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Quantifying Mineralization Utilizing Bone Mineral Density Distribution in the Mandible

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Abstract

Background—Microcomputed Tomography (μ CT) is an efficient method for quantifying the density and mineralization of mandibular microarchitecture. Conventional radiomorphometrics such as Bone and Tissue Mineral Density are useful in determining the average, overall mineral content of a scanned specimen; however, solely relying on these metrics has limitations. Utilizing Bone Mineral Density Distribution (BMDD), the complex array of mineralization densities within a bone sample can be portrayed. This information is particularly useful as a computational feature reflective of the rate of bone turnover. Here we demonstrate the utility of BMDD analyses in the rat mandible and generate a platform for further exploration of mandibular pathology and treatment.

Methods—Male Sprague Dawley rats ($n=8$) underwent μ CT and histogram data was generated from a selected volume of interest. A standard curve was derived for each animal and reference criteria were defined. An average histogram was produced for the group and descriptive analyses including the means and standard deviations are reported for each of the normative metrics.

Results— M_{peak} (3444 Hounsfield Units, SD =138) and M_{width} (2221 Hounsfield Units SD =628) are two metrics demonstrating reproducible parameters of BMDD with minimal variance. A total of eight valuable metrics quantifying biologically significant events concerning mineralization are reported.

Conclusion—Here we quantify the vast wealth of information depicted in the complete spectrum of mineralization established by the BMDD analysis. We demonstrate its potential in delivering mineralization data that encompasses and enhances conventional reporting of radiomorphometrics. Moreover, we explore its role and translational potential in craniofacial experimentation.

Introduction

The examination of Bone Mineral Density Distribution (BMDD) in human bone biopsies has advanced the understanding of how bone turnover correlates with various known pathologies and treatments aimed to enhance bone density.⁽¹⁻¹⁵⁾ Although the utility of BMDD in human bone biopsies has benefitted from an evolution in techniques, the examination of the same in a small animal model has been slow in development,

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specifically, in the craniofacial skeleton.^(16,17) Although the use of Micro-Computed Tomography (μ CT) in the murine mandible has become more commonplace, a correlating method for examining changes in BMDD has not been defined.⁽¹⁸⁻²¹⁾ This unique ability to detect subtle changes in mineralization characteristics would substantially complement the current examination of mandibular bone in various settings. The benefit of utilizing BMDD is that it delivers a wealth of information beyond conventional metrics such as Bone Mineral Density (BMD) and Tissue Mineral Density (TMD); values that calculate the overall average mineralization in a sample. Reliance on only those metrics may mask a wealth of information about bone biodynamics that is easily calculated and readily delivered in a BMDD analysis. Furthermore, this is a convenient standard feature that is readily available with MicroView software. Based on these assertions, we aimed to utilize this powerful analysis to generate normative quantifiable data in our experimental models designed to study mandibular pathologies.

Selecting reference criteria

Bone formation is a process by which newly deposited, non-mineralized matrix is gradually replaced by mineralized bone. This represents a maturation process, which is constitutively occurring in normal bone. The process of bone formation continues well beyond normal growth as a result of remodeling. Remodeling is the net result of bone resorption and formation brought on by a dynamic interplay between osteoclasts and osteoblasts in the maintenance of bone homeostasis. At any given time within the cortico-cancellous unit or osteosome there are quiescent, activation and formation phases; the net result is continuous maintenance of the bone and bone marrow organ and of the skeleton. Remodeling occurs in response to three distinct biological events: first, as a stochastic process that replaces old bone; second, as a process to repair bone in response to accumulation of damage; and third, as component of the generation of bone in normal growth and development.⁽²²⁻²⁴⁾ An important consideration is that these processes may occur independently of each other and at different rates, producing an environment where bone is at varied stages of development. Due to this remodeling dynamic, heterogeneity of mineralization densities will exist within any given volume of interest, and the distribution of bone mineral will be non-uniform. Hence, relying solely on metrics that describe average mineralization density can limit the full understanding of these events. Therefore, applying a more encompassing method of mineral density analysis is a valuable complement. What a BMDD histogram represents, is a snapshot at a given moment in time portraying this dynamic array of biological processes relating to the deposition and resorption of mineral in bone matrix.^(25,26) This information provides insight regarding the biodynamics of bone turnover. A BMDD analysis delivers a calculated means of visualizing and quantifying this diversity radiographically. We utilized previously established, relevant criteria to fully encompass these events.^(1,4)

Materials and Methods

8 Male Sprague-Dawley rats underwent μ CT and radiomorphometric analysis was conducted with MicroView 2.2 software. BMDD histogram data was generated from a pre-defined volume of interest (VOI) using a threshold that identifies voxels as being bone within this volume. This threshold is a Hounsfield Unit range of 800-4000, and is typically used in our models for the normal distribution of bone density. An average histogram was generated for the group, and the following metrics were derived in accordance with the calculations of Roschger et al.^(7,17):

- Mineralization Peak (M_{peak})
- Mineralization Width (M_{width})
- Mineralization Low (M_{low})

- Mineralization High (M_{high})
- Mineralization Weighted Mean (M_{wmean})

Metrics Derived from Our Laboratory

- Mineralization Slope of Rise (M_{sor})
- Mineralization Slope of Decline (M_{sod})
- Mineralization Total Number of Voxels (M_{tnv})

Definitions/Equations:

- M_{peak} = HU @ maximum f
- M_{width} = the width of the curve @ half of M_{peak}
- M_{wmean} = (HU x f) / Total f yields the weighted HU; then (HU x f) / Total HU yields weighted f the width of the curve
- M_{low} = the integral for the area under the curve at 5% of the M_{TNV}
- M_{high} = the integral for the area under the curve at 95% of the M_{TNV}
- M_{sor} = the slope of a line ($\Delta y / \Delta x$) intersecting the lowest point of mineralization (here, the f at 800HU) and M_{peak} .
- M_{sod} = the slope of a line ($\Delta y / \Delta x$) intersecting M_{peak} and the highest point of mineralization (here, the f at 4000HU).
- M_{tnv} = of f

Interpreting BMDD Metrics

The M_{peak} is an indicator of bone quality identifying the most frequent level of mineral density achieved in a sample.

M_{width} reflects the array of Hounsfield units corresponding to the diversity of mineralization densities that are represented in a sample. The width encompasses the range of Hounsfield values at half of the mineralization peak. This parameter describes differences in heterogeneity of mineralization. This becomes important in processes where bone turnover is limited and the range of possible mineralization densities may become restricted; in other words, in scenarios where bone becomes less heterogeneous or more homogeneous.

The M_{wmean} is the average weighted frequency per Hounsfield unit represented in the sample. Incidentally, this calculation is related to the calculation for the classic radiomorphometric TMD, and is used as an indicator of the average mineralization density represented in a volume of interest.

M_{low} and M_{high} correspond to the 5th and 95th percentile of the whole BMDD histogram curve. In general, these values reflect the overall lowest and highest mineralization densities and add sensitivity by quantifying these important ranges of mineralization.

M_{sor} and M_{sod} are more specific indicators of diversity of mineralization before and after peak mineralization respectively. In general, steeper slopes are indicative of less heterogeneity in mineralization.

Volumetric changes can be compared using M_{tnv} . This is a simple calculation indicating the overall volume of mineral being analyzed. This metric may be used to observe small

differences in the quantity of mineral being scanned or to quickly note sizable mineral volume changes due to experimental exposures or treatment strategies.

The means, standard errors and standard deviations from the means were calculated using PASW Statistics 18 software for each of the reported metrics.

Results

A graphic representation of the BMDD histogram generated from this experiment is shown in Figure 1. Computational analyses are reported for each Normative BMDD parameter in Table 1. Each metric is graphically depicted in the figures that follow (Figures 2-7). As shown, a wealth of information is readily available from this standard curve, further, quantifiable metrics with minimal variance are reported and described.

Discussion

Calculating conventional mineralization metrics such as BMD or TMD from a scanned VOI is a commonly used method for radiologic bone analysis.^(18-21, 27-34) Although these metrics are useful, they may mask some important information regarding bone mineralization throughout that volume. In essence, investigators may be able to glean more information about bone turnover by examining the full spectrum of mineralization as opposed to a focused average.^(30,35,36) For example, while examining osteoporotic risk of fracture, McCreadie and Goldstein reported a large overlap between normal and diseased populations while using BMD.⁽²⁷⁾ Roschger et al. proposed a solution to this limitation in analysis by using the process of BMDD. He examined a variety of disorders such as osteogenesis imperfecta, osteomalacia and osteoporosis using human bone biopsy samples in conjunction with high-resolution imaging and showed that unique characteristic patterns of mineralization with quantifiable differences were apparent specific to those pathologies.^(9, 13, 14, 17)

While BMD and TMD are indeed indicators of bone mineralization, they are simply reflective of the overall average mineralization. It is important to consider that this may mask an abundant dataset reflecting important quantifiable characteristics of bone biology. Figure 9 graphically depicts the TMD generated from our volume of interest in the rat mandible, and Figure 10 displays this TMD value superimposed on its corresponding BMDD curve. These figures illustrate the wealth of information surrounding the TMD that is delivered in the BMDD analysis.

Our experience utilizing conventional radiomorphometrics in the setting of radiotherapy-induced changes to mandibular bone yielded limitations that illuminated the need for a comprehensive means by which to measure subtle changes in mineralization biology.^(34,1) Figure 11 demonstrates cortical thinning in a murine mandible after radiotherapy (XRT). While a corresponding decrease in TMD was expected, in most instances TMD was actually higher in radiated samples than in non-radiated samples. A conventional analysis would have ended there with a puzzling result; however, the histogram analysis carries a great deal more information that is often overlooked. Disregarding or omitting that pertinent information carries the risk of coming to an erroneous conclusion. Comparison of the BMDD histograms revealed several obvious differences due to XRT.

Shown in Figure 12 are histograms representing BMDD in a non-radiated control vs. three groups that had varying doses of radiation (LD-Low Dose, MD-Middle Dose, HD-High Dose). While the weighted mean of mineralization, or TMD was actually higher with radiotherapy than in control, other characteristics, such as a notable decrease in bone of low

mineralization (Figure 13), and a limitation of the range of mineral densities produced (Figure 14) were more telling of radiation-induced changes.

Reflecting on these experiences, we recognized the value of generating a standardized BMDD curve in the rat mandible with our current imaging modality, μ CT. We hypothesized that the characteristics noted in the curve could be represented as quantifiable and comparable metrics of biological significance as reported by others in the human long bone literature.⁽¹⁷⁾ It is worth mentioning here that there are potential obstacles to consider when conducting this method with μ CT. Beam hardening due to scan setup can impact densitometry measurements in both cortical and trabecular bone; however, beam filtration is a means to avoid this issue should it arise.⁽³⁷⁾ This then becomes a comparatively inexpensive, efficient tool for analyzing BMDD in mandibular pathology.

Conclusions

We report five normative metrics representing quantifiable aspects of bone turnover derived from our BMDD analysis in the rat mandible. We also report three additional metrics that examine mineral heterogeneity before and after peak mineralization and total mineral content within a specimen.

We strongly advocate the inclusion of this powerful analysis when using radiomorphometrics to evaluate bone, particularly because it is a practical, computational tool that is widely available and largely under-reported. The scope of this tool extends far beyond the craniofacial skeleton and may be of value in the analysis of skeletal pathology regardless of origin.

Future directions include quantifying the effects of pathologic processes on mandibular BMDD in rat models as well as pushing the boundaries of BMDD analysis by examining its utility with Computed Tomography (CT) imaging in the clinical setting. Doing so may enable a non-invasive means to correlate clinical bone pathologies with experimental models designed to test and remediate those pathologies at the bench top.

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Figure 1.
BMDD histogram in the normal rat mandible

Figure 2.
Mineralization total number of voxels depicted as the total area under the curve

Figure 3.
Mineralization peak depicted as the intersection between the red line and the curve

Figure 4.
Mineralization width depicted as the width of the curve at half peak

Figure 5. Mineralization slope of rise and slope of decline depicted as the slope of the curve before and after the peak respectively

Figure 6.
Mineralization weighted mean depicted by the star

M_{low} and M_{high}

Figure 7.
Mineralization low and high depicted by the shaded areas under the curve

Figure 8.
μCT scan of a left murine hemimandible with volume of interest highlighted

Figure 9.
Graphic representation of TMD on a Hounsfield Unit per frequency scale

Figure 10.
BMDD Histogram on a Hounsfield Unit per frequency scale with TMD superimposed for comparison

Figure 11.
Evident cortical thinning demonstrated after radiotherapy to the hemimandible (left), as compared to the non-radiated sample (right).

Figure 12.
Control vs. XRT BMDD Histograms

Figure 13.
Focused and enlarged representation of Figure 12 highlighting radiation induced depletion on the early mineralization of bone.

Figure 14.

Focused and enlarged representation of Figure 12 demonstrating a limitation of the width of the curve implying a loss of mineral heterogeneity of the irradiated samples

Table 1

Bone Mineral Density Distribution Metrics

BMDD Metrics	N	Mean	Standard Deviation
Total Number of Voxels (M_{TNV})	8	278,724	9,807
Peak (M_{peak})	8	3,444	138
Width (M_{width})	8	2,221	628
Slope of Rise (M_{sor})	8	0.034	0.006
Slope of Decline (M_{sod})	8	-0.141	0.035
Weighted Mean (M_{wmean})	8	2,643	65
5th percentile (M_{low})	8	1,051	20
95th percentile (M_{high})	8	3,966	58