Gender differences in mandibular bone mineral distribution with aging

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By
Jie Liu, B.D.S, PH.D
Graduate Program in Dentistry

The Ohio State University
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Thesis Committee
Dr. Do-Gyoon Kim, Advisor
Dr. Allen Firestone
Dr. William Johnston
Dr. Jahanzeb Chaudhry
ABSTRACT

Objectives: The purposes of this study were to determine 1) if cone beam computed tomography (CBCT) can determine relative differences in bone mineral density distribution using clinical images of patients’ mandibular bone and 2) if the relative differences can be used to detect the effects of gender and age on bone mineral density distribution.

Methods: Sixty six clinical CBCT images from patients (36 females and 30 males) were identified. Three age groups (40, 50, and 60 years) were identified for male and female patients. Alveolar bone (AB) and basal cortical bone regions were digitally isolated. A histogram of gray levels, which are equivalent to degrees of bone mineralization, was obtained from each region of the CBCT images. Mean, standard deviation (SD), coefficient of variation (COV), fifth percentile low (Low₅) and high (High₅) of gray levels were obtained. Percentage differences of gray level parameters between alveolar and basal cortical bones were computed.

Results: The alveolar bone region had significantly higher SD and COV, but significantly lower Mean, Low₅ and High₅ than the basal cortical bone region for all CBCT images (p<0.001). Significantly higher percentage differences of SD, COV, and Low₅ were observed when the over 50 years old female group was compared to the male group of the same age (p<0.042).
Conclusions: Significant gender differences in gray level variability observed within the postmenopausal age group suggested that the current approach to oral bone mineral density assessment using 3D clinical CBCT images can provide additional information for early diagnosis of postmenopausal osteoporosis.
DEDICATION

This dissertation is dedicated to my family and friends who have supported and encouraged me so much.
Firstly, I would like to acknowledge my thesis committee, Dr.Kim, Dr.Johnston, Dr.Firestone, Dr.Chaudhry. I thank Dr.Kim for his guidance and help for this project, thank Dr.Johnston for his valuable suggestions for statistics, his caring for graduate students; thank Dr.Firestone for his genuine support and valuable input for my research, thank Dr.Chaudhry for his valuable input for image analysis.

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VITA

2000……………………………………………………B.D.S  Binzhou Medical College
  Shandong, China

2004……………………………………………………M.S.  Oral Science
  Capital Medical University
  Beijing, China

2009……………………………………………………Ph.D  Oral Biology
  The Ohio State University
  Columbus, OH

2013…………………………………………………… M.S., Cert. in Orthodontics
  The Ohio State University
  Columbus, OH

Fields of Study

Major Field: Dentistry

Specialty: Orthodontics
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Physiology of Bone, jaw bone and remodeling

Bone as loading bearing structures should be stiff enough to resist bending, but also flexible enough to avoid fracture (Seman E, 2003). Therefore, mean mineral content of the bone matrix and the heterogeneity of the mineralization are important factors for the mechanical properties of bone (Ruffoni et al, 2007). The remodeling activity of bone is the biological determination of mineralization (Grynpas et al, 1993). Remodeling is a coupled process of bone resorption followed by bone formation without a net change in bone volume (Ruffoni et al, 2007).

Bone is composed of osteons and bone packets, which are produced at different moments during modeling or remodeling cycles (Roschger et al, 2008). The mineralization kinetics for newly deposited bone consists of two stages. Primary mineralization of bone occurs shortly after the formation of basic structural unit (BSU) and proceeds rapidly with as high as 50% of mineral content increased within a few days. Then it is followed by gradual increase in mineral component, which is named secondary mineralization and it may last for months or years (Roschger, et al, 2008). Thus individual BSUs are produced at different points in time, resulting in heterogeneity in bone mineralization (Ruffoni et al, 2007; Roschger et al, 2008). Therefore regional
variation of bone mineral content naturally occurs in bone due to bone turn over. The amount of less mineralized immature bone is increased by active bone remodeling, thus reducing bone mineral density (BMD) (Boivin et al, 2003; Ames et al, 2010).

Alveolar bone (AB) as teeth supporting bone plays a very important role in oral functions. It undergoes constant remodeling due to masticatory force transferred through periodontal ligament. Masticatory forces produce constant stress and strain within the bone, leading to micro-damages in bone (Seeman et al, 2006). Thus constant remodeling was observed in alveolar bone compared with non-teeth bearing bone, resulting in higher variation and lower mineral density in alveolar bone compared with basal bone (Chun et al, 2009; Ames et al, 2010).

Postmenopausal Osteoporosis and Jaw Bone

Osteoporosis is defined as low bone mass and micro-architectural deterioration of the bone scaffold leading to increased bone fragility in vertebrae and long bones (NIH consensus panel). Women are at greater risk for osteoporosis after menopause. Of the estimated 10 million Americans with osteoporosis, about eight million are women. Low-trauma fractures associated with osteoporosis have significant effects on people’s quality of life. Therefore, effective preventive methods should be established, especially earlier diagnostic procedures to evaluate the risk of osteoporosis.
Bone loss accelerates in women at menopause because estrogen withdrawal increases the rate of bone remodeling. Osteoclastogenesis is amplified by estrogen deficiency via cytokines and growth factors. Osteoblast activity is also increased, but in a much lower extent (Martin et al, 1994). Thus, increased bone remodeling occurring after menopause causes less bone deposited in each basic multicellular unit (BMU) and more significant negative bone balance, leading to trabecular and cortical thinning that characterize post-menopausal osteoporosis (Seeman E, 2003).

Although the systematic effect of estrogen deficiency has been known for years, its relationship to jaw bone has been examined only recently. Current studies suggest that jaw bones are also vulnerable to osteoporosis (Klemetti et al, 1993; Bollen et al, 2000; Wactawski-Wende et al, 2001; Dervis E et al, 2005; Lerner et al, 2006). Positive correlation between osteoporosis and periodontal diseases, such as periodontal bone loss and tooth loss was reported (Wactawski-Wende et al, 2001; Yoshihara et al, 2005). Estrogen deficiency significantly enhanced the alveolar bone loss in an experimental animal model of periodontitis, indicating that postmenopausal osteoporosis may enhance the risk of periodontitis associated with inflammatory alveolar bone resorption (Kobayashi et al, 2012). Bone mineral density of condyles decreases after menopause as those in the vertebrae (Yamada et al, 1997). Erosion of inferior border of mandible and decrease in number and thickness of trabecular plates were more often seen in panoramic images of patients with osteoporosis than that of controls (Klemetti et al, 1993; Bollen et
Estrogen deficiency also increased the amount and speed of orthodontic tooth movement in ovariectomized rats (Arslan et al, 2007). Histomorphometric analysis indicated that osteoclast count was significantly increased, and in contrast, osteoblast count was relatively reduced in regions of tension in ovariectomized group compared with control (Arslan et al, 2007). All these findings indicate that mandibular BMD is correlated with skeletal bone density.

**Gender-related Osteoporosis**

Osteoporosis is more common in women than in men. In female estrogen deficiency is responsible for bone loss after menopause, however, in males, osteoporosis is mainly associated with hypogonadism (Kanis et al, 1997; Anderson et al, 1992). Age related cortical changes, such as relatively thin cortical changes tend to be more marked in females than in males (Von Wowen N, 2001). One study indicated that edentulous males have significantly higher alveolar bone height than edentulous females (Bollen et al, 2004).

Although there is not enough information about the correlation of systematic osteoporosis with oral conditions, bone mineralization of the mandible was reported to have a significant correlation with those of the lumbar vertebra and femur neck (Horner et al, 1996; Wactawski-Wende et al, 1996). Therefore, dental radiographs may offer valuable information for the screening of individuals at risk for osteoporosis.
Methods to Evaluate Bone mineralization

Bone mineral density (BMD) measurement was proposed as golden standard for diagnosis of osteoporosis and has been defined to be the mineral content within a mixed bone and soft tissue region, but does not give information about the material density of the bone itself. However, density measurement in tissue mineral density (TMD) is restricted within the volume of calcified bone tissue, thus providing information about the mineral density of the bone itself, and ignoring surrounding soft tissue (Bouxsein et al, 2010). Therefore, compared with BMD, TMD is considered a better parameter to evaluate mechanical properties of bone, such as stiffness, strength and toughness (Follet et al, 2004; Yao et al, 2007). Studies have shown that tissue mineral distribution can be easily quantified as histograms of gray level frequency distribution (Roschger et al, 2003, Ruffoni et al, 2008). Therefore, measurement of degree of bone mineral density (DBM), which is equivalent to tissue mineral density, will provide better insights about the state of bone. In addition, DBM is considered as a parameter to reflect bone turnover. Therefore, any changes in bone turnover rates or disturbance in mineralization kinetics will affect distribution of bone mineral density.

Micro-CT as a non-destructive 3D imaging method has been widely used to measure bone mineral density in research (Mulder et al, 1987). Regional variation of alveolar bone at rat mandible was successfully measured by micro-CT (Ames et al, 2010).
However, due to its high radiation dose, micro-CT cannot be widely used in clinical patients.

*Cone Beam CT and Its Clinical Application*

Cone beam CT (CBCT) is a new technology specially designed for dental clinical use. It quickly gains more popularity compared with micro-CT in measurement of density for mineralized tissue due to its adequate image quality and relatively lower radiation dosage. The effective dose of CBCT varies depending on devices and FOV (field of view) (Ludlow et al, 2006). Although radiation from CBCT is much lower compared with CT, it is still at least about 7-8 times higher than conventional panoramic radiographs (Ludlow et al, 2006). Other advantages of CBCT include high resolution level, low cost, fast scan and compact size for space saving (Arai et al, 1999).

Image quality of CBCT scans is influenced by a number of variables, such as the scanning unit, the field of view (FOV), scanning time, tube voltage and current, and voxel size (Kamburoglu et al, 2011). Voxel size can influence the characteristics of the final image in several ways: the smaller the voxel size, the greater the noise, the higher the resolution (Al-Rawi et al, 2010). Studies have shown that density values obtained by CBCT images varied depending on devices (Isoda et al, 2012). CBCT has been shown to be a reliable and appropriate tool for linear measurement of bony anatomic structures. However, presence of soft tissue and different voxel size affect the precision of the data.
Study by Sun et al indicated that measurements of bone thickness in CBCT images with a voxel size of 0.25 mm were closer to the direct measurements than 0.4 mm images (Sun et al, 2011).

Hounsfield unit (HU) values, defined as linear transformation of measured x-ray attenuation coefficient of the material with reference to water, is still the standard method to evaluate bone mineral density. HU represents the relative density of body tissues according to a calibrated gray-level scale, based on values for air (-1000 HU), water (0 HU), and bone density (+1000 HU) (Nackaerts et al, 2011). It is known that HU from CBCT are not valid due to limitations (Endo et al, 2001; Naitoh et al, 2004; Hua et al, 2008). Firstly, larger amount of scattered x-rays than conventional spiral CT is produced in CBCT, thus its ability to detect low-contrast tissue is reduced due to enhanced noise in reconstructed images (Endo et al, 2001). Beam hardening is another limitation of CBCT. The increase of mean energy of x-ray beam occurs when x-rays passes through an object, resulting in altering the HU values of certain structures, such as bone tissue. Because beam width from CBCT detector is larger than conventional multi-detector CT, thus causing non-uniform angular distribution of x-ray beam intensity, which is known as heel effect, thus HU from CBCT has no uniformity either. Therefore, there were concerns about the accuracy of using CBCT to evaluate bone density. However, some recent studies suggest that it was possible to estimate BMD of objects using voxel values from

**Overall Objective**

The objectives of this study were to determine 1) if the cone beam computed tomography (CBCT) can determine relative differences in bone mineral density distribution using clinical images of patients’ mandibular bone and 2) if the relative differences can be used to detect the effects of gender and age on bone mineral density distribution. In particular, this study focused on the gender difference during the transitional years of menopause.
CHAPTER 2
MATERIALS AND METHODS

After approval of the Institutional Review Board (IRB) at the Ohio State University (Protocol #: 2011H0128), 66 clinical CBCT images were retrospectively selected from a pre-existing 350 CBCT scans taken during routine clinical practice at the College of Dentistry. The patients’ jaw were using a CBCT scanner (iCAT, Imaging Science International, Hatfield, PA, USA) at 300 micron voxel sizes under a scanning energy (120 kV and 5 mA) with 8.9 seconds scanning time. These patients (36 females and 30 males, mean 54.6±8.1 years) were all healthy individuals without any medical complications or medications when they were scanned.

The analysis process of the 3D clinical CBCT images was consistent with the previous study (Taylor et al, 2013). The images were imported into image analysis software (ImageJ, National Institutes of Health, Bethesda, MD, USA). An oral region at mandibular left first premolar tooth were digitally dissected from each CBCT image following the previous study (Figure 1) (Taylor et al, 2013). Restored teeth were excluded. The tooth was identified and separated from the surrounding periodontal ligament and bone using Livewire® (Institute of Computing, State University of Campinas, Brazil), a semi-automated segmentation software that was used to isolate the
tooth in CBCT images as introduced in the previous study (Taylor et al, 2013; Agbaje et al, 2007). The tooth image was saved as a separate image file. Following removal of tooth, digital segmentation of bone voxels from non-bone voxels including the periodontal ligament voxels of the mandibular bone image was performed using a heuristic algorithm as used in the previous studies (Ames et al, 2010; Zauel et al, 2004). Then, the isolated tooth image was imported to the imageJ software and three-dimensionally dilated up to 1 mm (corresponding to about 3 voxels) from the tooth roots. A 3D alveolar bone (AB) region within 1 mm from the tooth roots was obtained by overlapping the dilated tooth image on the separated mandibular bone image (Figure 1).

A core region of basal cortical bone (CB) region was isolated by three-dimensionally eroding the separated mandibular bone image by 0.6 mm (corresponding to 2 voxels) from periosteal and endosteal surfaces of basal bone. This core region of basal cortical bone represents a bone region that has stable normal bone remodeling excluding the marginal basal bone at which an active bone modeling occurs (Huja et al, 2008).

The gray level of each bone voxel, which is equivalent to degree of bone mineralization (DBM), was maintained during the bone voxel segmentation process (Taylor et al, 2013; Ames et al, 2010; Kim et al, 2012). A gray level histogram was obtained for each alveolar and basal cortical bone region (Figure 2a). A mean value (Mean) was computed by dividing the sum of gray levels by the total count of voxels (Figure 2b). The Mean and standard deviation (SD) of the gray level histogram were used
to compute a coefficient of variation (COV=SD/Mean). The SD and COV account for the variability of gray levels. The 5\textsuperscript{th} and the 95\textsuperscript{th} percentiles of voxel counts in the histogram were assigned to Low and high gray levels (Low\textsubscript{5} and High\textsubscript{95}). Percentage (%) difference of the gray level parameters between alveolar and basal cortical bone regions \(((AB-CB) / ((AB+CB)/2)) \times 100\) was computed for each CBCT image.

Three age groups (40, 50, and 60 years) were identified for male and female patients. The age ranges of male patients were 45.4±2.7 years for 9 males (M40), 54.2±3.5 years for 11 males (M50), 63.9±3.5 years for 10 males (M60), and those of female patients were 45.9±3.2 years for 12 females (F40), 53.7±2.9 years for 12 females (F50), and 64.3±2.6 years for 12 females (F60). All the measurements were blindly performed by three raters (JL, HC, and HD) using the randomly coded CBCT images. Reliability measures of significant gray level histogram parameters (SD, COV, and Low\textsubscript{5} for AB and CB) were made on intra- and inter-rater determinations with the intra-class correlation coefficient (ICC) single score method of Shrout and Fleiss. A paired t-test was used to compare the gray level parameters between alveolar and basal cortical bone regions in the same individual image. Analysis of variance (ANOVA) and Fisher’s PLSD tests were used to compare the percentage differences of gray level parameters between the age groups for male and female patients. Pearson’s correlation coefficients were used to examine correlations between the percentage differences of gray level parameters.
Analysis of covariance (ANCOVA) was used to test the effect of groups on the correlations. Significance was set at ≤0.05.
CHAPTER 3

Manuscript

Gender differences of mandibular bone mineral distribution with aging

Jie Liu\textsuperscript{1}, Huan-Yu Chen\textsuperscript{1}, Hamza DoDo\textsuperscript{1}, Jahanzeb Chaudhry\textsuperscript{2}, Allen R. Firestone\textsuperscript{1}, William M. Johnston\textsuperscript{3}, Do-Gyoon Kim\textsuperscript{*}\textsuperscript{1}

\textsuperscript{1} Division of Orthodontics, College of Dentistry, Ohio State University, Columbus, OH, USA; \textsuperscript{2} Division of Radiology, College of Dentistry, Ohio State University, Columbus, OH, USA, \textsuperscript{3} Division of Restorative and Prosthetic Dentistry, College of Dentistry, Ohio State University, Columbus, OH, USA

\textsuperscript{*}For correspondence

Do-Gyoon Kim, Ph.D

Assistant Professor
Research Program Director
Division of Orthodontics
College of Dentistry
The Ohio State University
4088 Postle Hall
305 W. 12th Ave
ABSTRACT

Objectives: The purposes of this study were to determine 1) if the clinical cone beam computed tomography (CBCT) can determine relative differences of bone mineral density distribution using mandibular bone images of patients in clinic and 2) if the relative differences can be used to detect the effects of gender and ages on bone mineral density distribution.

Methods: Sixty six clinical CBCT images from patients (36 females and 30 males, mean 54.6± 8.1 years) were identified. Three age groups (40, 50, and 60 years) were identified for male and female patients. Alveolar bone (AB) and basal cortical bone regions were digitally isolated. A histogram of gray levels, which are equivalent to degrees of bone mineralization, was obtained from each region of the CBCT images. Mean, standard deviation (SD), coefficient of variation (COV), fifth percentile low (Low5) and high (High5) of gray levels were obtained. Percentage differences of gray level parameters between alveolar and basal cortical bones were computed.

Results: The alveolar bone region had significantly higher SD and COV but significantly lower Mean, Low5 and High5 than the basal cortical bone region for all CBCT images (p<0.001). The significantly higher percentage differences of SD, COV, and Low5 were observed for over 50 years old female than male (p<0.042).

Conclusions: The significant gender differences of gray level variability at the postmenopausal ages suggested that the current approach of oral bone mineral density
assessment using the 3D clinical CBCT images can provide additional information for early diagnosis of postmenopausal osteoporosis.

**Keywords:** Cone Beam Computed Tomography; Bone Mineralization; Percentage Difference; Menopause
Introduction

As cone beam computed tomography (CBCT) delivers a relatively lower dose of radiation and provides a higher imaging resolution than conventional medical computed tomography (CT), it has been widely used to provide detailed information for diagnosis of oral complications (Scarfe et al, 2006; Benavides et al, 2012). The X-ray based imaging technology of CBCT provides a non-destructive method to describe maxillofacial structures and assess bone mineral density. While three dimensional (3D) CBCT images have been used mainly to detect structural changes before and after oral treatment, relatively few studies have been performed to determine bone mineral density. Many previous studies indicated that CBCT can be used to assess bone mineral density by evaluating the gray levels of the CBCT images (Parsa et al, 2012; Reeves et al, 2012; Norton et al, 2001; Taylor et al, 2013; Huang et al, 2012). However, its clinical applicability is still controversial because of CBCT scanning artifacts (Hua et al, 2009; Hsu et al, 2010; Naitoh et al, 2009; Katsumata et al, 2009).

It has been well accepted that bone mineral density are different between male and female with aging (Seeman et al, 2003; Yamada et al, 1997). This difference is of great importance when considering oral health. For instance, prevalence of tooth loss increases more with aging in women than in men (Hugo et al, 2007; Musacchio et al, 2007). Furthermore, the risk of tooth loss in women with osteoporosis is three times greater than that in healthy women (Buencamino et al, 2009; Kribbs et al, 1990; Kaye et al, 2007).
Since about 50% of the postmenopausal female population shows symptoms of osteoporosis (Melton et al, 1993; Ross PD, 1997; Nevitt et al, 1999), there has been a growing interest in examining whether female sex hormone (estrogen) deficiency after menopause contributes to the edentulism. Many studies observed that estrogen deficiency induced active bone remodeling, leading to alteration of bone mineral density distribution (Yao et al, 2007; Busse et al, 2009; Ames et al, 2010; Kim et al, 2012). However, oral bone mineral density changes associated with menopause have not been fully understood.

A new approach was introduced to compare the relative values of mandibular bone mineral density between different individual CBCT images in previous studies (Taylor et al, 2013). The relative differences in gray level distribution obtained using clinical resolution CBCT were comparable to those using high resolution microcomputed tomography (micro-CT). Based on previous results, we hypothesized that the mandibular bone mineral distribution that can be assessed with CBCT images will provide detectable diagnostic results for patients in the clinic. Thus, the aims of this study were to determine 1) if the cone beam computed tomography (CBCT) can determine relative differences in bone mineral density distribution using clinical images of patients’ mandibular bone and 2) if the relative differences can be used to detect the effects of gender and age on bone mineral density distribution. In particular, this study focused on the gender difference during the transitional years of menopause.
Materials and Methods

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periodontal ligament voxels of the mandibular bone image was performed using a heuristic algorithm as used in the previous studies (Ames et al, 2010; Zauel et al, 2004). Then, the isolated tooth image was imported to the imageJ software and three-dimensionally dilated up to 1 mm (corresponding to about 3 voxels) from the tooth roots. A 3D alveolar bone (AB) region within 1 mm from the tooth roots was obtained by overlapping the dilated tooth image on the separated mandibular bone image (Figure 1). A core region of basal cortical bone (CB) region was isolated by three-dimensionally eroding the separated mandibular bone image by 0.6 mm (corresponding to 2 voxels) from periosteal and endosteal surfaces of basal bone. This core region of basal cortical bone represents a bone region that has stable normal bone remodeling excluding the marginal basal bone at which an active bone modeling occurs (Huja et al, 2008).

The gray level of each bone voxel, which is equivalent to degree of bone mineralization (DBM), was maintained during the bone voxel segmentation process (Taylor et al, 2013; Ames et al, 2010; Kim et al, 2012). A gray level histogram was obtained for each alveolar and basal cortical bone region (Figure 2a). A mean value (Mean) was computed by dividing the sum of gray levels by the total count of voxels (Figure 2b). The Mean and standard deviation (SD) of the gray level histogram were used to compute a coefficient of variation (COV=SD/Mean). The SD and COV account for the variability of gray levels. The 5th and the 95th percentiles of voxel counts in the histogram were assigned to Low and high gray levels (Low5 and High5). Percentage (%) difference
of the gray level parameters between alveolar and basal cortical bone regions \( \frac{(AB-CB)}{(AB+CB)/2} \times 100 \) was computed for each CBCT image.

Three age groups (40, 50, and 60 years) were identified for male and female patients. The age ranges of male patients were 45.4±2.7 years for 9 males (M40), 54.2±3.5 years for 11 males (M50), 63.9±3.5 years for 10 males (M60), and those of female patients were 45.9±3.2 years for 12 females (F40), 53.7±2.9 years for 12 females (F50), and 64.3±2.6 years for 12 females (F60). All the measurements were blindly performed by three raters (JL, HC, and HD) using the randomly coded CBCT images. Reliability measures of significant gray level histogram parameters (SD, COV, and Low5 for AB and CB) were made on intra- and inter-rater determinations with the intra-class correlation coefficient (ICC) single score method of Shrout and Fleiss. A paired t-test was used to compare the gray level parameters between alveolar and basal cortical bone regions in the same individual image. Analysis of variance (ANOVA) and Fisher’s PLSD tests were used to compare the percentage differences of gray level parameters between the age groups for male and female patients. Pearson’s correlation coefficients were used to examine correlations between the percentage differences of gray level parameters. Analysis of covariance (ANCOVA) was used to test the effect of groups on the correlations. Significance was set at ≤0.05.
**Results**

The alveolar and basal cortical bone regions were successfully isolated from the 3D CBCT images of 66 patients, providing the gray level histograms to obtain all parameters of interest (Figure 1, 2). Inter-rater reliability among raters JL, HC and HD was 0.99 (AB SD), 0.99 (AB COV), 0.99 (AB Low₅), 0.79 (CB SD), 0.98 (CB COV), and 0.99 (CB Low₅), for the gray level parameters.

The gray level histogram of alveolar bone region showed lower and wider distribution of values than that of basal cortical bone region (Figure 2). Thus, the alveolar bone regions had significantly lower means of Mean, Low₅ and High₅ but higher means of SD and COV than the basal cortical bone regions independent of gender and ages (p<0.001) (Table 1).

The lower gray levels of alveolar bone region than those of basal cortical bone region provided negative values of percentage differences of Mean, Low₅ and High₅ (Figure 3). All of the percentage difference parameters in each male and female group were not significantly different between age groups (p>0.064). All of the percentage difference parameters of 40 years age group were not significantly different between male and female (p>0.332)(Figure 3). However, female of 50 and 60 years age groups had significantly higher percentage differences of SD, COV, and Low₅ than male (p<0.042) while those of Mean and High₅ were not different (p>0.093).
To avoid any confusion for the correlations, absolute values for the negative values of percentage differences of Mean, Low$_5$ and High$_5$ were tested while the original positive values of percentage differences of SD and COV were used. Absolute value of the percentage difference of Mean had significant positive but weak correlations with SD and COV but negative correlations with those of Low$_5$ and High$_5$ (p<0.023) (Table 2). A strong positive correlation was found between the percentage differences of SD and COV (p<0.001) (Table 2 and Figure 4a). The percentage difference of SD and COV had a significant positive correlation with absolute value of the percentage difference of Low$_5$ but significant negative correlations with that of High$_5$ (p<0.007). These correlations were stronger with Low$_5$ than High$_5$ (Table 2 and Figure 4b). No significant correlation were found between absolute values of the percentage differences of Low$_5$ and High$_5$ (p=0.318).

Discussion

Consistent with the previous study, the alveolar bone region had significantly lower Mean, Low$_5$ and High$_5$ values, but significantly higher SD and COV than the basal cortical bone region, independent of gender and ages. This result demonstrated that the imaging analysis protocol developed for in vitro cadaver bone specimen using the high resolution images from 3D micro-CT in the previous study (Taylor et al, 2013), were successfully applied to examining older patients using clinical CBCT images, as shown
in the current study. The significantly higher percentage differences of SD, COV, and Low₅ for females of advancing ages than males at a comparable age suggested that variability of bone mineralization in jaw bone increased for females older than 50. Increase in the relative difference of new bone formation, represented by Low₅, is likely responsible for the higher variability of female jaw bone mineral distribution.

It was indicated that the measures of CBCT based bone mineral density are unstable mainly because of the variations of scanning conditions, scanned region in mouth and dense material artifacts (Katsumata et al, 2006 and 2007; De Vos et al, 2009). To reduce these CBCT artifacts, we used the same scanning conditions and the same mandibular left first premolar region excluding restored teeth. In addition, it was also indicated that there were some patient specific variations, including different CT attenuation coefficients of soft tissue and patients’ head positions during scanning that can influence the gray levels of CBCT image (Katsumata et al, 2007; Bryant et al, 2008). These inter-patient variations were addressed using the percentage differences computation that normalized the difference between the gray levels of alveolar and basal cortical bone regions by averaging the values of the two regions. Hence, the current percentage difference method was able to provide the relative values of gray level allowing for comparison of the bone mineral density distributions between different individual CBCT images.

The Mean of gray levels is equivalent to the averaged degree of bone mineralization in the isolated volume of alveolar and basal cortical bone regions. The SD and COV of gray
levels account for the variability of bone mineralization that was produced as a result of bone remodeling at different time points (Ames et al, 2010; Kim et al, 2011). Activated bone remodeling gives rise to rapid bone turnover by formation of new tissue following resorption of pre-existing tissue (Yao et al, 2007; Turner et al, 1994; Marcus et al, 1996; Boivin et al, 2005; Garnero et al, 1996). Since newly forming bone matrix has less bone tissue mineral density than old pre-existing bone tissue, and subsequent bone mineralization continuously increases for years, active bone turnover results in increasing variability of bone mineralization compared to that of normal bone remodeling (Yao et al, 2007; Marcus et al, 1996; Roschger et al, 2008). The Low_5 and High_5 account for the gray levels of the 5th percentile of newest and oldest bone tissue portions, respectively. Many studies observed an increase in the variability of bone mineralization for postmenopausal bone that had undergone estrogen-deficiency dependent active bone remodeling (Yao et al, 2007; Busse et al, 2009; Ames et al, 2010; Kim et al, 2012).

The alveolar bone region consistently showed higher variability (SD and COV) of gray levels and lower Mean, Low_5 and High_5 than the basal cortical bone region, using high resolution micro-CT and clinical CBCT images from animal and clinical models (Taylor et al, 2013; Huang et al, 2012; Ames et al, 2010). These results supported the theory that a high impact dynamic (cyclic) masticatory loading on teeth, which is directly transmitted to the jaw bone through the periodontal ligament, stimulates active alveolar bone remodeling. In agreement with the previous results, the current findings
demonstrated that the percentage difference analysis of gray levels can be applied to the analysis of clinical CBCT images of patients to examine the effects of gender and aging on mandibular bone mineral distribution.

The relative difference of measures between the alveolar and basal cortical bone regions was tested within the same CBCT image. The effects of gender and age on the gray level distribution were examined using the values of percentage differences between gender and age groups. We found no effects of aging on all parameters of gray level percentage difference, which was similar to a previous study that observed no correlation between mandibular bone density and age using a clinical multi-slice CT (Celenk et al, 2008). However, females over 50 had significantly higher percentage differences of gray level variability than males in the same age group. This result indicated that more bone remodeling likely occurred in the alveolar bone region than in the basal cortical bone region (the internal reference region) for the females, as opposed to the males. The average age of a woman going through menopause is estimated to be 51 (Wallace et al, 2010). Thus, the current finding could be associated with the postmenopausal estrogen deficiency induced active bone remodeling in the alveolar bone. The strong positive correlations of the percentage difference of Low5 with those of gray level variability suggested that increases in new bone formation were mainly responsible for the high variability of alveolar bone mineral density.
The main limitation of the current study was that the number of patient CBCT images may not be sufficient to draw solid conclusions. Another limitation was that we included only the mandibular left second premolar region, while regional variations of oral bone mineral density were observed (Cha et al, 2010; Santiago et al, 2009) Nevertheless, we found significant results based on the current sample size. Thus, the current results can provide strong support to future studies that will use the percentage difference approach to compare different CT images in a large cohort, including more age groups, different oral bone regions and complications, etc.

In conclusion, this study successfully demonstrated that the percentage difference of gray levels obtained from clinical CBCT images is applicable to the assessment of oral bone mineral distributions for both cross-sectional and longitudinal comparisons between different individual images. The significant gender differences of gray level variability observed in the postmenopausal age range suggested that the current approach of oral bone mineral density assessment using the 3D CBCT images can provide additional information for early diagnosis of postmenopausal osteoporosis. Further evaluation with a larger number of clinical images is required to be able to apply the current method to a clinical practice for the diagnosis of oral osteoporosis.
Acknowledgements

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References


CHAPTER 4
DISCUSSION

Consistent with the previous study, the alveolar bone region had significantly lower Mean, Low5 and High5 values, but significantly higher SD and COV than the basal cortical bone region, independent of gender and ages. This result demonstrated that the imaging analysis protocol developed for in vitro cadaver bone specimen using the high resolution images from 3D micro-CT in the previous study (Taylor et al, 2013), were successfully applied to examining older patients using clinical CBCT images, as shown in the current study. The significantly higher percentage differences of SD, COV, and Low5 for females of advancing ages than males at a comparable age suggested that variability of bone mineralization in jaw bone increased for females older than 50. Increase in the relative difference of new bone formation, represented by Low5, is likely responsible for the higher variability of female jaw bone mineral distribution.

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Appendix A: Tables

Table 1 Measures of gray level parameters at each region for gender and age groups (mean ± standard deviation). All values were significantly different between alveolar and basal cortical bone regions (p<0.001).

<table>
<thead>
<tr>
<th>Age</th>
<th>Mean</th>
<th>SD</th>
<th>COV</th>
<th>Low5</th>
<th>High5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AB</td>
<td>CB</td>
<td>AB</td>
<td>CB</td>
<td>AB</td>
</tr>
<tr>
<td>40</td>
<td>1767.27±113.04</td>
<td>2478.15±91.99</td>
<td>284.52±50.24</td>
<td>135.19±35.18</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>50</td>
<td>1717.81±170.83</td>
<td>2441.44±139.94</td>
<td>312.71±41.89</td>
<td>131.99±18.27</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>60</td>
<td>1719.42±151.17</td>
<td>2411.69±141.11</td>
<td>315.47±49.72</td>
<td>130.96±21.79</td>
<td>0.18±0.03</td>
</tr>
<tr>
<td>40</td>
<td>1697.11±143.94</td>
<td>2303.34±92.87</td>
<td>277.20±39.01</td>
<td>144.88±40.17</td>
<td>0.17±0.03</td>
</tr>
<tr>
<td>50</td>
<td>1737.06±171.28</td>
<td>2374.23±129.99</td>
<td>276.48±37.59</td>
<td>184.58±61.27</td>
<td>0.16±0.03</td>
</tr>
<tr>
<td>60</td>
<td>1621.24±93.99</td>
<td>2288.20±141.66</td>
<td>286.82±43.70</td>
<td>178.00±443.33</td>
<td>0.18±0.03</td>
</tr>
</tbody>
</table>
These slopes of significant correlations were not different between gender and age (ANCOVA, p>0.175). Absolute values were used for the negative values of percentage difference. Negative r represents an inverse correlation. Strong correlations were highlighted by bold font.

<table>
<thead>
<tr>
<th>X</th>
<th>Y</th>
<th>r</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (%)</td>
<td>SD (%)</td>
<td>0.280</td>
<td>&lt;0.023</td>
</tr>
<tr>
<td>Mean (%)</td>
<td>COV (%)</td>
<td>0.455</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean (%)</td>
<td></td>
<td>Low&lt;5&gt; (%)</td>
<td>0.767</td>
</tr>
<tr>
<td>Mean (%)</td>
<td></td>
<td>High&lt;5&gt; (%)</td>
<td>0.478</td>
</tr>
<tr>
<td>SD (%)</td>
<td>COV (%)</td>
<td>0.981</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD (%)</td>
<td></td>
<td>Low&lt;5&gt; (%)</td>
<td>0.729</td>
</tr>
<tr>
<td>SD (%)</td>
<td></td>
<td>High&lt;5&gt; (%)</td>
<td>-0.455</td>
</tr>
<tr>
<td>COV (%)</td>
<td></td>
<td>Low&lt;5&gt; (%)</td>
<td>0.828</td>
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<tr>
<td>COV (%)</td>
<td></td>
<td>High&lt;5&gt; (%)</td>
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<tr>
<td>Low&lt;5&gt; (%)</td>
<td></td>
<td>High&lt;5&gt; (%)</td>
<td>0.125</td>
</tr>
</tbody>
</table>
Appendix B: Figures

**Figure 1** a) A lower left first premolar region in the sagittal view from a 3D CBCT image slice and b) isolation of alveolar bone (AB) and basal cortical bone (CB) regions of a human mandible using the 3D CBCT image.
Figure 2  a) Comparison of gray level histograms between alveolar bone (AB, gray) and basal cortical bone (CB, black) regions using 3D CBCT image (300 micron voxel size) and b) gray level parameters determined using a typical histogram.
Figure 3 Comparisons between percentage (%) differences of gray level parameters a) Mean, b) SD, c) COV, d) Low5, and e) High5, for females and males in different age groups. The negative percentage difference arose from the lower values in the alveolar bone region than in the basal cortical bone region. The error bars represent standard deviation of each parameter. Significant difference is marked by *; p<0.05.
Figure 4  a) Strong positive correlation between the percentage (%) differences of SD and COV ($SD=1.017$ \ COV-28.792, $r=0.981$, $p<0.001$) and b) between the percentage difference of COV and absolute value of the percentage difference of $Low_5$ (COV=2.266 $|Low_5|-23.031$, $r=0.828$, $p<0.001$). These correlations were not different between gender and age groups (ANCOVA, $p>0.728$).