Preliminary Clinical Study to Evaluate the Relationship between Systemic Bone Turnover and the Microstructure of the Alveolar Bone

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Abstract: The objective of this study was to assess the possibility of developing a clinical minimally invasive and standardized method to evaluate the relationship between the microstructure of the jaw bone and systemic bone turnover. For this purpose, we performed standardized bone biopsy of the alveolar bone, and compared the 3D bone microstructure using micro-computed tomography (micro-CT) with bone mineral density (BMD) of the lumbar spine and biochemical markers of bone turnover. We evaluated a total of 9 samples taken from 6 patients by standardized biopsy using a trephine bur. BMD was evaluated using dual energy X-ray absorptiometry (DXA). Regarding the biochemical markers of bone turnover, serum bone-specific alkaline phosphatase (BAP) and serum osteocalcin (OC) were used as bone formation markers, and urinary cross-linked N-telopeptides of type I collagen (NTx) and urinary deoxypyridinoline (DPD) were selected as bone resorption markers. We scanned micro-CT images of these samples. Bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular spacing (Tb.Spac), fractal dimension, trabecular bone pattern factor (TBPf) and node-strut (Nd.Nd/TV, TSL/TV) were measured. Regarding the correlations between the parameters of bone microstructures, TB/TV, Tb.N, fractal dimension, and node-strut seemed to be positively correlated and Tb.Spac and TBPf seemed to be negatively correlated with each other, but Tb.Th seemed to have a low correlation with other parameters. OC and/or BAP showed a significantly high correlation with many structural parameters (p < 0.05%). In conclusion, some microstructural parameters may change according to the systemic bone turnover.

Key words: alveolar bone, micro-computed tomography (micro-CT), bone microstructure, bone mineral density (BMD), biochemical marker of bone turnover

We used the following abbreviations for the parameters

<table>
<thead>
<tr>
<th>Systemic parameters</th>
<th>Structural parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone mineral density (BMD)</td>
<td>Bone volume fraction (BV/TV)</td>
</tr>
<tr>
<td>Osteocalcin (OC)</td>
<td>Trabecular thickness (Tb.Th)</td>
</tr>
<tr>
<td>Mean of young adults (% YAM)</td>
<td>Fractal dimensions (fractal)</td>
</tr>
<tr>
<td>Bone-specific alkaline phosphatase (BAP)</td>
<td>Trabecular number (Tb.N)</td>
</tr>
<tr>
<td>Cross-linked N-telopeptides of type I collagen (NTx)</td>
<td>Trabecular bone pattern factor (TBPf)</td>
</tr>
<tr>
<td>Deoxypyridinoline (DPD)</td>
<td></td>
</tr>
</tbody>
</table>

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**Introduction**

Recently, there have been many reports that systemic turnover affects resorption of the jaw bone\(^1\)\(^2\). In addition, some studies have shown that X-ray evaluation of the jaw bone could be useful for estimating the progression of osteoporosis\(^3\)\(^4\). Thus, the changes in the jaw bone and systemic bone turnover are closely related. On the other hand, jaw bone surgery and osseointegrated implants have been performed recently for elderly and ill people, who frequently have abnormalities of systemic bone turnover\(^5\). These conditions are likely to affect the long-term results of surgery, and yet the relationship between bone surgery or osseointegrated implant and systemic bone turnover has not been clinically evaluated.

The aim of this preliminary study was to assess the possibility of developing a clinical minimally invasive and standardized method to evaluate the relationship between the microstructure of the jaw bone and systemic bone turnover. For this purpose, we performed standardized bone biopsy of the alveolar bone with a trephine bur (inner diameter 2.8 mm) from the implant insertion area. Bone microstructures of samples were measured using micro-CT. The results were compared with bone mineral density (BMD) obtained using dual energy X-ray absorptiometry (DXA) of the lumbar spine and biochemical markers of bone turnover.

**Material and Methods**

**Cases**

A total of 9 samples were taken from 6 patients by standardized biopsy at the time of implant insertion at the Department of Oral and Maxillofacial Surgery of Tokyo Medical University Hospital. All patients were Japanese, and their age ranged from 32 to 57 years old (average 49.7), and 5 were women (Table 1). None of the patients had cardiovascular disease, diabetes or metabolic bone disease. They also had not had radiation or bone

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Sex</th>
<th>Age</th>
<th>Biopsy site</th>
<th>Number of samples</th>
<th>Dual energy X-ray Absorptiometry*</th>
<th>Biochemical markers of bone turnover</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>BMD (g/cm(^2))</td>
<td>% YAM</td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>56</td>
<td>Lower Molar</td>
<td>1</td>
<td>0.822</td>
<td>-1.54</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>57</td>
<td>Upper Anterior</td>
<td>1</td>
<td>0.706</td>
<td>-2.35</td>
</tr>
<tr>
<td>3</td>
<td>F</td>
<td>48</td>
<td>Lower Anterior</td>
<td>1</td>
<td>0.866</td>
<td>-1.22</td>
</tr>
<tr>
<td>4</td>
<td>F</td>
<td>47</td>
<td>Lower Anterior</td>
<td>1</td>
<td>0.758</td>
<td>-1.99</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>57</td>
<td>Upper Anterior</td>
<td>4</td>
<td>1.053</td>
<td>0.11</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>32</td>
<td>Lower Molar</td>
<td>1</td>
<td>0.799</td>
<td>-1.75</td>
</tr>
<tr>
<td><strong>Average</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.834</strong></td>
<td><strong>10.4</strong></td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td><strong>0.120</strong></td>
<td><strong>5.1</strong></td>
</tr>
</tbody>
</table>

General information and the results of the systemic parameters of each case are described in the figure. The details of each parameter are described in the footnote.

*Bone mineral density (BMD) was compared from the mean of young adults (% YAM) using dual energy X-ray absorptiometry (DXA).
augmentation of the jaw bone previously. All patients were fully informed about the procedure, including the surgery, and gave written informed consent. The ethics committee of Tokyo Medical University has approved the research protocol.

Measurements of systemic bone metabolism

DXA (Norland, Fort Atkinson, WI, USA) of the lumbar spine (C2-C4) was performed and biochemical markers of bone turnover were measured before bone biopsy. We calculated BMD of the lumbar spine (average of C2-C4), and compared it with the mean of young adults (Young Adult Mean) using DXA. Regarding the biochemical markers of bone turnover, serum bone-specific alkaline phosphatase (BAP) and serum osteocalcin (OC) were used as bone formation markers. Urinary cross-linked N-telopeptides of type I collagen (NTx) and urinary deoxypyridinoline (DPD) were selected as bone resorption markers (Table 1).

Bone biopsy methods

Standardized bone biopsy of the implant insertion area was performed using a trephine bur (inner diameter 2.8 mm, outer diameter 3.2 mm). An osseointegrated implant was inserted immediately after bone biopsy in all cases (Fig. 1).

Micro-CT scanning

We scanned these samples using a micro-CT system (Elescan, Nittetsu Elex Co., Ltd., Kitakyushu, Japan). The apparatus is based on fan-beam tomography and is able to function in multislice mode. An X-ray tube with a microfocus (spot size of 6 × 8 μm) is used and a maximum resolution of 4 μm (in pixel size) is attainable. Scanning was conducted at 35 kVp and 100 mA. Consecutive tomographic slices with a slice thickness of 20 μm were acquired. The digital data were reconstructed to obtain CT images with pixel size of 17.91 μm in a 512 × 512 matrix. We created a 3D reconstruction image using the volume rendering method for morphological observations using computer software (TRI/3D Bon, Ratoc Co., Ltd., Tokyo, Japan).

Evaluation of the bone microstructures

All bone microstructures were measured directly from the 3D reconstructed images using the same computer software. At first, compact and cancellous bone were distinguished morphologically by conducting a visual check, tracing the border and distinguishing the trabecular bone in the bone marrow and the external compact bone. Then, a cylindrical area 2 mm in height of cancellous bone was selected for the measurement. Parameters included in the analysis were obtained as follows. Bone volume (BV) was calculated using tetrahedrons corresponding to the enclosed volume of the triangulated surface. Total tissue volume (TV) was the entire volume of the analysis. Bone volume fraction (BV/TV [%]) was calculated from these values, and trabecular
thickness (Tb.Th [um]) was determined according to the method by Hildebrand and Rugesegger. Trabecular number (Tb.N [1/mm]), and trabecular spacing (Tb.Spac [um]) were estimated based on the plate model. Fractal dimensions of the trabecular bone were measured as a representative of complexity using the box-counting method, which was developed three-dimensionally. Biomechanical stability of cancellous bone was determined not only by the amount of bone as expressed by BV/TV, but also by the orientation and degree of the interconnection of trabeculae. Trabecular bone pattern factor (TBPf) was evaluated using the relation of concave to convex surface, and node-strut (Nd.Nd/TV, TSL/TV) was evaluated using trabecular connectivity (Fig. 1).

Statistical evaluation

Four specimens were obtained from one case (case 4). The average values of the 4 specimens were taken to represent the values for this case. We calculated the statistical mandibular and maxillary values individually, but did not compare the statistical differences because of the small number of samples. We evaluated Pearson's correlation among structural and systemic parameters in 6 cases. P < 0.05% was considered to indicate a significant difference.

Results

Measurements of systemic bone metabolism

Average BMD was 0.834 ± 0.120, and YAM over 2 SD was found in only one case (2.35). Average BAP was 29.6 ± 11.9, and OC was 10.4 ± 5.1. Average NTx was 36.8 ± 13.7, and DPD was 5.5 ± 1.5. The lowest BMD case (2.35) also showed increases in both bone formation and resorption markers. This case was recognized as high-turnover osteoporosis (Table 1).

Survival rate of the inserted implants

All implants have survived for over 3 years.

Morphological observations

Compact bone was clearly distinguished from cancellous bone. Specimens harvested using a trephine bur seemed to be almost undeformed, but mild deformation of marginal areas was observed in cases of poor bone quality. Bone trabeculae seemed wider in the mandible than in the maxilla also by morphological observation (Fig. 2).
Microstructures of the jaw bone

We calculated the average of all parameters, and then compared them with the maxilla and mandible. There were two cases of poor quality in which most microstructural parameters indicated low bone fragility. The details of each parameter are described in the footnote.

Correlations between the parameters of bone microstructures

Tb.Th had low correlation with other parameters. On the other hand, most residual parameters indicated high correlation with each other (p < 0.05%). TB/TV, Tb.N, fractal dimension, and node-strut had positive correlations with each other. On the other hand, Tb.Spac, and TBPf had negative correlations with the other parameters (Fig. 3, Table 3).

Correlation between bone microstructures, BMD and biochemical markers

Regarding the bone formation markers, OC and/or BAP showed a high correlation with Tb.N, Tb.Spac, fractal dimension, TBPf and node-strut (p < 0.05%). BMD showed moderate correlation with many parameters. On the other hand, bone

![Trabecular number (Tb.N) and Node-strut analysis (TSL/TV)](image)

Table 2 Microstructures of jaw bone

<table>
<thead>
<tr>
<th>n</th>
<th>Maxilla</th>
<th>Mandible</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BV/TV</td>
<td>Tb.Th</td>
<td>Tb.N</td>
</tr>
<tr>
<td>Maxilla</td>
<td>2</td>
<td>MEAN</td>
<td>12.51</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.99</td>
<td>55.50</td>
</tr>
<tr>
<td></td>
<td>MAX</td>
<td>14.63</td>
<td>158.81</td>
</tr>
<tr>
<td></td>
<td>MIN</td>
<td>10.40</td>
<td>80.32</td>
</tr>
<tr>
<td>Mandible</td>
<td>4</td>
<td>MEAN</td>
<td>30.38</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>14.49</td>
<td>49.31</td>
</tr>
<tr>
<td></td>
<td>MAX</td>
<td>42.00</td>
<td>253.36</td>
</tr>
<tr>
<td></td>
<td>MIN</td>
<td>9.40</td>
<td>139.99</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>MEAN</td>
<td>24.42</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>14.59</td>
<td>58.03</td>
</tr>
<tr>
<td></td>
<td>MAX</td>
<td>42.00</td>
<td>253.36</td>
</tr>
<tr>
<td></td>
<td>MIN</td>
<td>9.40</td>
<td>80.32</td>
</tr>
</tbody>
</table>

Poor bone quality

(Case 1) 1 9.40 139.99 0.67 1494.62 2.14 2.70 4.86 2.70

Poor bone quality

(Case 2) 1 10.40 158.81 0.66 1525.94 2.17 5.11 7.42 3.30

We evaluated the microstructures of maxilla and mandible individually. There were two cases of poor quality in which most microstructural parameters indicated low bone fragility. The details of each parameter are described in the footnote.

Fig. 3 Correlation between the parameters of bone microstructures.
This figure indicates the correlation between trabecular number (Tb.N) and node strut analysis (TSL/TV). Y = 0.1176x + 0.4793, R²: 0.8805
resorption marker NTx showed moderate to low correlation with structural parameters, and the tendency was the same as for the formation markers. However, DPD showed no correlation with any structural parameter. Concerning the structural parameters, TB/TV, Tb.N, fractal dimension, and node-strut showed negative correlations with systemic parameters, but Tb.Spac, and TBPF showed positive correlations. BMD showed relatively high correlation with both formation and resorption markers. Tb.Th showed no correlation with any systemic parameters (Table 4).

### Discussion
In general, bone strength is determined by bone mineral density, bone microstructures, mineralization, collagen composition and organization, and micro-damage. Clinically, bone mineral density and bone microstructures are mainly used to estimate the progression of systemic bone disease\(^1\). Many clinical studies of bone quality regarding implants have been reported\(^{12,13}\), but none is based on this theory. In addition, many studies concerning long-term bone resorption and implant survival rate have measured only bone height\(^{14,15}\). Only Blomquist\(^{16}\) assessed BMD of the forearm using single-photon gamma absorptiometry, and recognized a significant difference in success rate with long-term bone graft. To evaluate the jaw bone quality in conjunction with systemic bone turnover clinically, analyzing microstructures or mineralization is a reasonable approach. On the
other hand, the relationship between the jaw bone and osteoporosis has clinically been evaluated in many studies, and a significant correlation was found between BMD of the spine and cortical bone width of the mandible. However, there was no consistent finding between BMD of the spine and trabecular bone of the mandible. White pointed out the possibility of evaluating the relationship between the trabecular structure of the alveolar bone and systemic bone turnover. This study suggests the possibility of evaluating the relationship between systemic bone turnover and implant insertion area, but used indirect evaluation using dental X-rays. pQCT is the most precise method of estimating the bone microstructure on a clinical basis, but the resolution is several hundred µm, while 20 µm resolution is needed to estimate the microstructure of human bone, because the thickness of the human iliac bone is 132. ± 27.9 µm. Micro-CT and histological examination are the main methods of doing this, but both methods require bone biopsy. Recently, micro-CT has greatly developed, because it is possible to evaluate 3D bone microstructures without distraction of any specimens, and additional histological and biological evaluation can be performed freely. Micro-CT has already been used for animal or cadaver studies to evaluate the microstructures of the jaw bone, but not for clinical study yet. Molly stated that micro-CT examination is not possible on in vivo subjects. On the other hand, bone biopsy of the jaw bone using a trephine bur has already been done in previous studies, but only 2D sectional specimen staining H-E or truidin blue was evaluated in all studies. We considered that this method could be developed to allow 3D evaluation of jaw bone microstructures using micro-CT.

Many parameters have been used for measuring bone microstructures. In general, depending on the bone resorption, the number (Tb.N) and thickness (Tb.Th) of the trabecular bone decrease, the shape of the trabecular bone becomes convex (TBPf), and the space between the trabecular bone (Tb.Spac) increases. Bone volume (BV/TV) and connectivity (node-strut) decrease and the structure becomes simple (fractal dimension). 2D morphometrical measurements of the maxillary alveolar bone reveal the same tendency. In this study, almost all parameters indicated this tendency, including two cases of poor bone quality. In addition, alveolar bone microstructures of cadavers using micro-CT also correspond with our results. However, one question remains. Tb.Th does not correlate with any other parameters. We suspect that this finding is due to the difference between the mandible and the maxilla, because the trabecular thickness of the maxilla and mandible are very different, both in morphological and morphometrical observations in our study. Other studies presented different results, but we could not make any conclusions due to the small sample sizes of those studies. Further studies with large sample sizes are needed to reveal the aerial difference of the jaw bone structures.

Recently, biochemical markers have been widely used to determine bone turnover. They are measured using blood or urine, and it is possible to eliminate local influences and surgical intervention. It is also possible to comprehend the dynamics of bone turnover. This method allows detection of the bone formation and resorption individually, and estimation of the situation of bone turnover from the composite of some formation and resorption markers. On the other hand, DXA is the most common and reliable method of determining bone density, and many studies use this method to compare jaw bone quality. The lumbar spine is usually used to evaluate a systemic index, but the results have to consider the differences among the locations. Biochemical markers of bone turnover and DXA are frequently used together to improve the prognostic assessment of metabolic bone diseases. In our study, bone formation markers showed a high negative correlation with microstructures. Most cases in our study were postmenopausal women, and one case showed high-turnover osteoporosis and two cases were osteopenia. These findings suggested that microstructures of the jaw bone were affected by high-turnover osteoporosis in postmenopausal women. Taniguchi reported that both ALP and NTx well correlated with the parameters of osteoporosis at the lower border of the mandible in postmeno-
pausal women. However, bone resorption markers NTx and DPD showed moderate to low correlation with structural parameters in our study. These differences may be due to the influence of local factors, because our samples were taken from the alveolar area, and Kimble suggested that local inflammation might accelerate systemic bone resorption.

It is suspected that some microstructural parameter may have changed according to the systemic bone turnover in this study. In addition, all implants have survived for over 3 years. Based on these, we conclude that this method is useful and safe to evaluate the relationship between systemic bone turnover and bone quality of the alveolar bone clinically. This method could be developed into two clinical applications in the future. One would be to evaluate the bone quality to predict long-term implant stability and the bone strength after jaw bone surgery. The other would be a minimally invasive bone biopsy method to evaluate systemic bone disease.

Reference


