nonspecific alkaline phosphatase (TNSALP) and is characterized by impaired mineralization of bones caused by low levels of alkaline phosphatase (ALP) activity [1–4]. Primary symptoms in the diagnostic criteria of HPP are bone mineralization disorders and premature loss of primary teeth (before the age of 4 years) [5].

HPP is classified into six types by the age of onset and symptoms [1–5]. Perinatal severe HPP is the most severe type with obvious symptoms at birth, whereas a child with infantile HPP presents with symptoms before 6 months of age. Perinatal severe HPP patients and half of infantile HPP patients have poor life prognoses without treatment because of respiratory complications [6]. In childhood type HPP, the first symptoms occur between 6 months and 18 years of age, while adult type HPP is diagnosed after the age of 18 years. Odonto type HPP is the mildest form, featuring dental complications at any age without bone symptoms. Early exfoliation of the primary incisors before 4 years of age sometimes leads to the diagnosis of mild HPP, such as childhood type HPP [7–12]. Odonto type HPP occasionally shifts to childhood or adult type HPP with bone symptoms as the patients grow up [13,14].

Enzyme replacement therapy (ERT) using bone-targeted recombinant ALP has improved the prognosis of severe type HPP patients [15–21]. The main dental symptom of HPP is early exfoliation of primary teeth caused by the disturbed formation of cementum [7–12]. Since the establishment of ERT in recent years, severe type HPP patients who did not survive until they were old enough to adapt to cooperative dental management are now able to visit dentists for dental treatment [22,23]. In addition to disturbed cementum formation, many dental manifestations are detected as the patients grow up, such as

hypomineralization of enamel and dentine, thin alveolar bone, malalignment, and occlusal problems [24]. However, the dental effects of ERT remain to be elucidated [24–26].

Presently, there is no standard index available for evaluating alveolar bone hypomineralization in HPP patients. The aim of this study was to establish a quantitative method for evaluating alveolar bone hypomineralization using orthopantomography to compare HPP patients with the standard values of systemically healthy subjects and to reveal differences between HPP patients and systemically healthy subjects.

Methods

Medical details of HPP patients

The following patient details were assessed and recorded: chronological age at the time of orthopantomography, sex, phenotype (perinatal severe, perinatal benign, infantile, childhood, odonto), age at onset of HPP, age at diagnosis, serum ALP values (Japanese Society of Clinical Chemistry method [40] at diagnosis), treatment with or without ERT, and type of *ALPL* mutation.

Collection of orthopantomographic images

Systemically healthy patients were invited to participate in the study when they required orthopantomography as part of their treatment for dental caries, periodontal disease, or occlusal problems. Informed consent was obtained from all parents or legal guardians of patients. The healthy subjects (aged 2–15 years) were classified into five age groups (2–4, 5–7, 8–10, 11–13, and 14–15 years). When informed consent was obtained from 40 subjects per group, orthopantomographic images were taken with a special step wedge constructed for the present study. Images were obtained from 200 systemically healthy subjects. However, 16 subjects were excluded because radiolucent or radiopaque periapical lesions were observed near the ROI on the orthopantomographic images. Sixteen more subjects were then recruited. A total of 17 HPP patients aged 2–15 years who came to our clinic for oral management were also invited to participate in this study.

Orthopantomography

A step wedge was fabricated for use as an indicator when applied to the chinrest of the orthopantomographic device (Hyper X, Asahi Roentgen Industries Co., Ltd, Kyoto, Japan) (Fig. 1A, B). Orthopantomographic images were taken with 12 mA of tube current and 60 kV of tube voltage.

Alveolar bone density

The ROI was manually placed at the distal side of the second mandibular premolar root or the primary second mandibular molar (Fig. 1C). The size of the ROI was 20 × 20 pixels. The average pixel value of this ROI was measured. If radiolucent or radiopaque lesions were recognized in this area, the patient's data was excluded.

Wedge brightness correction

The brightness of the mandible and the wedge on orthopantomographic images varies depending on the imaging model and the subject. Therefore, it is necessary to correct for differences in brightness as much as possible. We determined the boundary between eight steps of wedge brightness on each orthopantomographic image (blue vertical lines) (Fig. 1D). We measured the brightness inside the wedge of each step (yellow line, measured as close to the upper edge of the wedge as possible). We also measured the brightness of the background just outside the wedge of each step (red line). Finally, we subtracted the measured value of the red line from the measured value of the yellow line in each step. Thus, we determined the approximate value of the brightness of the wedge steps only, excluding the soft tissue brightness.

ROI brightness correction

Difference in the brightness of the ROI also needed to be corrected on orthopantomographic images. We therefore performed the following operations and calculations. Orthopantomographic images of a phantom were taken 10 times as a reference image using the same method as in Fig. 1, and the average brightness of the wedge was measured according to the method in Fig. 1D. The average brightness value inside the ROI was measured and defined as parameter A. The number of steps of the wedge on the same image closest to the average brightness of the ROI was determined. The average brightness of the step was defined as parameter B. The average brightness of the wedge of the phantom image at the same step was defined as parameter C. Thus, the ROI corrected brightness was calculated as follows:

ROI correction density = $A - (B - C)$

Dental age

Dental ages were calculated using the method described by Haavikko [39], which has been shown to be valid for application in Japanese subjects [41]. Haavikko reported age medians in years for 12 tooth formation stages for boys and girls separately as well as for the maxilla and mandible. One pediatric dentist assessed the formation stages of all permanent teeth from the orthopantomographic images. The formation stages were converted to chronological age, and the average of those chronological ages was the dental age of the individual.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 (GraphPad Software Inc., La Jolla, CA, USA). Intergroup differences were compared using analysis of variance (ANOVA). Bonferroni correction was used for post-hoc analysis. Differences with $P < 0.05$ were considered statistically significant. Differences in inheritance patterns were assessed by Fisher's exact test. A Pearson's correlation analysis was performed to find the correlation between chronological age and dental age, and between pixel value and dental age.

Ethical approval

This study was conducted in full adherence to the Declaration of Helsinki (64th World Medical Association General Assembly, Fortaleza, Brazil, 2013) and the Ethical Guidelines for Medical and Health Research Involving Human Subjects. The study protocol was approved by the Ethics Committee of Osaka University Graduate School of Dentistry (approval no. H29-E26). Other institutions were approved by their own Ethics Committee as participating facilities based on our approval. All volunteers and patients were informed in writing and gave written informed consent to participate. All data were fully anonymized before they were accessed in this study. This trial was registered (UMIN000033623).

Results

Background of subjects

Orthopantomographic images were obtained from 200 systemically healthy subjects (110 males, 90 females) and 17 HPP patients (8 males, 9 females) from our clinic (Supplementary Table 1).

Orthopantomographs were taken for the purpose of treatment for dental caries, periodontal disease, and occlusal problems in the systemically healthy subjects and HPP patients. Table summarizes the medical details of the HPP patients in the present study. The most frequent phenotype of HPP reported was the odonto type, followed by perinatal severe and childhood, perinatal benign, and infantile. There were no significant differences between the average age at onset for patients with odonto type (range, 14.4–39.6 months) and childhood type (range, 8.4–20.4 months). There were also no significant differences between the average age of diagnosis for patients with odonto type (range, 20.4–70.8 months) and childhood type (range, 25.2–75.6 months). One patient with infantile type and two patients with childhood type were initially diagnosed based on the dental manifestation of early exfoliation of primary teeth. The mean serum ALP values for patients with odonto type were significantly greater than those for patients with perinatal severe type ($P=0.0016$) and perinatal benign type ($P=0.0198$). There were no significant differences between mean serum ALP values for patients with odonto type and childhood type, or between odonto type and infantile type. All patients with perinatal severe and infantile types were receiving ERT. Information regarding the *ALPL* mutations for 15 cases showed that autosomal recessive inheritance was significantly more frequent in the non-odonto types than in the odonto type ($P=0.007$).

Reproducibility of adjusted pixel value

A step wedge was fabricated for use as an indicator when applied to the chinrest of the orthopantomographic device and was placed to create vertical height below the inferior edge of the mandible during orthopantomography (Fig. 1). Reproducibility was checked 10 times with phantom scans on different days. The pixel values of each step wedge and the right and left region of interest (ROI) were calculated and adjusted (Supplementary Table 2). The reproducibility determined with the variation coefficient of the 10 phantom scans ranged from 1.7–3.9%, suggesting that the present method has high reproducibility.

Corrected pixel value in systemically healthy subjects

The corrected pixel values in the systemically healthy subjects were 77.8 ± 4.5 (average $\pm$ SEM) in the 2–4 year group ($n = 40$), 75.5 ± 4.7 in the 5–7 year group ($n = 40$), 84.9 ± 4.9 in the 8–10 year group ($n = 40$), 93.2 ± 4.8 in the 11–13 year group ($n = 40$), and 110.9 ± 4.5 in the 14–15 year group ($n = 40$) (Fig. 2). In the systemically healthy subjects, the corrected pixel values of the 2–4, 5–7, 8–10, and 11–13 year groups were significantly lower than that of the 14–15 year group (2–4, 5–7 and 8–10 year groups versus 14–15 year group: $P < 0.0001$, 11–13 year group versus 14–15 year group: $P = 0.0038$) and increased with age.

Chronological age, dental age and corrected pixel values in systemically healthy subjects

Dental ages were evaluated in the orthopantomographic images according to the stages of the permanent teeth. However, the crown of the maxillary central incisor was not formed completely in one case, and the tooth germ of the third molars could not be observed in another case, demonstrating the difficulty in determining the dental age. These two cases were therefore excluded from the analyses in the present study. A significant positive correlation was found between chronological age and dental age in the systemically healthy subjects ($P < 0.0001$) (Fig. 3). Additionally, dental age was demonstrated to be positively and significantly correlated with the corrected pixel values in systemically healthy subjects ($P < 0.0001$) (Fig. 4).

Corrected pixel values and dental age in HPP subjects

The corrected pixel values of two odonto type patients aged 2–4 years, one odonto type patient aged 5–7 years, two odonto type patients aged 8–10 years and one odonto type patient aged 11–13 years were lower than the mean values of the systemically healthy subjects (Fig. 5). However, the corrected pixel values of all of the HPP patients without ERT were higher than the mean values of systemically healthy subjects. Additionally, the corrected pixel values of one-third of the HPP patients treated with ERT were higher than the systemically healthy subject group of the same age.

Correlation between chronological and dental age in HPP patients

The dental age of four patients with HPP could not be evaluated because the crown of the maxillary central incisor was not completely formed. The dental age of all HPP patients treated with ERT was shown to be lower than the mean age of the systemically healthy subjects (Fig. 6). A significant positive correlation was found between chronological and dental age in the HPP subjects ($P < 0.0001$) (Fig. 7). However, the gradient of the linear equation of HPP subjects was smaller than that of the systemically healthy subjects, suggesting that HPP patients had a lower dental age than that of the systemically healthy subjects.

Discussion

The frequency of severe type HPP, mostly the perinatal and infantile types, has been estimated to be 1 per 150,000 in Japan and North America, and 1 per 300,000 in European countries [27–30]. However, the frequency of heterozygous HPP is estimated to be 1 per 6370 in European countries [4,31]. In the present study, patients with odonto type HPP made up half of the total patients and had mostly dominant inheritance [24]. It is estimated that many patients with odonto type remain undiagnosed. There was no significant difference in serum ALP values at diagnosis between odonto type and childhood type HPP [24]. Additionally, dental manifestations were the first symptoms of HPP in three cases of infantile and childhood type HPP in this study. HPP is known to be a progressive disease. Patients diagnosed with odonto type HPP with only dental manifestations sometimes transition to childhood type or adult type HPP with bone symptoms as they grow up [2,13,14,24]. Early diagnosis and management of growth and development are important for HPP patients. Dentists are in a position to make an early diagnosis based on the early exfoliation of the primary incisors.

This is the first study to devise a method for quantitatively evaluating alveolar bone mineralization in orthopantomographic images taken in HPP patients. To achieve this goal, it is important to know the standard values for alveolar bone mineralization in systemically healthy subjects. Therefore, we collected data from systemically healthy subjects who came to our clinic for dental treatment or periodical examinations. When healthy patients required orthopantomographic images for treatment of dental caries, periodontal disease, or occlusal problems, the images were taken with a special step wedge constructed for the present study. Orthopantomographic images were obtained from 200 systemically healthy subjects aged 2–15 years to compare with those of 17 HPP patients.

A novel method was devised to quantitatively evaluate pixel values in orthopantomographic images using the step wedge as a reference. Different quantitative and qualitative indices calculated on orthopantomography have been proposed to screen for reduced skeletal bone mineral density (BMD) in osteoporosis [32,33]. BMD was predicted by quantitative analysis of the trabecular pattern on dental radiographs by Geraets *et al.* [32]. A systematic review of the linear and quantitative orthopantomographic measures to assess the accuracy of these indices was performed by Calciolari *et al.* [33]. They described limitations related to differences in the experience and agreement between different operators and the different image quality and magnification of the orthopantomography. They also concluded that standardized orthopantomography and controlling for magnification and distortion are needed in detecting reduced skeletal bone density. The indicator we developed solves the problem of orthopantomography not having quantitative gray values like those of multi-detector computed tomography images by attaching a simple indicator to the chinrest.

The corrected pixel values in the systemically healthy subjects were significantly lower in the younger group than in those aged 14–15 years and increased with age. We chose the distal side of the mandibular left second premolar tooth germ in the primary or mixed dentition or the root apex of the second premolar in the permanent dentition as the measurement point for quantification of the corrected

pixel values. Stable values were obtained in this study because this region is little affected by changes in the dentition. Several studies have evaluated BMD using mandibular cortical width in children with osteogenesis imperfecta, which is the most common skeletal disease reported in children [34,35]. Our method can be applied in future studies to measure the condition of the mandible in bone diseases that are accompanied by dental symptoms, such as osteogenesis imperfecta or X-linked hypophosphatemia.

Studies involving quantitative evaluations with orthopantomography for clinical osteoporosis screening have used the mandibular cortical index and the mandibular inferior cortical width below the mental foramen [36,37]. Evaluation of the alveolar trabecular bone pattern of the mandible using orthopantomography and intraoral radiography has also been reported [32]. It has also been reported that the density and fractal analysis of orthopantomography can be used to detect osteoporosis [38]. These studies used aluminum balls of different diameters to standardize brightness. Because these methods do not fully consider the change in brightness caused by overlapping soft tissues, we attempted to devise a modified quantitative evaluation method using orthopantomography.

A significant positive correlation was found between chronological age and dental age in systemically healthy subjects. Dental age corresponds to the formation of the permanent tooth germs, which is an important index for assessing tooth mineralization. However, the actual dental age calculated in the present study should be regarded as lower than the chronological age in each case. This is because no data has been collected from Japanese children in recent times; therefore, we used data obtained from Scandinavian children 50 years ago [39] when measuring dental age in our clinical practice. The gap between chronological and dental ages in systemically healthy subjects may have been a result of the use of dental ages of different races or eras. A positive correlation was also found between dental age and corrected pixel values in the systemically healthy subjects, indicating that corrected pixel values increase with dental age because both the tooth and the bone are calcified tissues.

Patients with odonto type HPP, the mildest form of HPP with only dental symptoms, exhibit relatively higher serum ALP values than the other types of HPP except for childhood type HPP, including perinatal severe, perinatal benign, and infantile type HPP [24]. Early exfoliation of primary teeth, which is the main dental manifestation, is caused by disturbed cementum formation [7,8,11]. Because HPP is a progressive disease, bone symptoms may appear as patients grow up [2,13,14,24]. The corrected pixel values of three-quarters of the odonto type patients were lower than the mean values of the systemically healthy subjects in this study, suggesting that early exfoliation of primary teeth could be caused not only by disturbed cementum formation, but also by hypomineralization of alveolar bone. This finding also demonstrates that the reduction in ALP activity influences the mandibular bone of odonto type patients who do not have skeletal bone symptoms or signs. Taken together, the lower corrected pixel values in odonto type patients is a possible indicator of disease progression from the dental region to the whole body. Additionally, the values may be a criterion for estimating the prognosis of odonto type HPP.

One-third of the HPP patients treated with ERT recorded higher corrected pixel values than the systemically healthy subject group of the same age. Serum ALP values at birth in perinatal severe type

HPP patients were almost 0, and they cannot survive without treatment due to respiratory failure caused by severe bone hypomineralization [6]. Their corrected pixel values were close to those of systemically healthy subjects, indicating that ERT reduces hypomineralization in mandibular bone to the same extent as skeletal bone.

The dental ages of HPP patients were lower than those of the systemically healthy subjects, indicating that tooth development in HPP patients could be slower than that of systemically healthy children. No studies were found investigating tooth formation speed as a dental manifestation of HPP patients. We previously reported in a nationwide survey that hypomineralization of enamel and dentine was detected in HPP patients, especially in those with severe type HPP [24]. Delayed tooth formation is a novel dental finding in the HPP patients observed in the present study. We consider that low serum ALP values influence not only bone hypomineralization but also tooth hypomineralization in HPP patients. The dental age of all HPP patients treated with ERT was lower than the mean dental age of the systemically healthy subjects, suggesting that ERT may not reduce tooth hypomineralization to the same extent as skeletal bone hypomineralization. A limitation of this study is that it is a cross-sectional study of a rare disease. A longitudinal study of HPP patients is necessary to study the effect of ERT in the oral region, and more cases should be included to confirm any statistically significant differences.

A major limitation of the study is the small number of HPP patients included because of the rarity of this disease. In the present study, only 17 HPP patients were included, which was not sufficient for statistical analysis. However, compared with the systemically healthy subject groups of the same age, the corrected pixel values of three-quarters of the odonto type patients were lower, and those of one-third of the HPP patients treated with ERT were higher. These tendencies may be proved to be statistically significant with the accumulation of many more cases in the future.

We devised an innovative method of using orthopantomography for quantitative assessment of alveolar bone mineralization. This method revealed that odonto type HPP is sometimes accompanied by hypomineralization of the alveolar bone and teeth, and that ERT is effective in reducing hypomineralization of the alveolar bone in HPP patients.

Declarations

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Author contributions

RO and KN designed the entire study under the supervision of NK. RO, TN, and SM performed the experiments, and data interpretation was conducted by RO and TN. RO and TN wrote the manuscript, which all authors read and approved.

Additional information

The authors have no competing interests associated with this study.

Data availability statement

The original contributions presented in the study are included in the article; further inquiries can be directed to the corresponding author.

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Table

1. Table. Characteristics of HPP patients

HPP form	Perinatal severe	Perinatal benign	Infantile	Childhood	Odonto	Total
Total no. of patients	3	2	1	3	8	17
(rate for all patients)	(18%)	(12%)	(6%)	(18%)	(47%)	(100%)
Age at onset	0.0	0.0	0.0	15.6 ± 3.7	25.7 ± 2.8	14.8 ± 3.2
Mean ± SEM (months)	[0.0]	[0.0]	[0.0]	[18.0]	[22.8]	[18.0]
[median]						
Ages of diagnosis	0.0	0.0	19.2	42.4 ± 16.6	41.0 ± 5.5	27.9 ± 5.9
Mean ± SEM (months)	[0.0]	[0.0]	[19.2]	[26.4]	[37.8]	[26.4]
[median]						
ALP value (U/L)	12.7 ± 6.7	52.0 ± 13.0	167.0	199.3 ± 86.3	278.8 ± 18.1	184.5 ± 30.9
Mean ± SEM						
Total no. of patients who received ERT	3	1	1	1	0	6
(rate for all patients in each phenotype)	(100%)	(50%)	(100%)	(33%)	(0%)	(35%)
Age at start of ERT administration	0.0	0.0	20.4	142.8	-	27.2 ± 23.4
Mean ± SEM (months)	[0.0]	[0.0]	[20.4]	[142.8]		[0]
[median]						
Duration of ERT administration	47.7 ± 9.2	41.0	14.6	34.2	-	38.8 ± 6.7
Mean ± SEM (months)	[40.0]	[41.0]	[14.6]	[34.2]		[38.5]
[median]						
Inheritance patterns	3/0	2/0	1/0	1/2	0/6	7/8
AR/AD					NA 2	NA 2

ALP, alkaline phosphatase; ERT, enzyme replacement therapy; HPP, hypophosphatasia; AR, autosomal recessive; AD, autosomal dominant; NA, not available; SEM, standard error of the mean.

Figures

Figure 1

Methods for taking orthopantomographic images with the original indicator. A. A step wedge (arrow) was fabricated for use as an indicator when applied to the chinrest of an orthopantomographic device. B. Taking the orthopantomographic image using the special chinrest with the step wedge. The red dotted circle indicates the step wedge. C. Taking the orthopantomographic image with the special chinrest. The step wedge was projected under the left side of the mandibular bone (red rectangle). The yellow dot on

the orthopantomograph is the region of interest (ROI), which is a square area of 20 × 20 pixels. D. Enlargement of the red square of Fig. 1C, showing the wedge brightness correction. The blue vertical lines indicate the boundaries between the eight steps of wedge brightness for each orthopantomographic image. The yellow line shows the site where brightness was measured inside the wedge of each step. The red line shows the site where brightness was measured outside the wedge of each step.

Fig. 2

Figure 2

Distribution of corrected pixel values for each age group in years. Significant differences were determined using ANOVA with Bonferroni correction. **P < 0.01 and ****P < 0.0001 versus 14–15 year group.

Fig. 3

Figure 3

Correlation of chronological age and dental age. A significant positive correlation was found between chronological age and dental age (correlation coefficient = 0.9640).

Fig. 4

Figure 4

Correlation of the corrected pixel value and dental age. A positive correlation was found between the corrected pixel value and dental age (correlation coefficient = 0.4968).

Fig. 5

Figure 5

Comparison of the corrected pixel value of hypophosphatasia patients in each phenotype with or without enzyme replacement therapy (ERT) and in systemically healthy subjects. Circles represent treated groups and triangles represent untreated groups. Black horizontal lines shown in each chronological age group indicate average $\pm$ SEM of the healthy subject.

Fig. 6

Figure 6

Comparison of dental age of hypophosphatasia patients in each phenotype with or without enzyme replacement therapy (ERT) and in systemically healthy subjects. Circles represent treated groups and triangles represent untreated groups. Black horizontal lines shown in each chronological age group indicate average $\pm$ SEM of the healthy subject.

Fig. 7

Figure 7

Correlation of chronological age and dental age. A significant positive correlation was found between chronological age and dental age in hypophosphatasia patients (correlation coefficient = 0.9642).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable1.docx](#)
- [SupplementaryTable2.docx](#)