Title: A study of the relationship between congenitally missing permanent teeth and alveolar bone density during the growth stage using a computer-assisted measurement system for intraoral radiography

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Running title: The relationship between congenitally missing permanent teeth and alveolar bone density

Key words: congenitally missing permanent teeth (CMT), alveolar bone mineral density (al-BMD), computer-assisted detection/diagnosis, intraoral radiography

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Abstract

Background

Patients with congenitally missing permanent teeth (CMT) (excluding the third molars) often experience delayed eruption and weak eruptive forces, which can result in difficulty with bite raising. Therefore, to understand the characteristics of the alveolar bone, which is where permanent teeth develop, the alveolar bone mineral density (al-BMD) of the mandible was quantitatively compared between patients with CMT and patients with all permanent teeth except for the third molars using a computer-assisted measurement system for intraoral radiography (DentalSCOPE).

Material and methods

The participants were 40 patients aged 11 to 14 years who had not yet undergone orthodontic treatment. Ten males and ten females with CMT were selected as the CMT group, and ten males and ten females without CMT were selected as the control group. Intraoral dental X-ray imaging was performed using an indicator with internal reference objects to measure bone density.

Results

The al-BMD was the lowest in males in the CMT group, with a significant difference compared with males in the control group ($p<0.05$). A significant difference in the ratio of bone age to chronological age was observed in males ($p<0.05$).

Conclusion

The finding of decreased al-BMD in the CMT group using DentalSCOPE is considered to provide useful information for orthodontic clinical practice.

Introduction

The prevalence of congenitally missing permanent teeth (CMT) (excluding the third molars) in Japanese orthodontic patients has been reported to be between 6.2% and 15.5%[1-6], and it is one of the most common developmental anomalies in humans. Recent reports suggest that the frequency has been increasing even more[1-3]. CMT can lead to problems such as malocclusion, aesthetic issues, and functional disorders. Furthermore,

delayed tooth formation and eruption during the growth stage[7-12] and insufficient vertical growth of the alveolar bone have been suggested[13,14]. This suggests that the initiation and progress of orthodontic treatment may be delayed. Dental treatment for patients with CMT is frequently comprehensive, and it is very important for orthodontists to understand not only bone quantity, but also bone quality when considering orthodontic treatment, final prosthetics, and the use of implants.

Quantitative computed tomography (QCT) and dual-energy X-ray absorptiometry (DEXA) are generally used for quantitative assessment of bone density and diagnosing bone diseases. However, these methods require specialized equipment, and it is difficult to apply the measuring devices to the jaw bone area.

In the field of dentistry, the method of measuring alveolar bone mineral density (al-BMD) up to now using

photodensitometry involved attaching a reference to a dental film, photographing the region of interest (ROI), and measuring the bone mass by comparing it to the density obtained from the X-ray film. Aluminum [15,16], lead foil[17], and copper plates [18-20] have been used as reference materials.

Regarding al-BMD during the growth period, Mayama[18,19] previously reported al-BMD measurements using photodensitometry in patients with CMT aged 7 to 16 years. However, the photodensitometry method applicable to the intra-oral method required a complicated procedure to convert the density of the reference and the mandibular bone into a relative exposure dose using the characteristic curves for dental films produced for each lot and further substituting these values into equations to derive the bone mineral content ($CaCO_3$).

In recent years, the digitalization of intraoral X-ray imaging has advanced, and Katsumata et al. [21,22] developed DentalSCOPE, which can quantitatively evaluate and digitize al-BMD. Technology that can quantitatively evaluate al-BMD will greatly contribute to clinical dentistry.

This study aimed to use DentalSCOPE to obtain quantitative values for al-BMD in a specific disease condition involving CMT and to compare al-BMD between a group with CMT and a control group during the growth period.

Materials and Methods

The study included 40 patients between the ages of 11 and 14 years who had visited the Orthodontic Department of the Iwate Medical University Dental Medical Center but had not yet started orthodontic treatment. Of these patients, ten males (average age 13.2 years, number of

CMT 1–8) and ten females (average age 13.0 years, number of CMT 1–12) with CMT were assigned to the CMT group (Table 1). Ten males (average age 13.5 years) and ten females (average age 13.6 years) without CMT were assigned to the control group. Both groups were required to have heights and weights within ±1 standard deviation of the average for their age[23]. Cases with cleft lips or palates, systemic diseases, or bone sclerosis in the area where bone density was measured were excluded from the study.

After obtaining informed consent from the patients and their guardians to participate in this study, intraoral dental X-ray photographs (tube voltage, 70 kV; tube current, 10 mA; 0.12 to 0.14 seconds, Max-DC70, Morita, Tokyo, Japan) were taken using an X-ray indicator that contained calcium carbonate reference objects designed for the dental X-ray diagnostic device program

(DentalSCOPE; Media Co, Tokyo, Japan)(Fig. 1).

The BMD value was automatically calculated using a computer-aided measurement system (DentalSCOPE) for intraoral radiography. The ROI was defined as the area between the second premolar and the first molar on the main chewing side of the mandible. In cases in which the second premolar was congenitally missing and the second deciduous molar remained, the ROI was defined as the alveolar bone between the second deciduous molar and the first molar.

Furthermore, since the patients were in the growth phase, bone age was evaluated using hand-wrist X-ray images obtained from a series of examinations for orthodontic treatment. Bone age was evaluated using the Tanner-Whitehouse 2 (TW2) method, which is the standard for Japanese children[24], based on the RUS (radius, ulna, and short bones) maturity stage of bone, and converted to

bone age.

Statistical analysis was performed using unpaired *t*-tests for age and sex between groups. A paired *t*-test was used to examine the relationship between chronological age and bone age. Comparisons of al-BMD by sex between two groups were assessed using the Mann-Whitney U test, whereas comparisons among the four groups were evaluated using the Kruskal-Wallis test. A covariance analysis was conducted to examine the factors that affect bone density. Analyses were performed using SPSS version 30 (IBM JAPAN, Tokyo, Japan), with a significance level of 5%.

Results

1. There was no significant difference in age and sex between the groups.

2. The average value of al-BMD was the highest in control group males, at 1012.6 ± 36.3 mg/cm^2, followed by the control group females at 980.9 ± 46.4 mg/cm^2, the CMT group females at 922.5 ± 47.4 mg/cm^2, and the CMT group males at 813.9 ± 48.0 mg/cm^2. A significant difference was found between the males in the control group and those in the CMT group ($p<0.05$) (Fig. 2).
3. No correlation was observed between the number of missing teeth and al-BMD (Fig. 3).
4. There was a significant difference between males in the CMT group and control group in the relationship between chronological age and bone age. Specifically, males in the CMT group had a bone age approximately 1% lower than their chronological age, whereas the control group had progressed by 7% (paired *t*-test; $p<0.05$) (Table 2).

Discussion

The development of DentalSCOPE eliminates the need for the complex procedures of photodensitometry, and by quantifying bone density from images, it has become possible to obtain quantitative evaluations quickly and accurately. The clinical studies using DentalSCOPE have reported the measurement of al-BMD in the lower molar region of healthy young adult men and women[25], quantitative evaluation of apical inflammatory diseases in the lower molar region[26], assessment of al-BMD in osteoporotic patients before tooth extraction who were administered bone resorption inhibitors[27], evaluation of changes in apical lesions of lower molars[28], and assessment of age-related changes in al-BMD in the lower incisor region[29], among others.

As in these studies, previous research has frequently

used the mandibular molar region as the ROI. The present study also set the ROI in the mandibular molar region. The reasons for this include that this positioning facilitates reproducible imaging because it allows the intraoral X-ray detector to be placed nearly parallel to the tooth axis, the ability to use the mental foramen as a landmark for the measurement site even if teeth are missing, and the relatively uniform thickness of the surrounding soft tissues with minimal interindividual variation[17]. DentalSCOPE's default settings are also optimized for measuring mandibular premolars[21]. Based on these factors, the ROI set in this study was considered appropriate.

There have been no evaluations of al-BMD during the growth period using DentalSCOPE. Since there are currently no reports from the same age group for comparison, the present study provides the obtained al-

BMDs as reference values (Table 2).

Regarding the effects of sex differences on al-BMD, Ohashi et al[25] reported that they measured the al-BMD of healthy men and women aged 22 to 25 years, and they found that the al-BMD of men was significantly higher than that of women. In the present study targeting 11- to 14-year-olds, no significant differences between the sexes were observed. Regarding bone mineral content (BMC) during the growth period, Maki et al[15] reported that there was no significant difference between males and females in BMC measured by the photodensitometry method for ages 8 to 10 years. Maeda et al[16] also reported that, using the photodensitometry method, measurements of BMC in males and females aged 15 to 64 years showed that, in the 15 to 19 year age group, females tended to have higher values. However, they did not mention the reasons for this.

Previously, the authors[18,19] reported that, by using the photodensitometry method, they compared the BMC of a CMT group aged 7-15 years with a control group without missing teeth, and they found no significant sex difference in the CMT group or the control group. Although the present study targeted a different age group, 11 to 14 years, no significant difference between the sexes was observed in this study as well. One possible reason for this is the timing of the onset of secondary sexual characteristics.

Generally, in present-day Japan, boys start puberty at ages 10 to 12 years, whereas girls start at ages 9 to 11 years, and it is said to be completed in 2 to 3 years[30]. One of the most important events during the secondary sexual characteristics stage is the increase in bone mineral density[30]. It has been suggested that the fact that girls in the age group of 11 to 14 years begin

puberty earlier than boys is one of the factors that contributed to the lack of a significant difference in bone density between the sexes.

In the comparison between the CMT group and the control group, a significant difference in al-BMD was observed in males, but not females. When examining the ratio of bone age to chronological age, males in the CMT group had a bone age approximately 1% lower than their chronological age, whereas the control group had progressed by 7%, showing a significant difference (Table 2). This suggests that the secretion status of growth hormone, which affects bone age, is different.

The effects of sex hormones on bones involve estrogen playing a major role in both males and females. In males, testosterone is converted to estrogen by aromatase, playing an important role in bone metabolism. It has been reported that the increase in BMD is greatest from

the end of the development of secondary sexual characteristics to young adulthood[30]. From the present study, although it is possible that males in the CMT group may have had delayed onset of secondary sexual characteristics, some cases with high BMD were also observed, indicating significant individual variability. The control group showed low variance, whereas the CMT group showed high variance, suggesting that at least when al-BMD is low, there may be some bone metabolic events related to CMT (Figure 3).

Another factor that may contribute to the sparsity of trabecular bone is the reduction in physical stimulation due to decreased masticatory force associated with an insufficient number of teeth. Only two papers have assessed the impact of CMT on trabecular bone[31,32].

Temur et al[31] used panoramic X-ray images of cases with CMT of the second mandibular premolar to examine

the trabecular bone structure of the mandible through fractal analysis. They reported that the fractal dimension values obtained from the ROI area at the same site in this study were significantly lower in the CMT group than in the control group. This suggests that it may have an adverse effect on bone quality, concluding that CMT affects bone structure.

In the present study, no correlation was found between the number of CMT and al-BMD. This may be due to the small sample size of the study and individual variation. Based on the values measured in the present study, it seems significant to capture and compare the increase in bone density at regular intervals to understand the growth of mandibular alveolar bone.

CMT and delayed eruption of permanent teeth have been reported previously. Garn et al[9] reported the delayed formation and eruption of premolars and molars

in cases of CMT including the third molars. Rune et al[10] showed that, in a group with 6 to 7 CMT including the third molars, there was an average delay in tooth formation of 1.8 to 2.0 years compared with chronological age. Van der Weide[11] noted that significant delays in tooth formation were more apparent in males than in females, and that there is considerable individual variation in tooth formation in cases of oligodontia. Medina et al[8] reported that, in cases of CMT there were significant delays in the formation of the second molars, maxillary incisors, and second premolars, and that dental age was significantly delayed.

The causes of delayed formation and eruption are not clear, but Garn et al[12] suggested that there are genes related to calcification delay and eruption, and that extreme manifestations of these genes are thought to be linked to CMT. Murakami et al[33] also identified a

missense mutation (P20L) in the PAX9 gene from the genes of patients with oligodontia associated with delayed eruption, suggesting that this gene may interfere with the development of teeth and could be a cause of the onset of oligodontia. This indicates that the gene is related to CMT and delayed eruption.

However, considering that the movement of teeth during orthodontic treatment increases when bone density decreases[34], and that in comparison with adults, children's teeth move faster[34], it may be that the correlation between natural eruption force and bone density is low.

In the present study, the al-BMD of both males and females in the CMT group was lower than that of the control group. Although no significant difference was observed in females, a significant difference was noted in males. This may be related to the reports that the

delay in dental formation is more pronounced in males than in females.

Tooth development is under genetic control, and bone density is highly related to sex hormones. The results of the present study suggest that genetic factors and hormones, which are endogenous factors in growth and development, interact with each other.

This report demonstrated that, when al-BMD is low during the growth stage, a more in-depth clinical evaluation may be required.

Conclusion

In the present study, al-BMD was lower in the male CMT group than in the control group. The discovery of decreased al-BMD in the CMT group using DentalSCOPE is considered to provide useful information for orthodontic clinical practice.

Acknowledgments

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Ethical Approval

This study was approved by the Ethics Committee of the School of Dentistry, Iwate Medical University (01375).

Author Contribution

Hisayo MAYAMA : Research conception, research planning, data collection, data analysis and interpretation, data management, informed consent acquisition, manuscript writing, finalization of submitted manuscripts.

Noriaki TAKAHASHI : Research planning, determination of imaging conditions, verification of acquired X-ray images.

Mituru IZUMISAWA : Research planning, determination of imaging conditions, verification of acquired X-ray images.

Ryouichi TANAKA : Research planning, research coordination, data interpretation, manuscript revision.

Kazuro Satoh : Research planning, research coordination.

Conflicts of interest

The authors declare no conflicts of interest.

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Figure Legends

Figure 1

A: Special dental X-ray beam indicators containing reference objects.

B: The reference objects have different amounts of embedded calcium carbonate : 20% (500mg/cm^2), 60% (1,000 mg/cm^2), and 100% (1,500 mg/cm^2) .

C: The region of interest (ROI) is represented as a yellow-colored rectangular area between the apex of mandibular second premolar and first molar tooth.

Figure2.

The results of the Kruskal-Wallis test showed a significant difference in bone density between the CMT group and the control group of males. Box plots of al-BMD for each group. The horizontal line inside the box

represents the median, while the ends of the box indicate the 25th percentile and the 75th percentile.

Figure3.

The relationship in the scatter plot between number of congenital absence and al-BMD. The number of congenital absence did not show a significant correlation with al-BMD. Compared to the distribution of al-BMD in the control group (number of missing=0, n=20), the group with congenital absences group(n=20) exhibited considerable individual variation regardless of the number of missing.

Table 1.

Distribution of congenitally missing teeth by quantity

Table 1 Distribution of congenitally missing teeth by quantity

No. of missing teeth	1	2	3	4	5	6	7	8	9	10	11	12
Males	4	1	1		2		2					
Females	4	2				1	1				1	1

Table 2. Summary of the chronological age, alveolar bone mineral density, the bone age, and the ratio of bone age to chronological age of all groups.

Table 2 Summary of the chronological age, alveolar bone mineral density, the bone age, and the ratio of bone age to chronological age of all groups.

	Chronological age (years) Mean ± SD	al-BMD (mg/cm²) Mean ± SD (range)		Bone age (years) Mean ± SD	(BA/CA)×100 (%)	
CMT group Male (n=10)	13.2±1.55	813.9±48.0 (557.0-1264.0)	*(1)	13.0±1.21	99.1±9.45	*
Control group Male (n=10)	13.5±0.99	1012.6±36.3 (788.5-1123.0)		14.5±1.01	107.4±9.45	
CMT group Female (n=10)	13.0±1.17	922.5±47.4 (541.5-1231.2)		13.5±1.30	104.6±4.56	
Control group Female (n=10)	13.6±0.69	980.9±46.4 (780.8-1144.6)		13.8±1.14	102.5±6.77	

CMT : congenitally missing permanent teeth, al-BMD : alveolar bone mineral density, BA : Bone age, CA : Chronological age, SD : standard deviation *:$p < 0.05$

*(1) : Results of the Kruskal-Wallis test

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*(1) : Results of the Kruskal-Wallis test

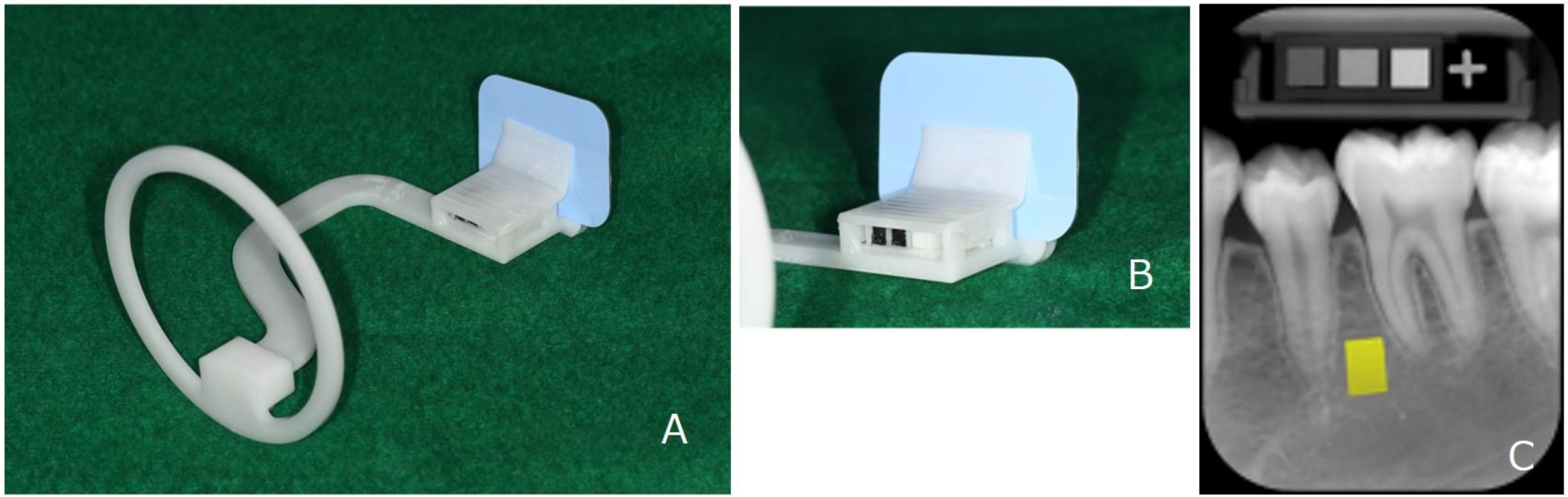

Fig. 1 A: Special dental X-ray beam indicators containing reference objects.
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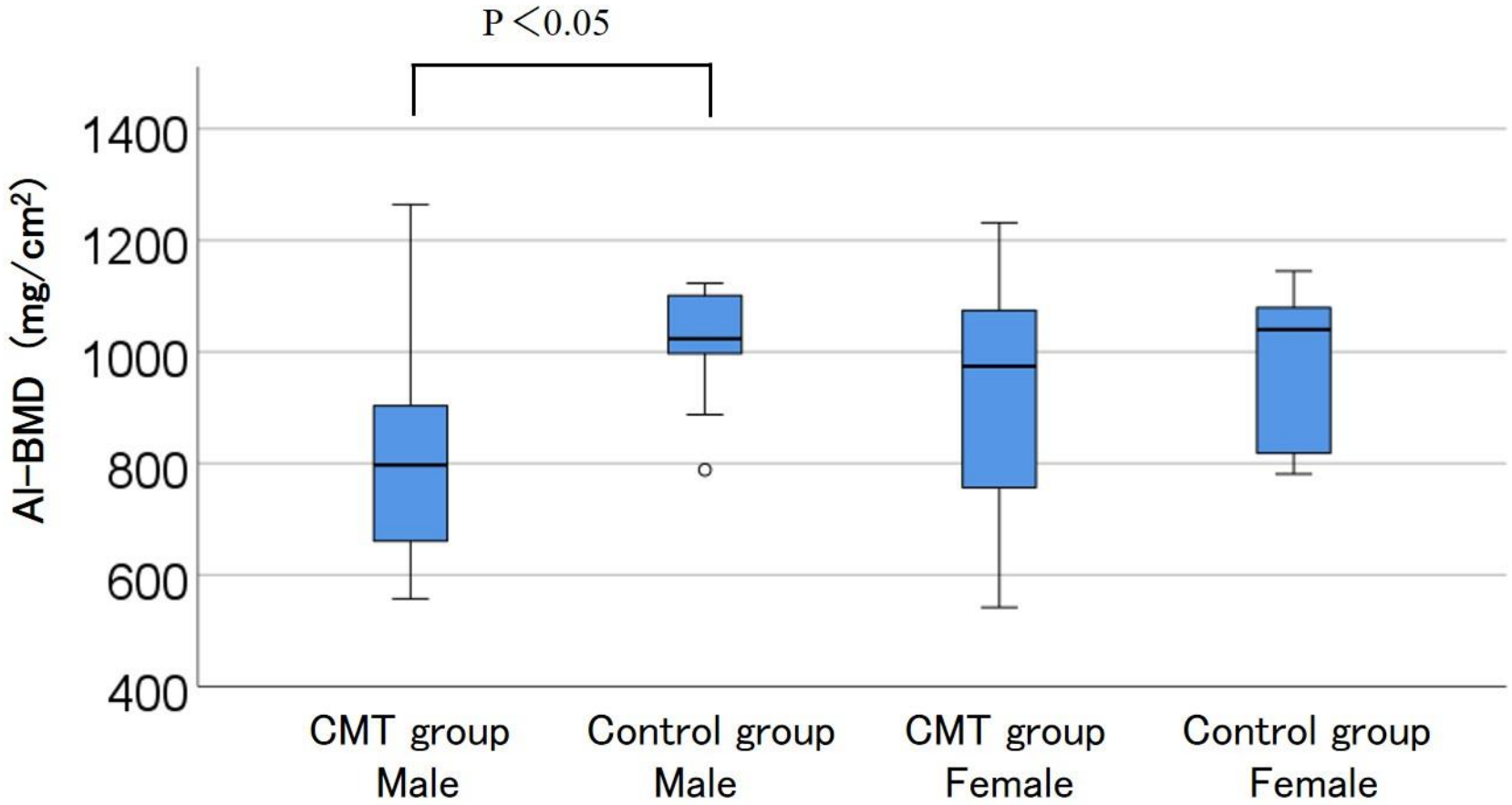

Fig. 2 The results of the Kruskal-Wallis test showed a significant difference in bone density between the CMT group and the control group of males . Box plots of al-BMD for each group. The horizontal line inside the box represents the median, while the ends of the box indicate the 25th percentile and the 75th percentile.

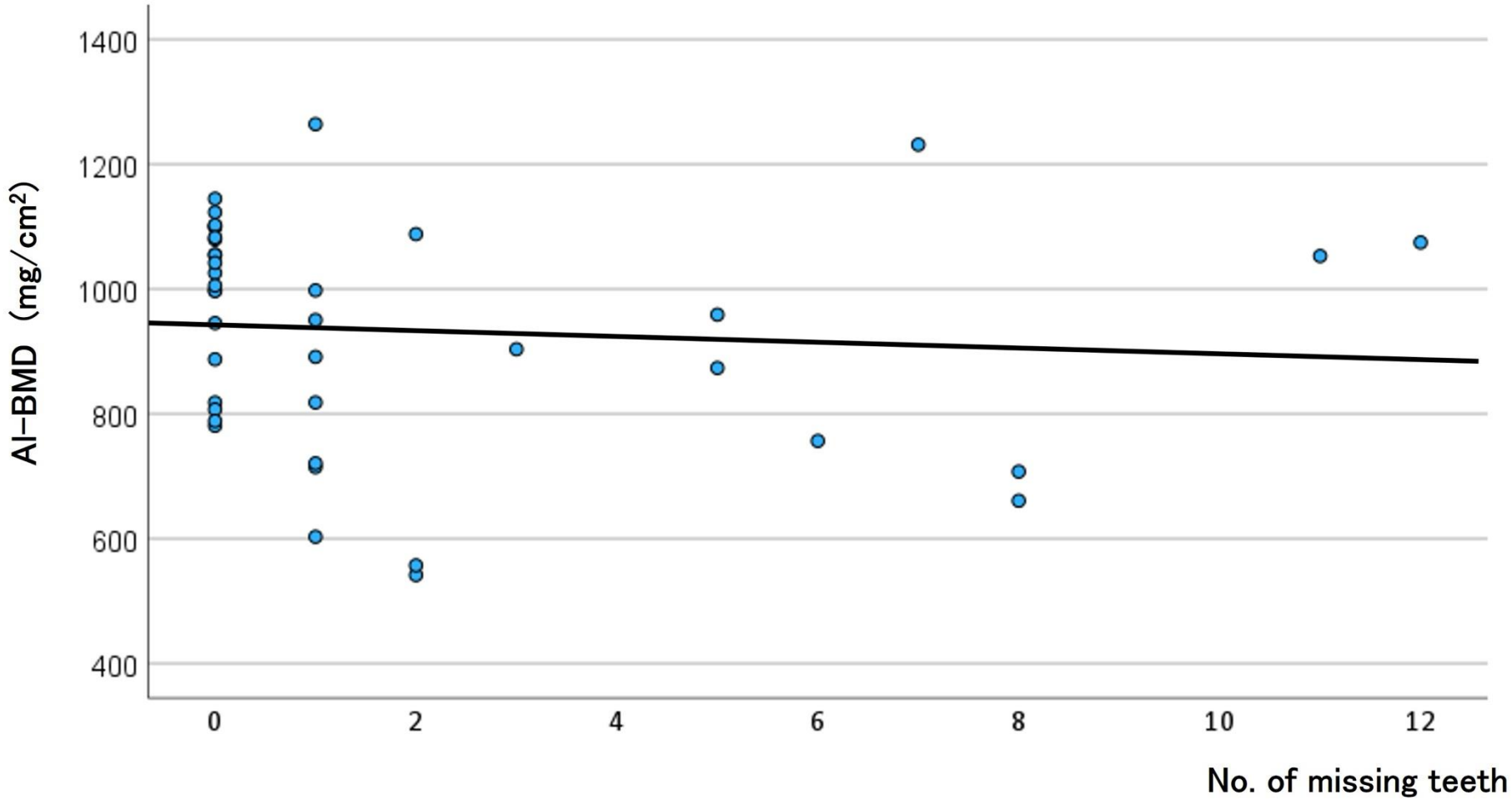

Fig. 3 The relationship in the scatter plot between number of congenital absence and al-BMD. The number of missing teeth did not show a significant correlation with al-BMD (R^2= -0.019, p = 0.610). Compared to the distribution of al-BMD in the control group (number of missing=0, n=20), the group with CMT group(n=20) exhibited considerable individual variation regardless of the number of missing.