Bone Mineral Density Analysis for

Evaluation of Cervical Vertebral Maturation

Thesis
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By
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ABSTRACT

Objective: The cervical vertebral maturation (CVM) method is a useful tool for detecting the status of mandibular growth. However, the current CVM method based on two dimensional cephalometric radiographs may not fully include all factors that may be involved in bone maturation. For instance, the current CVM cannot account for changes of bone mineral density (BMD), which result from active bone remodeling during growth. Thus, the objective of this study was to describe bone density changes in the cervical vertebrae occurring during the period of adolescent mandibular growth and development.

Materials and methods: Cone beam computerized tomography (CBCT) images were obtained from 41 patients before and after orthodontic treatments. Patients were 14.47±1.42 years and 16.15±1.38 years before and after treatments, respectively. IRB approval was obtained for this retrospective CBCT analysis. Two cervical vertebrae (C2 and C3) were digitally isolated using the three dimensional CBCT. Gray level histogram of each vertebra was obtained using CT attenuation values of voxels. Mean and standard deviation (SD) of gray level histogram were obtained. Percentage (%) differences of gray level parameters between C2 and C3 ((C3-C2)/(C2+C3)/2)*100) were computed. A paired t-test was utilized to compare the % differences between before and after treatments.
**Results:** The % difference of Mean significantly increased after treatment (3.06±1.11%) (p<0.001) compared to before treatment (1.22±1.76%) while the SD before treatment (-6.62±6.27%) was significantly greater than after treatment (-1.97±5.62%) (p<0.002).

**Conclusions:** The Mean and variability (SD) of gray levels account for a degree of bone mineralization and active remodeling, respectively. It was found that the growth rate of C2 is about twice that of C3 at 14.5 years but the rates of both cervical vertebrae decreased to the same level at 16.5 years old. Taken together, the current results indicate that the active bone remodeling of C2 decreased while bone mineralization of C3 which undergoes less bone remodeling increased during vertebral maturation.

**Keywords:** Cervical vertebral maturation, bone density, CBCT
DEDICATION

Dedicated to the many devoted educators who have been a part of my life.

Thank you!
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CHAPTER 1

INTRODUCTION

Growth Assessment in Orthodontics

It has long been important for orthodontists to have the capacity to evaluate a patient’s facial growth status. This knowledge can be useful when attempting to predict the best time to begin treatment that can influence a patient’s facial growth. For example, if potential growth remains, the orthodontist may take advantage of this by using a growth modification appliance. Growth modification appliances work by enhancing, restricting, or redirecting growth, but they are only effective if the patient hasn’t finished growing. In addition, they may be more effective during and near the patient’s peak adolescent growth spurt. For example, Class II functional appliances have been shown to be more effective when used during or slightly after the peak mandibular growth spurt rather than before the peak (Baccetti 2000), and it has also been demonstrated that Class II treatment with cervical headgear aimed at restricting maxillary growth is more likely to produce favorable results if undertaken during a period of higher maxillary growth velocity (Kopecky 1993). Baccetti et al. observed that growth modification aimed at the mandible appears to be most effective during the circumpubertal growth spurt while maxillary growth modification appears to be more effective during the prepubertal growth stages (Baccetti 2005).
In other cases, the orthodontist may wish to wait until the patient’s facial growth is complete or nearly complete to begin treatment. In orthognathic surgery cases, for example, it is imperative to postpone surgery and sometimes pre-surgical orthodontic treatment until the end of the growth phase in order to prevent untoward relapse. Without an accurate way to assess facial growth, we may recommend treatment at a less than optimal time. It is reasonable to assume that the more accurately we can evaluate growth status, the more predictable, efficient, and successful orthodontic treatment could be.

There are many methods of assessing growth currently, but many of these methods are effective only for measuring somatic or general body growth. These somatic growth assessment methods include the use of height/weight charts, observation of secondary sex characteristics, chronologic age, and hand-wrist radiography to assess general skeletal age (Fishman 1982). The cervical vertebral maturation stage (CVMS) method was originally developed to assess general skeletal growth by correlating it to results found via hand-wrist radiography, and it was later determined that this method was also useful to assess mandibular growth status in particular (Lamparski 1972). Finally, if radiographic exams are performed at multiple time-points, serial cephalometric superimposition may be used to compare facial and mandibular growth and to determine whether a given patient’s facial growth is complete or ongoing. While some methods may be appropriate in certain cases (e.g. the use of height/weight charts in the pediatrician’s office to evaluate whether a young child is thriving), they may be inadequate when more precise prediction is required. For example, the same method (the use of height/weight charts) would be inappropriate to determine whether a patient has
declined in mandibular growth enough to recommend orthognathic surgery. Although general body growth is modestly correlated to facial growth, a higher level of precision is desired. For example, for a skeletal Class III surgical patient, only serial cephalometric superimposition could give an accurate enough evaluation of growth completion.

The Cervical Vertebral Maturation Stage Method of Growth Assessment

Perhaps the most popular method of mandibular growth assessment for most purposes in orthodontics today is the CVMS method. Lamparski in his 1972 thesis, first concluded that the cervical vertebrae were as valid a tool as the hand-wrist radiograph for assessing skeletal age. He developed a six-stage set of standards for evaluating growth based on the two-dimensional morphology of the vertebrae (C2, C3, and C4) (Lamparski 1972). These stages were further described by Hassel et al. and Hassel and Farman. The first stage, s1, was classified as the initiation stage in which significant adolescent growth occurs. In s2, the acceleration phase, growth accelerates, and an additional large increment of adolescent growth occurs. During s3, the transition phase, peak pubertal growth rate is achieved. Stage s4 represents the deceleration phase in which the growth rate declines and a smaller increment of growth is added, s5 is the maturation stage in which a very small amount of growth occurs, and finally s6 is the completion stage in which growth is complete and within which the patient will remain for the rest of life (Hassel 1991, Hassel 1995).

In orthodontics, it is more important to be able to assess the amount and timing of remaining jaw growth than to assess remaining statural growth. O’Reilly and Yaniello in
1988 demonstrated that a direct relationship could be found between these CVM stages and mandibular growth specifically rather than simply between the CVM and general body growth (O’Reilly 1988). In 2000, Franchi and Baccetti evaluated individual patients using Lamparski’s original method and found the CVMS system to have high validity for the evaluation of skeletal maturity and the identification of pubertal peak growth rate for the mandible (Franchi 2000). In 2002, Baccetti and Franchi improved upon the initial six stage method proposed by Lamparski, converting Cvs 1-6 into a condensed CVMS I-V, essentially by consolidating stages 1 and 2. Improvements included the fact that a single cephalometric film could be used to assess the growth stage, rather than relying on comparisons between consecutive films, and the analysis relied only on C2-C4 which should be easily visible even with a protective radiation collar in place (Baccetti 2002). One study by Chen et al. in 2004 even demonstrated a reasonable predictive value for mandibular length increases during the pubertal growth period based on analysis of single cephalometric films (Chen 2004).

Most recently, Baccetti and Franchi in a 2005 study revisited this concept to further refine the method, maintaining their previous goals. They once again expanded the analysis to include six stages, concluding that at CS1-CS2, a patient is prepubertal. Upon attainment of stage CS2, the patient is likely to begin a growth spurt in one year, and once CS3 has been observed, the pubertal growth spurt should occur within the year that follows. In the same study, they review literature that describes the usefulness of this knowledge, explaining that desired growth changes in the maxilla (e.g. protraction, expansion) are best achieved during the pre-pubertal stage, and those in the mandible
(e.g. restriction, enhanced vertical or anteroposterior growth) are best achieved during the peak pubertal growth spurt (Baccetti 2005).

The CVMS method is not yet perfect. The five-stage method was found to excellently represent the window containing peak mandibular growth between stage II and stage III (Soegiharto 2008). However, the reliability of the CVM method is not as well established. As the method relies upon subjective assessment, it opens the window to disagreement, and studies to evaluate reliability have included design flaws (Ballrick et al.). One study examining intra- and inter-rater reliability concluded that the CVM method has poor reproducibility and is not suitable for clinical use. Another study compared reliability when evaluating all five stages to that used when evaluating only pre- vs post-pubertal growth spurt and found similarly poor reliability for the five stage method but excellent intra- and inter-rater reliability when participants were asked to assign a patient to one of only two stages. Hence, the CVM method may only be clinically useful for distinguishing between pre- and post-mandibular growth. (Gabriel 2009)

CBCT Imaging in Orthodontic Diagnosis and Treatment Planning

In recent years, with increasing frequency, some orthodontists have begun to routinely or for a number of indications, expose a patient to cone-beam computed tomographic (CBCT) imaging for diagnostic purposes. Available in dentistry since 1998, CBCT imaging has continued to increase in popularity. There remains debate regarding whether it is appropriate to expose a CBCT image as a part of routine treatment planning.
for each orthodontic patient or whether this imaging technique should be reserved for limited use only according to specific indications (e.g. cleft palates, impacted canines, etc.) (Isaacson 1994, Kapila 2011). Much of this debate is couched in the “as low as reasonably practicable” (ALARP) standard.

Standard two-dimensional radiographs pose a number of limitations in their usefulness in diagnosis and treatment planning. For example, they often contain magnification errors, images may be superimposed over one another, rotated structures cannot always be viewed in the desired plane of space, and images can be distorted by elongation or foreshortening errors depending on patient positioning. (Tsao 1983, Adams 2004). In contrast, 3-D CBCT images allow the practitioner a more realistic view of a patient’s anatomy.

There are, of course, disadvantages associated with the use of CBCT imaging. In terms of radiation exposure, a single orthodontic CBCT image exposes the patient to as much or slightly more radiation than a combined series including a lateral cephalometric film, panoramic, and full mouth series (Silva 2008). Additionally, CBCT imaging requires a larger financial investment by the doctor should he or she choose to purchase a machine, and that cost is likely to be passed on to the patient. Finally, image reading for CBCTs requires additional expertise above and beyond that which doctors have historically used to evaluate two-dimensional x-rays. This may require additional training to avoid errors in interpretation. Doctors should be aware as well that they will be responsible for reading any exposed images to review for pathology, and if they are not qualified to do this, they may wish to contract with a radiologist to review the images.
and relieve the orthodontist of liability, further increasing costs. (Ahmed 2012) In fact, one study estimated that the incidence of incidental findings of potential pathology may be as high as 25% (Cha 2007).

CBCT images provide a number of advantages as well. By creating a three-dimensional image, the practitioner receives as much information as they would have obtained from their standard series and a great deal more, and often the information is of a higher quality. The image can be converted into a panoramic view and a standard two-dimensional lateral cephalometric view which can be analyzed as usual. For cephalometric analysis, the two image types are approximately equal, but when compared with the information gleaned from a panoramic radiograph, the CBCT image provides more and better information on localizing impacted teeth, evaluating root resorption, and assessing cleft lip and palate patients (Adams 2004, Korbmacher 2007). In addition, the three dimensions allow for precise examination of pathology throughout the head and superior neck and a better view of the dentoalveolar tissues pertinent to treatment. For example, when limited alveolar bone is present in the mandibular anterior region and further proclination may not be desirable, the doctor is aware of the degree of severity of the situation before treatment begins (Lund 2010, Leung 2010).

The availability of three dimensional analysis leads to another important question for orthodontics. If we are currently using the two-dimensional morphology of the cervical vertebrae to analyze the patient’s growth status, then what additional information could we obtain by including the third dimension? It is certainly possible that the two-dimensional morphology when viewed from another angle may reveal additional
diagnostic clues. However, another diagnostic technique made possible with three-dimensional imaging is an assessment of tissue mineral density (TMD) distribution. Since we know that the cervical vertebrae are going through predictable remodeling procedures during the stages leading up to, during, and after the pubertal growth spurt, we hypothesize that the TMD distribution and/or bone turnover may change predictably during this time frame as well. There has, however, been significant controversy surrounding the use of CBCT imaging to assess tissue mineral density. This will be discussed in further detail below.

**Pubertal Growth Changes in the Cervical Vertebrae**

Throughout life, bone undergoes modeling and remodeling procedures via the apposition and resorption of bone. Frost defined bone modeling as an uncoupled process of resorption or formation, causing changes in the size or shape of a bone. Modeling occurs during periods of growth when the bones are increasing in size and changing in shape as well as during aging and in disease states (e.g. osteoporosis) in which more bone is resorbed than is deposited. Bone remodeling, however, occurs consistently throughout life and is defined as a coupled process of resorption followed by formation. It affects only the quality of bone, leaving the morphology unaffected. This remodeling process provides a baseline degree of bone metabolism to which we can compare other changes in bone turnover. (Frost 1990)

Based on our knowledge of the morphologic changes in the cervical vertebrae during growth, we know that some modeling is taking place at this location, especially
during the pubertal growth spurt. This is apparent because we can observe changes in the morphology of the vertebrae even in two-dimensions. These changes have been observed and described in detail in numerous growth studies. The cervical vertebrae (specifically C2, C3, and C4) increased in overall height as well as length throughout childhood and adolescence with a spurt during puberty, coinciding with the general skeletal growth spurt. In addition, as the vertebrae increase in size, the inferior surface develops a concavity of increasing depth. (Altan 2012) One study using dual-energy x-ray absorptiometry (DEXA) scan in adolescent females demonstrated that significant changes in bone density occur in the spine during late puberty. Bone density increases significantly during puberty as it approaches its peak in early adulthood. (Bass 1999) Hence, we expect that an increase in bone turnover above the baseline remodeling level would accompany these changes in adolescents.

*Tissue Mineral Density Analysis Using CBCT*

As mentioned previously, there has been controversy surrounding the use of CBCT imaging for TMD distribution analysis. Currently, dual-energy x-ray absorptiometry (DEXA) constitutes the gold standard in bone mineral density (BMD) assessment. (Carey 2007) Since DEXA scans would not be otherwise useful in clinical orthodontics, they would not be indicated as a diagnostic tool to assess growth. In contrast, since CBCT imaging does provide value in orthodontic and dental treatment planning, researchers have been motivated to evaluate its potential use in evaluating BMD, and one study sought to determine the correlation between BMD assessment using
CBCT and DEXA. The results of this in vitro study revealed a strong positive correlation between the BMD of a total bone block verified by CBCT and the BMD of the same bone block verified by DEXA, indicating usefulness of CBCT as a tool for BMD analysis. (Marquezan 2012)

Naitoh et al. in 2008 conducted an in vivo study comparing CBCT and multi-slice CT (MSCT) using patient images and found that voxel values of CBCT images and BMDs of MSCT images were highly correlated \( r^2 = 0.965 \), suggesting that voxel values in CBCT could be used to evaluate bone density. A major difference between these two methods is that BMD analysis using MSCT scans utilizes CT values as measured in Hounsfield units (HU) to provide an absolute estimate of actual BMD. In contrast, BMD analysis undertaken with CBCT imagining is accomplished by using voxel values which are not directly comparable to HU. (Naitoh 2004, Naitoh 2009)

Nomura et al. in 2010 also demonstrated a high correlation between CBCT and MSCT voxel values, further verifying the competence of CBCT in evaluating degree of bone mineralization (DBM). DBM differs from bone mineral density (BMD) in that it accounts for only the mineral content of hard tissue, regardless of the apparent volume of the bone. This measurement eliminates soft tissues and voids or air spaces in its assessment but can be useful nonetheless in evaluating bone turnover, specifically when viewed in terms of variability rather than in absolute numbers. (Nomura 2010) Gonzales-Garcia et al. compared CBCT to micro-CT and found a strong positive correlation between the bone density measurements assessed by each method. (Gonzales-Garcia 2012) Most recently, Taylor et al. also found a high correlation
between CBCT and micro-CT both in vitro and in vivo for measuring relative differences in grey level distribution between alveolar and cortical bone. (Taylor 2013)

**Thesis Objective**

The purpose of this longitudinal study is to determine whether CBCT imaging may provide additional information about the growth status of a patient above and beyond that which can be gleaned from two-dimensional radiographs. We wish to investigate whether the changes in the bone mineral density of the cervical vertebrae or changes in the variability of the bone mineral density have a predictable relationship with the patient’s chronological age or to the growth status of the mandible. Since it will not be possible to accurately evaluate all of the information with our current number of subjects, we have limited this particular study to the specific aim below.

**Specific Aim**

We will measure the bone mineral density (BMD) distribution of the cervical vertebrae, C2 and C3, and make observations regarding the relationship between the BMD of C2 and C3 within single images and between longitudinal images. We will then seek to determine whether there exists a relationship between this information and the patient’s status as pre- or post-orthodontic treatment.
CHAPTER 2
MATERIALS AND METHODS

IRB Approval

This retrospective study was approved by the Institutional Review Board at The Ohio State University.

Patient Images

CBCT images included in this study were originally taken as diagnostic pre- and post-treatment records on routine orthodontic patients at Case Western Reserve University’s College of Dentistry, Department of Orthodontics. Patients received comprehensive orthodontic treatment with full fixed appliances and were excluded if they had craniofacial anomalies, facial asymmetries, orthognathic surgical treatment, rapid palatal expansion, headgear, or extraction (except third molar) treatment.

Each image was taken at 2 mA and 120 kV with a Hitachi CB MercuRay Scanner. The voxel size was either 292 microns or 377 microns. Eighty-two paired images from 41 patients before and after orthodontic treatment were included in this study. The patient population was comprised of 15 males and 26 females. Although efforts were made to equally distribute the sample by gender, due to the availability or lack of availability of complete data sets, these subjects were chosen. At the start of treatment, the average patient age was 14.47±1.42 years, and at the conclusion of treatment, the average patient age was 16.15±1.38 years. Treatment duration ranged from 9 to 26 months with an average duration of 20.17 months.
Two-Dimensional Analysis

Each 3D image was first converted to a 2-dimensional lateral cephalometric view using Dolphin software, and images were digitized and traced by one observer. From the tracings, the following measurements were recorded: SNB, Mandibular Length (Co-Gn), Mandibular Skeletal (Pg-Na Perp), ANB, ANS-Me, and LFH/TFH. Also using the two-dimensional lateral cephalometric view, the same researcher assigned a CVM stage to each patient image using the 5-stage method developed by Baccetti et al. (Baccetti 2002). The 5-stage method was chosen as it is the standard clinically used at the university.

The same researcher then recorded specific measurements of vertebral height, length and concavity using protocols established by Altan et al. (Altan 2012). Measurements made included the following: C2 angle, C2 height, C2 inferior width, C3 angle, C3 height, C3 superior width, C3 inferior width, C4 angle, C4 height, C4 superior width, and C4 inferior width. Measurements were recorded to scale, based on the inclusion of a 100 mm ruler embedded in the image. These measurements were not used for this study but rather will be retained for possible use upon collection of a larger sample size.

Three-dimensional Analysis

Two other researchers were responsible for all 3-dimensional assessments. Images were first converted to tagged image file (.tif extensions) format and uploaded into Image J software. Each whole image was first cropped to isolate a smaller field of
view surrounding the vertebra of interest, either C2 or C3. The cropped images were processed using ModelPrep (Finite Element Method, FEM processing program) to isolate the vertebra and eliminate other surrounding hard and soft-tissues. The images were further prepared using the envelope method in Image J until only the isolated vertebra itself remained, including both cortical and trabecular bone. Next, the image was rotated and further cropped to isolate only the vertebral body, cutting off the posterior process. To make the point of lateral separation repeatable, a standard was established to crop the image at 10 voxels on either side of the first point where the body separates from the lateral extensions.

A gray level histogram for the cropped image of each vertebra was constructed using CT attenuation values of voxels, and the mean, standard deviation, and covariance were calculated. This process was repeated for both C2 and C3 for all 52 images.

Statistical Analysis

Percentage (%) differences of gray level parameters between C2 and C3 ((C3-C2)/(C2+C3)/2)*100) were computed. A paired t-test was utilized to compare the % differences between before and after treatments.
CHAPTER 3
MANUSCRIPT

Cervical Vertebral Bone Mineral Density Changes in Adolescents during Orthodontic Treatment

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Abstract

Objectives The current study examined whether 1) cone beam computed tomography (CBCT) images can be utilized to detect changes in cervical vertebral volume and bone mineral density (BMD) distribution during adolescent growth and development and 2) those changes are associated with changes of the cervical vertebral maturation (CVM) stages and mandibular length.

Materials and methods Eighty two CBCT images were obtained from 41 patients before (T1, 14.47±1.42 years) and after (T2, 16.15±1.38 years) orthodontic treatment. Two cervical vertebral bodies (C2 and C3) were digitally isolated from each image and their volumes, mean and standard deviation (SD) of gray level histograms were measured. The CVM stages and mandibular length were also estimated after converting the CBCT image.

Results Significantly higher variability (SD) of gray level distribution for C2 before treatment (p<0.001) decreased to the same level as C3 after treatment (p=0.669). No correlations were found between changes of the CVM stage with those of cervical vertebral BMD parameters and volume, and mandibular length (p>0.135) while the measures of those parameters at T1 had significant correlations with those at T2 (p<0.001).

Conclusions The mean and variability (SD) of gray-levels account for BMD and active remodeling, respectively. The current results indicate that BMD distribution and volume of the cervical vertebral body changed due to active bone remodeling during maturation.
Clinical Relevance The 3D clinical CBCT images can provide quantitative information about the volume and BMD distribution of human cervical vertebra, which can be used for an estimation of skeletal maturity.

Keywords: Skeletal maturity, Cervical vertebral maturation, Bone mineral density, CBCT
Introduction

Evaluation of patients’ facial growth status is of importance in developing optimal orthodontic treatment plans [1, 2]. Skeletal maturity status should be considered to determine effective timing for the use of growth modification appliances such as Class II functional appliances and headgears. It has been demonstrated that the cervical vertebra is a valid anatomical reference for estimating skeletal maturation, providing comparable results to those obtained with hand–wrist radiographic assessment [3]. The cervical vertebral maturation (CVM) method has been widely used to estimate the skeletal maturity for orthodontists [3-6]. However, many clinical studies observed that the CVM method has poor reliability and repeatability for evaluation of bone maturity [7-9].

A major limitation of the CVM method is that it classifies the stages of maturation based on qualitative descriptions of cervical vertebral shape based on a two dimensional cephalogram. As such, the estimated CVM stages vary subject to observer dependent bias. On the other hand, a growing number of dental providers are using three dimensional (3D) images of clinical cone beam computed tomography (CBCT) for diagnosis and treatment planning [10]. As the CBCT image field of view can include the cervical vertebrae, recent studies examined applicability of CBCT to assessment of skeletal maturity [11-13]. However, those studies investigated only the morphology of the cervical vertebra while CBCT can provide additional information including bone mineral density (BMD).

It was observed that the BMD changes reflect the physiology of bone development during childhood and adolescence [14]. A clinical CT has been used as a standardized method to assess orthopedic BMD [15]. Many clinical studies indicated that the CBCT
image can be used for volumetric assessment of BMD [16-20]. Combining the observations from these previous studies, we hypothesized that the clinical CBCT based 3D morphological and volumetric BMD analyses for cervical vertebrae can provide quantitative information to estimate the skeletal maturity of patients. Thus, the objectives of the current study were to examine whether 1) the CBCT images can be utilized to detect changes of cervical vertebral volume and BMD distribution and 2) those changes are associated with changes of the CVM stages and mandibular length. The current study used a longitudinal comparison of those parameters measured in teens before and after orthodontic treatment.

**Materials and methods**

The institutional review board for this retrospective study was approved by a university-based IRB. The CBCT images of patients were originally taken as diagnostic pre- and post-treatment records on routine orthodontic patients at a collaborating university’s graduate orthodontic clinic. This is their standard record procedure. Patients received comprehensive orthodontic treatment with full fixed appliances and were excluded if they had craniofacial anomalies, facial asymmetries, orthognathic surgery, rapid palatal expansion, headgear, or extraction (except third molar) treatment. Each image was taken at 2 mA and 120 kV with a Hitachi CB MercuRay Scanner (Hitachi Medical Systems America Inc., Twinsburg, Ohio) (Figure 1). The voxel size of the three dimensional (3D) CBCT image was either 292 microns or 377 microns. Eighty-two paired images from 41 patients (15 males and 26 females) randomly selected before (T1) and after (T2) orthodontic treatment were included for this study. At the start of
treatment, the average patient age was 14.47±1.42 years, and at the conclusion of
treatment, the average patient age was 16.15±1.38 years. Treatment duration ranged from
9 to 26 months with an average duration of 20.17 months.

The 3D CBCT image was imported to image analysis software (ImageJ, National
Institutes of Health, Bethesda, MD, USA). Two cervical vertebrae (C2 and C3) in the
same CBCT image were digitally cropped, separated and saved as individual image files
(Figure 1b). Segmentation of bone voxels from non-bone voxels outside of the vertebra
was performed automatically using a heuristic algorithm as used in previous studies [21,
22]. Posterior processes were digitally removed at 10 voxels from either side of vertebral
endplate leaving only the vertebral body in the final image (Figure 1). Vertebral body
volume was estimated by multiplying the total bone voxel count by the volume per voxel.
Then, the changes in vertebral body volume between T1 and T2 were obtained using an
absolute difference obtained by subtracting before (T1) measures from after (T2)
measures. The gray level of each bone voxel, which is equivalent to bone mineral
density, was maintained during the segmentation process. Gray level histograms were
obtained for C2 and C3 vertebral bodies before and after treatment (Figure 2a,b). A mean
value (Mean) was computed by dividing the sum of gray levels by the total count of
voxels and a standard deviation (SD) of gray level distribution was also computed using
the histogram for each vertebral body (Figure 2c). To compare the gray level parameters
for the two different CBCT images before and after treatment, a percentage (%)
difference of the gray level parameters and vertebral volume between C2 and C3
vertebral bodies (((C2-C3)/(C2+C3)/2)×100) was computed for each CBCT image.
CVM stage and mandibular length were assessed by two dimensional (2D) cephalometric view by converting the same 3D CBCT images to their corresponding 2D lateral cephalometric view using orthodontic imaging software (Dolphin3D, DolphinImaging and Management Solutions, Chatsworth, CA, USA). The CVM stage was assigned following the 5-stage method developed in a previous study [6]. This method categorizes patients into one of five stages based on the shape of cervical vertebrae (C3 and C4) by assessing whether they are trapezoidal or rectangular in the horizontal dimension, square, or rectangular in the vertical dimension, and evaluating for the presence or absence of a concavity on the inferior borders of C2, C3, and C4. When using this method, peak mandibular growth is presumed to occur between stages II and III. The mandibular length was measured using the same 2D cephalometric view for each patient before and after treatment. The mandibular length measurement was based on the distance from condylion that was defined as the most posterior superior point on the condyle to anatomical gnathion that was defined as the midpoint between the most anterior and inferior point on the bony chin.

All CVMS stage evaluations were blindly performed by one examiner using the randomly coded CBCT images. Five images were randomly selected for repeated measure by the same examiner for an intra-rater reliability test. An additional five images were randomly selected and evaluated by a second examiner to determine the inter-rater reliability. Intra- and inter-rater agreements were analyzed with intra-class correlation coefficient (ICC) with Shrout-Fleiss random set method and single score method, respectively (SAS, Cary, NC) [23]. Although this statistical test is intended for
continuous rather than ranked data, this data set was perfectly ranked, making this an appropriate evaluation.

A paired t-test was used to compare between C2 and C3 vertebral bodies for the gray level parameters in the same individual image and the absolute amount of vertebral body volume difference (T2-T1). The same paired t-tests were utilized to compare between the two different CBCT images before (T1) and after (T2) treatments for the percentage (%) differences of Mean, SD, and vertebral body volume, as well as CVM stage and mandibular length.

Pearson’s correlation coefficients were used to examine correlations of the pre-treatment values for the percentage (%) differences of Mean, SD, and vertebral body volume, and CVM stage and mandibular length with the after treatment values for the same variables. Correlations were also examined between treatment period change (T2-T1) of the CVM stage and those changes (T2-T1) of all other parameters. Significance was set at ≤0.05.

**Results**

Inter-rater reliability between raters BC and EJ was 0.54 for CVM. Intra-rater reliability for rater BC was 0.90 for the same variable.

The cervical vertebrae were successfully isolated from the CBCT images (Figure 1) providing the gray level histograms before and after orthodontic treatment (Figure 2).

The Mean of the Mean gray levels for C2 vertebral body was significantly lower than that for C3 vertebral body both before (T1) and after (T2) treatment (p<0.001) (Table 1). In contrast, mean of the SD values for C2 vertebral body was significantly higher than
that for C3 vertebral body before treatment (p<0.001) while no significant difference was detectable after treatment (p=0.669). The C2 vertebral body volume significantly changes compared to C3 vertebral body volume after treatment (p<0.001).

The negative percentage (%) difference of the Mean of the gray levels was computed because the values of C2 were lower than those of C3. The percentage (%) difference of the Mean gray levels between C2 and C3 significantly increased whereas that of the SD and vertebral body volume values substantially decreased after treatment (p<0.001 for all) (Table 2). Both CVM levels and mandibular length significantly increased after treatment (p<0.001 for both).

The percentage (%) differences of SD and vertebral body volume, and CVM stage and mandibular length had significant correlations between before and after treatments (p<0.001) (Table3 and Figure 3) while percentage (%) differences of Mean did not have a significant correlation between the two time points (p=0.220).

Correlations of post-treatment change (T2-T1) of CVM stage with those changes (T2-T1) of all other parameters including the percentage (%) differences of Mean, SD and vertebral body volume, and mandibular length were not significant (p>0.135).

Discussion

The Means of gray levels, which are equivalent to bone mineral density (BMD), were significantly different between the second (C2) and third (C3) cervical vertebrae, and the percentage difference between C2 and C3 increased after treatment. In contrast, the higher variability (SD) of gray level distribution for C2 before treatment decreased to the same level as C3 after treatment. Consistently, it was found that the C2 vertebral body
volume changed significantly more than the C3 vertebral body volume over the course of the observation period. These findings imply that more bone remodeling occurred in the C2 vertebral body than in the C3 vertebral body during the observation period for growing adolescents resulting in the alteration of BMD distribution. We also found that the CVM level and mandibular length increased during the same period. Taken together, these results indicate that the 3D clinical CBCT-based analysis could provide information of bone mineral density distribution that is changing in concert with both skeletal maturation and facial growth.

Many studies have evaluated the applicability of CBCT for the assessment of BMD for patients in clinical practice [16-20]. However, the consistency of CBCT based BMD measurement is still open to debate due to questions regarding the variations of scanning conditions and target locations to scan [24-27]. The patient specific variations include thickness of soft tissue and head position during the scan [28, 29]. To alleviate these factors, we compared the gray level parameters of consecutive vertebrae (C2 and C3) in the same CBCT image. In addition, the relative values of percentage differences of BMD parameters between C2 and C3 could allow for comparison of the BMD distributions between the two different CBCT images taken before and after treatment. This percentage difference analysis was evaluated in a previous study that showed comparable values of bone mineral content obtained from clinical CBCT images with those from the high resolution of micro-CT images [19].

The Mean of gray levels is equivalent to averaged BMD of each vertebral body. The SD of gray levels accounts for variability of BMD resulting from bone modeling and remodeling [22, 30, 31]. Activated bone modeling is an uncoupled process by which
resorption of pre-existing bone tissue and formation of new bone tissue occur independently. The coupled bone remodeling process is comprised of bone formation following resorption [32-36]. As the newly forming bone tissue has less tissue mineral density than pre-existing bone tissue, the variability of tissue mineral density inherently increases. Prolonged progressive mineralization of bone tissue following new bone formation alters the variability of tissue mineral density. In this study, the C2 vertebral body had higher variability but lower Mean of gray levels indicating that more active bone modeling and remodeling occurred in the C2 vertebral body than in the C3 vertebral body during the observation period. The high degree of bone remodeling of the C2 vertebral body subsided after treatment, reaching a similar level to that of the C3 vertebral body. These findings were consistent with observations from a previous study that showed a growth rate approximately twice as high for C2 than for C3 in 14.5 year-old girls and progressively declining to the same level between C2 and C3 in the same patients at the age of 16.5 years [37]. Hence, this is an active area of bony change in adolescents and might be related to other maturational changes.

Meanwhile, the lower level of bone remodeling in the C3 vertebral body before treatment likely resulted from the development of greater bone mineralization with less resorption of the highly mineralized pre-existing bone and less formation of the less mineralized new bone, resulting in the high mean of BMD values for C3 after treatment. The greater change in vertebral body volume for C2 than C3 vertebral bodies after treatment supported these observations because this volume change could result from the active bone modeling and remodeling during the observation period.
The percentage (%) differences of Mean, SD and vertebral body volume between C2 and C3 vertebral bodies reflected the changes of BMD distribution for each vertebral body showing that the percentage difference of the Mean increased while those of the SD and vertebral body volume decreased after treatment. For the same duration, the skeletal maturity estimated using CVM increased along with the mandibular length. As the relative values of percentage differences can be used to compare the measures from CBCT images taken under different scanning conditions, we attempted to test whether changes in those CBCT based gray levels and other parameters can explain the CVM changes during the treatment period (T2-T1).

The CVM changes were not associated with any parameters examined in the current study. This result might arise from inaccuracy of CVM method to classify the maturity levels as indicated in previous studies [7, 8]. Furthermore, the current estimation of CVM values had the weak inter-operator reliability (0.54) based on only two cervical vertebrae (C2 and C3), which might not be as accurate as in the previous study that used three cervical vertebral levels (C2, C3 and C4) [6]. However, the values of gray level parameters were obtained semi-automatically and thus provided a more objective result. In addition, the values obtained before treatment had significant correlations with those after treatment (Table 3) suggesting that the progress of growth and maturity can be estimated by having the initial information of patient.

A limitation of the current study was that only two longitudinal groups before and after orthodontic treatment were examined. The age range examined in the current study could be expanded to include pre, peak and post peak changes. [37]. There were no gender effects on the results. Another limitation was that the bone voxels inside the
vertebral body were not segmented from non-bone voxels. The rough clinical CBCT image resolution did not allow for accurate separation between bone and non-bone voxels. Thus, the gray levels of this study represent BMDs that included bone marrow gray levels in part as the conventional BMD was measured [15]. Finally, the mandibular length was measured using the 2D cephalometric view of 3D CBCT image, but these 2D image based analyses might not produce comparable information with 3D image based BMD and volume measures. It was recommended the 3D based analysis include mandibular bone volume measurements in the future studies.

To our best knowledge, this study is the first work to examine the applicability of BMD distribution for the assessment of bone maturation using the 3D clinical CBCT images taken from dental patients. It is possible that the clinical CBCT image based analysis could be used to determine the association of biological activities including bone modeling and remodeling with cervical vertebral growth in vivo for human patients. The percentage difference method that was developed to reduce the CBCT scanning errors to assess gray values between the CBCT images successfully provided significant maturation dependent differences in BMD distribution. It was found that the initial measures of those parameters play a limited role in determining those measures afterward. These findings suggest that the 3D clinical CBCT images can help provide additional quantitative information related to the volume and BMD distribution of human cervical vertebrae, which can distinguish the age related changes.
Acknowledgments

We thank the Delta Dental Foundation for providing financial support for this research through the Dental Master’s Thesis Award Program. We thank the Craniofacial Imaging Center at Case Western Reserve University for providing us with the CBCT images used in this study.

Conflict of interest

The authors declare that they have no conflict of interest.
References


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CHAPTER 4
DISCUSSION AND CONCLUSIONS

The purpose of this study was to determine whether additional information in the form of BMD distribution would be useful in assessing skeletal maturity and growth potential. In fact, we found that the BMD does change predictably during the adolescent growth period. In the pre-treatment patient population the standard deviation of the gray levels of C2 was significantly higher than that for C3, indicating a higher level of bone turnover in C2 at this younger age. By the post-treatment time-point, there were no significant differences between the standard deviations for the two vertebrae, indicating similar levels of bone turnover, despite a higher mean gray scale value, indicating a higher degree of mineralization, for C3 at both time points. It is possible that this represents the attainment or approach of baseline values of bone turnover that the patients will continue to experience throughout life, and if so, it would indicate the conclusion of skeletal growth and the commencement of skeletal maintenance and remodeling activity. In order to determine whether this is the case, we would require data on patients of a wider range of ages and bone maturity levels.

In addition, the difference between the mean gray values for C2 and C3 was greater at the post-treatment time-point than at the pre-treatment time-point. As higher gray values indicate a higher level of mineralization, this indicates that there is a greater difference in mineralization between the two vertebrae after treatment than before
treatment. Since we did not attempt to assign absolute values for mineralization, we must evaluate these results carefully. As the difference became greater, it is likely that C3 underwent more mineral deposition while C2 maintained a stage of greater turnover. That C2 was undergoing greater bone turnover at the pre-treatment time-point is evident based on the differences in standard deviation as stated previously. Hence, we hypothesize that after the pre-treatment stage, C3 began a phase of greater mineralization, progressing towards ultimate peak bone mineral density (typically attained in early adulthood) and final maturation to adult levels of bone turnover. Despite the fact that C2 had not yet caught up to C3 in terms of mineralization by the post-treatment time-point, it may be the case that it was beginning a similar stage of final mineralization in approach of adult baseline levels, explained by the equivalent amount of bone turnover between the two vertebrae after treatment. Evaluation of adult patients may reveal a trend towards similar mineralization levels and maintenance of similar turnover levels, and we recommend that further research investigate this.

This method for assessing skeletal maturity is desirable because it incorporates more data than it was previously possible to include using only two-dimensional images. By analyzing the CBCT, a clinician can assess not only the CVM stage based on the two-dimensional output, but he or she may also assess the level of bone turnover in each vertebra, giving an indication of how close the patient may be to completion of growth. In order to use this information to make accurate predictions, however, we would require data on a larger number of patients and those from a wider range of ages and skeletal maturities. With this additional longitudinal data, it is possible that clinicians may be
able to more accurately time the onset of the peak growth spurt and time treatment accordingly.

In addition, the method we used of comparing C2 to C3 within the same image to compare the mean difference between the two before and after treatment, eliminates the complications associated with attempting to estimate an absolute value of BMD. Since the machine parameters, background noise, and other patient factors may affect the estimate of the absolute value of BMD, we elected instead to use relative values such as the percent difference. Essentially, we did not compare the absolute gray scale values in any pre-treatment image to those in its corresponding post-treatment image, but rather we compared the differences in the two vertebrae in any single image and the differences in the two vertebrae in the pre-treatment image to the differences in the two vertebrae in the corresponding post-treatment image. Comparing absolute values would be less accurate because of the potential for any parameters to have changed in the patient, the machine, or the atmosphere between exposures.

The findings of this study indicate that C2 reaches its final maturity later in the adolescent growth phase than does C3 as evidenced by higher levels of bone turnover in C2 relative to C3 at pre-treatment and converging levels of turnover at post-treatment. It appears that C3 reaches or will reach a baseline level of remodeling activity at some point prior to C2. The mean gray scale values between the two vertebrae diverge while the turnover converges. This may mean that C2 will complete its mineralization at a slight lag behind C3. In addition, it seems that by the time adolescent growth is complete, or what may correspond to a CVM stage of V, indicating completion of skeletal growth, both C2 and C3 will have reached final maturity in terms of bone remodeling.
This evidence supports previously published research by Altan et al. who found that at age 14.5, C2 experiences a higher growth rate, as evidenced by the addition of a significantly greater number of millimeters in height per year, than C1, C3, or C4. The Altan study also demonstrates that all four of these vertebrae examined converged in growth rate by approximately age 16.5. The age groups correspond approximately to the pre- and post-treatment age groups included in our study, but the data differ in that their study was limited to adolescent females, and ours included both genders in an approximately equal ratio. Together, these seem to indicate that C2 is the last of the cervical vertebrae to decline to the baseline growth rate and that all of the cervical vertebrae have declined almost to baseline by age 16.5 in females.

This study does involve significant limitations. Namely, we used only those CBCT images which were readily available which limited the patient population in terms of patient age, gender, and quantity. In addition, there has been a great deal of evidence presented both in favor of and opposing the use of CBCT images for the estimation of bone density. We attempted to circumvent this problem by using relative values as stated previously rather than absolute values. This method is preferred because it does not rely on a precise estimate of BMD, but rather on comparisons within an image. Also, the vertebrae we used in this study (C2 and C3) which were chosen due to their consistent presence in every image differed significantly in their size and shape. This likely affected the ratio of cortical to trabecular bone and thus the relative bone density of each vertebrae as a whole. If C3 and C4 had both been available for comparison, for example, the affect of this confounding factor could have been reduced. Finally, the process of isolating the vertebrae in 3 dimensions is somewhat imprecise. It is possible that some
voxels that included part of the vertebrae were excluded or that some voxels including only the surrounding tissue were inadvertently included in the final gray scale value histograms.

We chose to compare patients pre- and post-treatment for convenience, but they did not all begin and end treatment at the same CVM stages. It is likely that if patients were compared on a less arbitrary scale and a more objective one, e.g. by comparing only patients within a single CVM stage to those in another, differences may have been more meaningful. Although we could have grouped images based only on these CVM parameters, we wished to compare patients longitudinally, requiring that our groups be divided in terms of pre- and post-treatment. In the future, we would advise that with a larger patient population, more intricate comparisons may be made. An ideal RCT would randomly assign patients to the study prior to the treatment start, grouping them according to their CVM stage, and include a larger number of patients with longitudinal data that was also grouped by CVM stage. Another alternative would be to attempt a similar study in an animal model which would enable us to expose repeated images on the same subjects during the peak growth spurt and potentially to further define our hypothesis.

Conclusions

From this evidence, we can conclude the following:

1. During adolescence, not only does the morphology of the cervical vertebrae change, but the level of bone turnover changes as well.
2. At age 14.5, bone turnover for C2 is greater than that for C3.

3. As patients approach the end of their adolescent growth phase, the degree of bone turnover in C2 becomes equal with that of C3, but there remains a difference in level of mineralization with C3 being more mineralized on average at age 16.5.

4. As patients approach the end of their adolescent growth spurt, the difference in bone mineralization between C2 and C3 increases.

**Acknowledgments**

We thank the Delta Dental Foundation for providing financial support for this research through the Dental Master’s Thesis Award Program. We thank the Craniofacial Imaging Center at Case Western Reserve University for providing us with the CBCT images used in this study.

**Conflict of interest**

The authors declare that they have no conflict of interest.
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APPENDIX A:

TABLES AND FIGURES
Table 1. Comparison between C2 and C3 vertebral bodies for Mean and SD of gray levels, and absolute amount of vertebral body volume difference between before (T1) and after (T2) orthodontic treatments.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Parameters</th>
<th>C2 vertebral body</th>
<th>C3 vertebral body</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before (T1)</td>
<td>Mean</td>
<td>1948.312±81.873</td>
<td>1969.746±87.370</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>141.635±27.739</td>
<td>133.530±23.642</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After (T2)</td>
<td>Mean</td>
<td>1997.257±50.028</td>
<td>2054.788±52.916</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>121.063±13.299</td>
<td>120.504±13.829</td>
<td>0.669</td>
</tr>
<tr>
<td></td>
<td>T2-T1 (mm³)</td>
<td>Vertebral body volume</td>
<td>1436.676 ±1270.435</td>
<td>850.389 ±612.565</td>
</tr>
</tbody>
</table>
Table 2. Comparison between before and after orthodontic treatments for the percentage (%) differences of Mean, SD and vertebral body volume between C2 and C3 vertebral bodies, and the CVM stage and mandibular length. The negative percentage (%) difference of Mean represents that the values of C2 vertebral body were lower than those of C3 vertebral body.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Before (T1)</th>
<th>After (T2)</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (%)</td>
<td>-1.085±1.605</td>
<td>-2.838±1.291</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD (%)</td>
<td>5.467±6.901</td>
<td>0.515±6.655</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vertebral body volume (%)</td>
<td>60.381±9.156</td>
<td>55.295±8.461</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CVM stage</td>
<td>2.927±1.010</td>
<td>3.561±0.776</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mandibular length (mm)</td>
<td>115.827±5.261</td>
<td>119.022±5.813</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Table 3. Correlations of the percentage (%) differences of Mean, SD and vertebral body volume, and the CVM stage and mandibular length between before (T1) and after (T2) orthodontic treatments.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>r</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (%)</td>
<td>NS</td>
<td>0.220</td>
</tr>
<tr>
<td>SD (%)</td>
<td>0.547</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Vertebral body volume (%)</td>
<td>0.593</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CVM stage</td>
<td>0.692</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mandibular length (mm)</td>
<td>0.875</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Fig. 1 A typical CBCT image process to isolate the cervical vertebrae (C2 and C3). From the initial full field of view 3D image, vertebrae are cropped and viewed here as a single slice. Next, using the cropped image, the vertebral voxels are separated from non-vertebral voxels, and the vertebral body is cropped from the entire image.
**Fig. 2** Typical histograms of gray level (a) before (T1) and (b) after (T2) orthodontic treatment of C2 (black) and C3 (gray) vertebral bodies of the same patient.
Fig. 3 Correlations of a) vertebral body volume (T2=0.642 T1+24.892, r=0.593, p<0.001) and b) mandibular length (T2=0.792 T1+21.545, r=0.875, p<0.001) between before (T1) and after (T2) orthodontic treatments.