Study of microarchitecture of jaw bones with their histomorphometric study in patients with radicular cysts on the background of osteoporosis and without violations of mineral metabolism

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Abstract
Histomorphometry of bone tissue is still an important method of an objective evaluation of mechanisms of remodeling at cellular and tissue levels, which allows to reliably study the morphological manifestations of osteoporosis. We set the goal to study the microarchitecture of jaw bones with their histomorphometric study in patients with radicular cysts on the background of osteoporosis and without violations of mineral metabolism. Histological materials for histomorphometric study were obtained during radicular bone removal (cystectomy). After semi-automatic image transfer of histoparticles into black and white (binary masks), using the ImageJ software, the following parameters were measured: trabecular space, intertrabucular space, the area of the cross-section between the trabecular space ($\mu m^2$) and the histomorphometric coefficient of bone density. In response to the pathological effects of radicular cysts, irrespective of their size, an adaptive-compensatory reconstruction of the jaw bones occurs that morphologically manifests as a sealing of their trabecular structure, and the decrease of the intertrabecular space. The state of mineral metabolism in patients affects the osteo-regenerative potential in jaw bones.

Keywords: Microarchitecture of jaw, histomorphometry of bone, radicular cysts, osteoporosis

Introduction
In modern clinical dental practice, osteodensitometric diagnostic method is widely used to study the state of mineral metabolism of jaw bones [1-5]. Densitometry is a non-invasive method for determining the mineral density of bone tissue. This method of diagnostics also makes it possible to detect morphological disturbances in the jaw bones on the background of metabolic osteopathy (osteoporosis) [2-4]. For screening evaluation of morphological changes in jaw bones in these pathological conditions, mandibular cortical index, mandibular cortical width, panoramic mandibular index are determined on digital orthopantomograms, measurements of the optical density of the jaws are carried out using modern computer technologies [6-14]. With the help of the appropriate computer programs, the parameters (fractal sizes) representing the morphometric characteristics of the trabecular structure of the jaw bones are processed and calculated in digital orthopantomographs [15,19]. At the same time, histomorphometry of bone tissue is still an important method of an objective evaluation of mechanisms of remodeling at cellular and tissue levels, which allows us to reliably study the morphological manifestations of osteoporosis [20-22]. However, in the analysis of professional literature, we have not found data about the features of microarchitecture of jaw bones located in areas affected by radicular cysts, in patients with a violation of mineral metabolism (osteoporosis).

The purpose of the study: we set the goal to study the microarchitecture of jaw bones with their histomorphometric study in patients with radicular cysts on the background of osteoporosis and without violations of mineral metabolism.

Methods: 83 patients (45 males and 38 females) aged 20 to 70 years with radicular jaw cysts who were on an outpatient treatment in the surgical department of the medical stomatological center of the Danylo Halytsky National Medical University were examined. In 44 patients, tumor-like neoplasms were located on the upper jaw, and in 39 - on the lower jaw. The patients were conducted clinical examination and determined the bone mineral density (BMD)
using the LUNAR Corp. ultrasonic bone densitometer "Achilles" (USA) by measuring the time of passage of the ultrasound wave through the heel bone (a screening method for the identification of patients at risk for osteoporosis), which depends on its density and elasticity [23]. Mineral density of bone tissue in patients is analyzed according to the Z criterion (according to the WHO standard), which reflects on what percentage of the mean square deviation differs the index of strength of the bone tissue of the patient in comparison with the age norm. The normal mineral density of the bone tissue is estimated at Z values above 0 SD, signs of osteopenia at Z from -2.0 to -1.0 SD, and evidence of osteoporosis with Z is less than two standard deviations. All patients had standard orthopantomograms of the facial skeleton on Orthophos XG (Sirona) X-ray diagnostic apparatus. On the received radiograms we distinguished radicular cysts of small sizes, which are located within the alveolar segment of only one tooth (1-1.5 cm), radicular cysts of medium size, which are located within the alveolar segments of two or three teeth (2-3 cm), radicular large cysts that are located within the alveolar segments of more than three teeth (>3 cm). For a qualitative characterization of the cortical layer of the mandible, Klemetti and co-authors MCI index (mandibular-cortical index) was used [24]. Morphological characteristics of the cortical plate of the mandible, located below the mental opening, were evaluated. Depending on the morphological characteristics, three of its types are distinguished: the normal cortical layer C1 - the internal border of the cortical plate is clear and even, somewhat damaged cortical layer; C2 - the edge of the cortical layer has single crescent defects with bundle of the cortical plate from one or two sides, a significantly damaged cortical layer; C3 - the border is fuzzy, the uneven cortical plate is multilayered, porous, has many defects (fig. 1). The value of the MCI index is a manifestation in the maxillofacial region of the state of mineral metabolism in the human bone system [11-14].

Histological materials for histomorphometric study were obtained during radicular bone removal (cystectomy): after the trepanation of bone walls over tumor-like neoplasms, a bone tissue of the jaws (cortical plates and spongiform parts) sizes 1.0 × 0.5 cm was taken from the areas directly adjacent to the shells of radicular cysts (Fig. 2). Specimens of bone tissue (biopsy) were fixed in 10% - a neutral solution of formalin, carried out through special environments, paraffin sections with a thickness of 4-5 microns dyed with hematoxylin and eosin. Preparations dyed with hematoxylin-eosin were examined using a Meiji MT4300 LED microscope with an x20 lens, eyepiece x10. The histogram samples were photographed by Canon EOS 550D camera with adapter MA150 / 50 and adapter MA986 with an increase of x1.9, calibration for morphometry was performed using the Meiji MA285 slide with determination of the ratio of pixel to micrometer. All further morphometric studies were conducted exclusively with primary, non-edited Jpeg photos with a resolution of 5184 * 3456 with the same gauge data for each magnification.

For the morphometric analysis, ImageJ v.1.48u software was used. Each shot was investigated three times, the average area value for each shot was calculated. Semi-automatic method was used to calculate the studied parameters, for this the following sequence of commands was performed: 1. Window / level to the maximum contrast of the image, is determined visually (Figure 3.1); 2. Split channel division into colour channels and selection of the green channel (Fig.3.2); 3. Use the Threshold function to semi-automatic allocation of the maximum site with bone tissue and transformation the image into a binary mask (Figure 3.3). 4. Measurement of the fragment of the selected tissue.

Fig 1: Morphological characteristics of the cortical plate of the mandible on orthopantomogram according to the cortical index (MCI) Klemetti E. (1994).

Fig 2: A fence for histomorphometric study of the fragment of bone tissue of the upper jaw from the area adjacent to the shell of the radicular cyst (arrow shows the isolated bone biopsy)

Fig 3: Morphometric analysis of histological preparation of bone tissue with the application of software ImageJ v.1.48u. (Fig. 3.1-3.4 - sequential image changes in the process of processing tools ImageJ). The first two paragraphs were issued in the form of a macro
(the order of action that must be remembered for re-execution to achieve a certain goal) to optimize the process. However, the Threshold function does not automatically execute through a pre-configured macro, and its parameters are individually determined by each researcher. After that, the use of a number of additional functions of the ImageJ program can be used to more precisely separate the bone tissue. In particular, the use of the function - Fill Holes for filling small cleared areas on the surface of the bone section, using the Erode function to reduce the number of small artifacts (predominantly formed connective tissue) in the intervals between the areas of bone tissue and reducing the contact points of the artifact sites with bone tissue, using the Dilate function to fill in insufficiently painted gaps in the depth of the bone tissue (Fig. 3.4). In addition, the selective allocation of fragments by the Wand tool was used and in some cases, to remove the artifacts used the Paintbrush tool. After semi-automatic image transfer of histoparticles into black and white (binary masks), using the Image J software, the following parameters were measured: trabecular space (%) - the area of black colour that corresponds to the area of all the bone beams imaged on the histopreparatum; intertrabecular space (%) - area of white color, corresponding to the area of interbeams spaces; the area of the cross-section between the trabecular space (μm²) and the histomorphometric coefficient of bone density (HMM of the CBD) was calculated based on the formula we proposed:

\[ \text{HMM of the CBD} = \frac{\text{trabecular space} \%}{\text{intertrabecular space} \%} \]

Statistical processing of the obtained research results was carried out with the help of the computer program of statistical calculations "Statistica 8".

**Results of the research**

In the study of mineral density of bone tissue using the ultrasound densitometry of heel bone in 17 women aged 45-70 years, signs of osteopenia and osteoporosis were revealed. Osteoporosis was diagnosed in 9 patients, osteopenia was observed in 8 women, all of them were in the postmenopausal period. Indicators of BMD within the age range were in 8 observed women, aged under 45 years. In the observed men, the age norm of this indicator was more often preserved according to densitometry data - in 14 cases. Signs of osteopenia were diagnosed in 4 cases and osteoporosis in 2 cases. Indicators of the MCI index of the mandible in 87% of cases, both in women and in men, were directly correlated with the state of the mineral density of heel bones, determined by the method of ultrasonic densitometry. The morphological signs of osteoporosis were observed in patients on orthopantomograms: cortical plates of the mandible at the level of the masticatory group of teeth had structural features of C2 and C3 according to the MCI index (Fig. 4), which is confirmed with the literature data \[11-14\]. The morphological manifestations of this disease in periodontal tissues - resorption of alveolar bone were also radiologically visualized.

Depending on the state of mineral metabolism, patients were divided into two clinical groups: 1-clinical group: 44 patients (29 men and 15 women) were included in it, in which age-related or functional disorders of mineral metabolism were not detected; 2 - clinical group: it included - 38 patients (27 women and 11 men), in which age or functional disorders of mineral metabolism were found - osteoporosis.

In histomorphometric studies of bone biopsies taken from intact jaw sections, in patients with no mineral abnormalities, it was found that the density of bone tissue in the mandible statistically significantly differs from the upper jaws (Table 1). The histomorphometric coefficient of bone density of the lower jaws is 3,13 ± 0,71 units of account and the upper jaws are 2,26 ± 0,82 units of account, which is more than 1.38 times on average \( p<0, 01 \).

![Fig 4: Orthopantogram of a patient (65 years old) with radicular cyst in the area of the roots of 33, 34 teeth. X-ray signs of osteoporosis according to the cortical index (MCI) Klemetti E. - C2 (indicated by an arrow) are revealed.](Image)

**Table 1:** Histomorphometric indices of bone biopsies with intact sections of the jaws of patients without violations of mineral metabolism

<table>
<thead>
<tr>
<th>Histomorphometric indices</th>
<th>Anatomical sections of the fence biopsy of intact bone tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Maxilla (n=9)</strong></td>
</tr>
<tr>
<td>Square of intertrabecular space intersection(μm²)</td>
<td>1110,57 ± 8,40</td>
</tr>
<tr>
<td>Trabecular space (%)</td>
<td>69,30 ± 2,94</td>
</tr>
<tr>
<td>Intertrabecular space (%)</td>
<td>30,7 ± 1,06</td>
</tr>
<tr>
<td>Histomorphometric coefficient of bone density (units of account)</td>
<td>2,26 ± 0,82</td>
</tr>
</tbody>
</table>

Note: \* - the statistical significance of the difference between the indices obtained in intact bone tissues of the mandible was compared with those obtained in intact bone tissues of the upper jaw.

In the 1st group of patients during histomorphometry of bone biopsy samples taken from the jaw sections adjacent to the shells of small and medium sized radicular cysts, we found in them a significantly greater trabecular density in the spongy structure compared with intact bones: the size of the trabecular space was 75, 54 ± 1,29%, the size of the intertrabecular space is 24,46 ± 1,71%, respectively, the bone density index increased to 3,09 ± 0,75 units of account \( p<0,05 \). When comparing the morphometric indices obtained during the study of the maxillary bones affected by large cysts with those in the maxillary bones adjacent to the cysts of medium to small size, a tendency towards further
consolidation of bone tissue in response to increasing compression on the side of these tumor-like neoplasms was found: the size increased trabecular space from 75,54 ± 1,29% to 76,71 ± 1,17% (p > 0,05), while the area between the trabecular space decreased from 1614,5 ± 9,3 μm² to 1537,27 ± 9,4 μm² (p > 0,05), accordingly, the histomorphometric coefficient of bone density increased from 3,09 ± 0,75 c.u. to 3,29 ± 0,86 c.u. (p > 0,05) (Table 2).

Table 2: Histomorphometric indices of bone biopsy of the upper jaws of patients with radicular cysts

<table>
<thead>
<tr>
<th>State of the studied bones</th>
<th>Group 1. Without mineral metabolism disorders (n=23)</th>
<th>Group 2. On the background of osteoporosis (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone tissue adjoining to cysts of small to medium sizes (n = 12)</td>
<td>Bone tissue adjoining to cysts of big sizes (n = 11)</td>
<td>Bone tissue adjoining to cysts of small to medium sizes (n = 10)</td>
</tr>
<tr>
<td>Square of inter trabecular space intersection(μm²)</td>
<td>1614,54±9,3 p&lt;0,05**</td>
<td>1537,27±9,4 p&lt;0,05*</td>
</tr>
<tr>
<td>Trabecular space (%)</td>
<td>75,54 ± 1,29 p&lt;0,05**</td>
<td>76,71 ± 1,17 p&lt;0,05*</td>
</tr>
<tr>
<td>Inter trabecular space (%)</td>
<td>24,46 ± 1,71 p&lt;0,05**</td>
<td>23,29 ± 1,83 p&lt;0,05*</td>
</tr>
<tr>
<td>Histomorphometric coefficient of bone density (units of account)</td>
<td>3,09 ± 0,75 p&lt;0,05**</td>
<td>3,29 ± 0,86 p&lt;0,05*</td>
</tr>
</tbody>
</table>

Notes: * - the statistical significance of difference of indicators was compared in one study group; ** - the statistical significance of the difference between the indices of bone tissue adjacent to the cysts of small and medium sizes taken in patients without violations of mineral metabolism, were compared with indices of intact bone tissues; *** - the statistical validity of the difference in the rates was compared in the various studied groups: bone tissue adjacent to small and medium cysts taken in patients with a violation of mineral metabolism, was compared with bone tissues adjacent to cysts of small and medium sizes taken in patients without violations of mineral metabolism.

The process of bone tissue sealing was more intense on the lower jaw (Table 3). Compared to intact bones, there was a statistically significant (p<0,01) increase in the number of bone trabeculae in jaw regions undergoing chronic pathological effects from radicular cysts. When comparing the architecture of the spongy bone in the biopsy of the mandibular bones taken from the areas adjacent to the cysts of big sizes, with the data obtained in the study of bone tissues adjacent to smaller cysts, a statistically significant difference was established between them: the size of the trabecular space increased from 83,29 ± 2,17% to 86,14 ± 2,15%, while the area between the trabecular space decreased from 749,16 ± 4,5 μm² to 650,82 ± 4,1 μm² (p<0,05), respectively, the histomorphometric coefficient of bone density increased from 4,98 ± 0,65 USD. to 6,21 ± 0,59 units of account (p<0,05).

Table 3: Histomorphometric indices of bone biopsy of the lower jaws of patients with radicular cysts of the mandible

<table>
<thead>
<tr>
<th>State of the studied bone</th>
<th>Group 1. Without mineral metabolism disorders (n=21)</th>
<th>Group 2. On the background of osteoporosis (n=19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone tissue adjoining to cysts of small to medium sizes (n = 11)</td>
<td>Bone tissue adjoining to cysts of big sizes (n = 10)</td>
<td>Bone tissue adjoining to cysts of small to medium sizes (n = 10)</td>
</tr>
<tr>
<td>Square of inter trabecular space intersection(μm²)</td>
<td>749,16 ± 4,5 p&lt;0,01**</td>
<td>650,82 ± 4,1 p&lt;0,05*</td>
</tr>
<tr>
<td>Trabecular space (%)</td>
<td>83,29 ± 2,17 p&lt;0,01**</td>
<td>86,14 ± 2,15 p&lt;0,05*</td>
</tr>
<tr>
<td>Inter trabecular space (%)</td>
<td>16,71 ± 1,83 p&lt;0,01**</td>
<td>13,86 ± 1,72 p&lt;0,05*</td>
</tr>
<tr>
<td>Histomorphometric coefficient of bone density (units of account)</td>
<td>4,98 ± 0,65 p&lt;0,01**</td>
<td>6,21 ± 0,59 p&lt;0,05*</td>
</tr>
</tbody>
</table>

Notes: * - the statistical significance of difference of indicators was compared in one study group; ** - the statistical significance of the difference between the indices of bone tissue adjacent to the cysts of small and medium sizes taken in patients without violations of mineral metabolism, were compared with those of intact bone tissues; *** - the statistical validity of the difference in the rates was compared in the various studied groups: bone tissue adjacent to small and medium cysts taken in patients with a violation of mineral metabolism, was compared with bone tissues adjacent to cysts of small and medium sizes taken in patients without violations of mineral metabolism.

In the 2nd group of patients with osteoporosis during histomorphometric studies, it was found that in bone biopsies taken from the jaw regions adjacent to the radicular cysts, a statistically significant decrease in the density of the spongy bone was observed, regardless of the size of these odontogenic tumor neoplasms. During the studying of microarchitecture of the maxillary bones affected by the pathological process, we found that on the background of violations of mineral metabolism, the dilution of bone tissues increases in response to the increasing influence of odontogenic cysts in different sizes: the area of the trabecular space decreases from 66,84 ± 2,18% to 64,52 ± 2,25% (p<0,05), while the area between the trabecular space increases from 2156,73 ± 9,9 μm² to 2341,88 ± 10,5 μm² (p<0,05), respectively the histomorphometric coefficient of bone density decreases from 2,01 ± 0,78 c.u. to 1,82 ± 0,39 (p<0,05) (Table 2). On the lower jaw the dilution of the spongy bone structure,
adjacent to the radicular cysts of different sizes was less pronounced, however, the general tendency to decrease the density of the investigated sections of the jaw bones under the influence of increasing intensity, of the local pathological factor was preserved. Histomorphometric indices of bone tissues adjacent to cysts of small and medium sizes were as follows: the size of the trabecular space was 73.53 ± 2.49%, the size of the intrabrabucular space was 26.47 ± 2.51%, the histomorphometric coefficient of bone density was 2.78 ± 0.87 units of account histomorphometric indices of bone tissues adjacent to large cysts: the size of the trabecular space is 72.86 ± 2.48%, the size of the intrabrabucular space is 27.14 ± 2.43%, the histomorphometric coefficient of bone density is 2.68 ± 0.92 units of account ($p > 0.05$) (Table 3).

**Discussion**

The use of modern computer technologies for histomorphometric analysis of bone biopsy specimens with the use of special methods for counting the studied parameters can greatly simplify the work and reduce the time spent for the analysis of a significant number of histopreparations (100 samples), which is consistent with the literature data [25-27]. The results of our histomorphometrical studies of jaw bones obtained from the sites subjected to destructive effects of radicular cysts suggest that, in addition to the local pathological factor, bone remodeling is largely influenced by the general state of mineral metabolism in patients. Under the conditions of the experiment, it was revealed that systemic bone metabolism disorders occur on the background of osteoporosis, morphological changes in the trabecular structure of the tubular (femoral bones) and flat bones (lower jaw) are expressed [28,30]. In elderly women, the optical density of the bones of the alveolar branches of the jaws is lower than in males [31]. The changes we found in the microarchitecture of jaw bones in patients with osteoporosis confirm the influence of age factor on this process. In patients without violations of mineral metabolism, jaw bones located in the area of radicular cysts differ in their structure from other sites. However, in literary sources there is contradictory information about the density of bones directly in the areas of defect [30,32]. Some authors [31] believe that under the influence of radicular cysts there is a rarefaction of adjacent bone tissue, others that it has a more dense structure [33]. We support the opinion of the aforementioned author that one of the reasons for changing the morphological structure of the spongy substance of the jaw bone (thickening of the trabecula, reducing the intertrabecular space) is its compensatory reconstruction, which takes place in response to increasing pressure of the growing volume of cystic content, which is a morphological manifestation of local adaptational body reaction. On the background of systemic disorders of mineral metabolism, the bone remodeling process is disturbed, the higher activity of the resorption processes in the jawbone tissue appears, which may affect the course of the reparative osteoregeneration [32, 33, 34].

**Conclusions**

In response to the pathological effects of radicular cysts, irrespective of their size, an adaptive-compensatory reconstruction of the jaw bones occurs that morphologically manifests as a sealing of their trabecular structure, and the decrease of the intertrabecular space. The state of mineral metabolism in patients affects the osteo-regenerative potential in jaw bones. On the background of violations of mineral metabolism (osteoporosis) the adaptive osteo-regenerative potential of jaw bones in the affected areas of this tumor-affected neoplasm decreases, an imbalance of bone remodeling processes occurs, which morphologically manifests itself by an increase in intertrabecular space, a decrease in the thickness of trabeculae and bone density. This creates favorable conditions for the appositional growth of the radicular cysts.

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