Bisphosphonate-associated jawbone osteonecrosis: a correlation between imaging techniques and histopathology
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Objectives. Recently, jawbone osteonecrosis has been reported as a potential adverse effect of bisphosphonates administration. This paper considers and highlights histopathologic and radiologic features of this condition.
Study design. Eleven patients, owing to unresponsiveness to conservative treatment and uncontrollable pain, underwent surgical resection of diseased jawbone after extensive hyperbaric oxygen therapy. A thorough clinical, laboratory, and imaging study was performed. Surgical specimens underwent histopathologic and immunohistochemical evaluation.
Results. Computerized tomography (CT) scans showed increased bone density, periosteal reaction, and bone sequestration in advanced stages. With magnetic resonance imaging (MRI), exposed areas showed a low signal in T1- and T2-weighted and inversion recovery images, which suggests low water content and is histopathologically correlated with paucity in cells and vessels (osteonecrotic pattern). Unexposed diseased bone was characterized by T1 hypointensity and T2 and IR hyperintensity, which suggests high water content and inflammation, associated with hypercellularity, osteogenesis, and hypervascularity (osteomyelitic pattern).
Conclusions. Diseased bone extends beyond the limits of the bone exposed in the oral cavity. Histopathologic examination correlated well with CT and MRI, which are the choice for the evaluation of bisphosphonate-associated jawbone osteonecrosis.

Aminobisphosphonates play a central role in the treatment of malignancy-associated hypercalcemia and for the prevention of bone fractures in patients with metastatic bone disease or multiple myeloma.1-3 Among aminobisphosphonates, pamidronate and zoledronate have shown the most consistent effects for the treatment of bone metastases in cancer, with zoledronate being more potent in vitro than pamidronate.4 Aminobisphosphonates inhibit osteoclasts at different stages, binding selectively to hydroxyapatite and accumulating in sites of active bone remodeling. Once bisphosphonates are stored in bone, their release is dependent on the rate of bone remodeling.5 In addition, aminobisphosphonates have antiangiogenic properties both in vitro and in vivo.6,7

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Recent studies performed in cancer patients suggest that bisphosphonates, in some cases, may be responsible for jawbone lesions.8-10 Clinically, these lesions appear as nonhealing exposed bone areas, which can be accompanied by fistulization, purulent discharge, and pain.11-14 The current nomenclature for bisphosphonate-associated jawbone lesions reflects the prevailing hypothesis that such a condition is a form of osteonecrosis.11,15,16 However, the pathogenesis of jawbone disease in patients receiving bisphosphonates is largely unknown, and the biologic mechanisms by which bisphosphonates are responsible for bone remodeling and angiogenesis impairment in human jaws are still uncertain. Furthermore, very few data are available in the literature regarding histopathologic and radiologic features. In the present paper, we have addressed and reported data regarding histopathology and radiologic features of bisphosphonate-associated jaw osteonecrosis that occurred in a series of our patients.

METHODS

Patients

The study group (Table I) consisted of 11 patients (4 men and 7 women) whose ages ranged from 49 years to 84 years (mean age 75.9 years). Five patients (45.5%) were affected by multiple myeloma, 4 (36.4%) by breast cancer, 1 (9%) by prostate cancer, and 1 (9%) by kidney cancer. All of them received intravenous amnobisphosphonates for a mean period of 37.3 months (range 9 months to 60 months); in particular, 1 patient received pamidronate, 1 received zoledronate, and the remaining 9 were first administered with pamidronate and then with zoledronate. Eight patients also received concomitant therapy for the underlying malignancy as detailed in Table I. All patients developed jawbone osteonecrosis. It occurred in 2 cases without apparent precipitating events, and in 8 patients a history of tooth extraction was reported, whereas in 1 patient a trauma from poorly fitting removable denture was identified; the mandible and the maxilla were affected in 8 cases (73%) and 3 cases (27%), respectively. Owing to unresponsiveness to conservative treatment (antibiotics / superfical surgical debridement) and uncontrollable pain, in accordance with the oncologists and with patient’s consent, it was decided to perform surgical resection of the affected area of jawbone. Preoperatively, a thorough clinical, laboratory, and imaging technique study of patients was performed.

Radiologic evaluation

Patients underwent panoramic radiograph, spiral computerized tomography (CT), and magnetic resonance imaging (MRI) of the jaws. The CT scans (CT Brilliance 6 slices; Philips), based on 1-mm to 2-mm axial slices parallel to the hard palate, were evaluated with wide windowing levels. True cross-sectional images of the mandible and maxilla were obtained from CT data using multiplanar reformation software (Denta-scan®). No contrast media were used for CT imaging.

The MRI images of the jaws were obtained by using a magnet of 1.5 T (Magnetom Symphony 1.5 Tesla; Siemens). The MRI scans consisted of T1- and T2-weighted spin-echo axial and inversion recovery (IR) images, 4 mm in thickness and at 0.4-mm intervals.

Treatment

After discontinuation of bisphosphonates therapy, a 10-day cycle of antibiotic therapy (amoxicillin-clavu-
lanate and metronidazole) was prescribed. Furthermore, all patients underwent 25 preoperative sessions of hyperbaric oxygen therapy (HBO) (2.5 ATA, 90 minutes), and then a complete resection of the diseased bone with jaw reconstruction if needed was performed. Postoperatively, patients received a 10-day cycle of antibiotic therapy and 25 sessions of HBO.

Histopathology and immunohistochemistry

The specimens of the 11 patients who underwent bone resection were histopathologically evaluated. Every specimen was cut to obtain multiple bone sections consisting of the entire cross-sectional area of the resected jaw. The periosteal layer was included. Sampling for histopathologic evaluation was performed from areas with bone exposed in the oral cavity, from each margin of bone resection, and from areas of diseased bone but without intraoral exposure (Fig. 1).

Specimens were fixed in 4% formaldehyde, decalcified in Kristensen solution for 24-48 hours, and embedded into paraffin blocks. Tissue blocks were cut into 5-μm serial sections, stained with hematoxylin and eosin, and photographed (Leica DFC 280; Leica Microsystems Imaging Solution, Cambridge, U.K.). Immunohistochemical staining was used to evaluate blood vessels within specimens. In particular, specimens were subjected to immunoperoxidase stains (CD34 antibody clone QBEND 10, dilution 1:40; Immunotech, Marseilles, France) according to the manufacturer’s instructions.

RESULTS

Clinically, bone disease presented as a nonhealing extraction socket or areas of extensive bone exposure (9 patients) with variable purulent discharge (Fig. 2, A). Gingival fistulae were sometimes observed (Fig. 2, B-E) as well as cervical cutaneous fistulae in advanced bone disease (Fig. 2, F).

Radiologic findings

Panoramic radiographs showed delayed or absent bone remodeling of the extraction sockets (Fig. 3, A). Diffuse radiopaque areas were seen between radiolucent areas in advanced bone disease.

The CT scans showed large areas of increased medullary bone density extending beyond the limits of the bone exposed in the oral cavity (Fig. 3, B). Periosteal reaction and bone sequestration were predominant in advanced stages of disease (Fig. 3, C). Maxillary involvement was always associated with purulent sinusitis. The entire cross-sectional area of the jaw was involved in all cases (Fig. 3, D).
The MRI showed 2 patterns of bone disease in the studied cases. In the first pattern, characteristic of exposed areas of diseased bone, low signal was observed in T1- and T2-weighted images, with a relatively low signal in IR images, which was suggestive of low water content. The second pattern, typical of unexposed diseased bone, was characterized by T1 hypointensity and T2 and IR hyperintensity, suggesting high water content (Fig. 3, E-G). In advanced disease with extensive bone exposure, the 2 patterns coexist, with the second one being always present in a peripheral distribution (Fig. 3, E-G).

**Histopathologic findings**

The specimens obtained from the areas of exposed bone in the oral cavity were characterized by a large amount of nonvital bone, with rough margins and empty lacunae. Osteoblasts and multinucleated osteoclast-like cells were almost absent and vessels were scanty, no sign of bone remodeling was seen. Fungal contamination of the exposed necrotic bone was found in most cases. In contrast, the specimens obtained from the areas of diseased but unexposed bone were characterized, in all of the resected jaws, by hypervascular fibrous tissue and inflammatory infiltrate filling large intertrabecular spaces. Areas of lamellar bone with empty lacunae coexisted with areas of lamellar bone containing viable osteocytes surrounded by woven-fiber bone. In all cases, woven bone largely prevailed over lamellar bone (Figs. 4, A and B). At sites where osteogenesis was evident, bone trabeculae were rimmed by osteoblasts (Fig. 4, C). The formation of new bone was also detected below the periosteum (Fig. 4, D). In all of the specimens, intertrabecular spaces were rich in blood vessels of different size and multinucleated osteoclast-like cells detached from the bone surface (Fig. 4, E). The specimens obtained from the margins of resection showed normal bone structure and vasculature in 8 out of 11 jaws (Fig. 4, F). Three patients showed moderate signs of osteomyelitis at one margin of bone resection.

The vasculature of the affected tissue was constituted by a mixture of capillaries, round venule-like vessels, and small arterioles (Fig. 5, A). The capillaries had normal histologic features. Most CD34-positive arterioles within the fibrous tissue had a rounded or slightly cuboidal endothelium (Fig. 5, B), very similar to the picture observed in chronic osteomyelitis (Fig. 5, F). Most vessels contained pink proteinaceous material in their lumen and red blood cells agglutinated into a roleaux, which is probably a post-biopsy fixation artifact (Fig. 5, C and D).

**DISCUSSION**

In this study we evaluated and reported the radiologic features in a series of jawbones resected from patients affected by bisphosphonate-associated jaw-
bone disease; furthermore, we have attempted to define their related histopathologic features.

Bisphosphonate-associated jawbone disease encompasses a number of alterations involving macroscopic anatomy of jawbones, bone structure, bone cell populations and function, and vasculature. Modifications in bone morphology are clearly visible both from a clinical point of view and at CT scan (Figs. 2 and 3). It should be emphasized that the diseased bone goes much beyond the limits of the clinically exposed bone areas, and therefore clinical examination is not at all reliable in assessing lesion extent. Both CT scan and MRI are adequate in evaluating bone involvement, as confirmed by previous studies. The CT features of jawbone disease resembled those of a chronic osteomyelitic process, with predominant signs of osteosclerosis and periosteal reaction; osteolysis may be also evident, in accordance with other authors.

Diseased bone is always involved in all of its cross-section, including the periosteum, as confirmed by both cross-sectional reformatted images and histopathologic sections. In addition, diseased bone exhibits different features resulting from being exposed in the oral cavity or not. This was not reported in other studies, but in the
Table II. Histopathology of bisphosphonate-associated osteonecrosis and imaging features correlates

<table>
<thead>
<tr>
<th></th>
<th>Exposed affected bone</th>
<th>Unexposed affected bone</th>
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<tbody>
<tr>
<td><strong>CT scan</strong></td>
<td>Increased medullary bone density</td>
<td>T1 hypointensity</td>
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<tr>
<td></td>
<td>Thickening of trabeculae</td>
<td>T2 and IR hyperintensity</td>
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<tr>
<td></td>
<td>Periostal reaction</td>
<td>Large inter trabeculae spaces</td>
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<td></td>
<td>Bone sequestration (advanced stages)</td>
<td>Hypervascular fibrous tissue</td>
</tr>
<tr>
<td><strong>MRI</strong></td>
<td>Low T1- and T2-weighted signal</td>
<td>Inflammatory infiltrate</td>
</tr>
<tr>
<td></td>
<td>Low signal in IR</td>
<td></td>
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<tr>
<td><strong>Histopathology</strong></td>
<td>Empty</td>
<td></td>
</tr>
<tr>
<td>Inter trabecular spaces</td>
<td>Empty</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Empty</td>
<td></td>
</tr>
<tr>
<td><strong>Cell populations</strong></td>
<td>Osteoblasts and multinucleated osteoclast-like cells almost absent</td>
<td>Osteoblasts rim new bone trabeculae</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Multinucleated osteoclast-like cells detached from the bone surface</td>
</tr>
<tr>
<td><strong>Vasculature</strong></td>
<td>Almost absent</td>
<td>Capillaries</td>
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<tr>
<td></td>
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<td>Round venule-like vessels</td>
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<td></td>
<td></td>
<td>Small arterioles (rounded or slightly cuboid endothelium)</td>
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<tr>
<td></td>
<td></td>
<td>Pink proteinaceous endoluminal material</td>
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<td></td>
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<td>Red blood cells agglutinated into a roleaux</td>
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IR, Inversion recovery.

In the present series it is evident at MRI investigation and is further confirmed at a microscopic level by histopathologic examination. The first MRI pattern described above, suggestive of low water content, is typically associated with the areas of bone exposed in the oral cavity, whereas the second MRI pattern, suggestive of edema and inflammation, characterizes unexposed areas. These MRI patterns have specific histopathologic correlates (Table II). Paucity in cells and vessels is typically seen in the areas of exposed bone, making up the picture of necrosis; on the other hand, diseased but unexposed bone is characterized by hypercellularity, hypervascularity, and osteogenesis: histopathologic aspects that are also characterizing but not exclusive features of osteomyelitis. In particular, new bone trabeculae rimmed by osteoblasts and delimiting large inter trabecular spaces filled by hypervascular fibrous tissue and inflammatory infiltrate well fit the histopathologic picture of a chronic osteomyelitic process, but the same does not apply to multinucleated osteoclast-like cells. They are typically detached from the bone surface (Fig. 4, E), whereas in chronic osteomyelitis the osteoclasts are attached to bone and show erosive activity (Fig. 5, E). This detachment is of special interest because it gives us the opportunity to hypothesize that these multinucleated osteoclast-like cells are effectively osteoclasts whose alterations in cellular morphology can be attributed just to loss of bone contact; on the other hand, their detachment from bone may well reflect the functional impairment caused by bisphosphonates. In fact, it is well known that a key early event for bone resorption to be initiated is the attachment of the osteoclast to the bone matrix via integrins (mainly αVβ3), which results in the formation of the sealing zone that enables the osteoclast to isolate the subcellular resorptive microenvironment.

Aminobisphosphonates inhibit the mevalonate pathway and prevent post-translational prenylation of guanosine triphosphate–binding proteins, including Ras, Rac, and Rho; this causes unbalance in function of these proteins which regulates the assembly of focal adhesions and membrane ruffling and are involved in bone resorption by regulating cytoskeletal organization in osteoclasts. These events lead to alterations in cellular morphology and function and end up with the observed detachment of osteoclasts from bone, which may have a role in light of new proposed pathogenetic theories.

Furthermore, we found histologic evidence of osteoclasts’ inactivation in bisphosphonate-associated jaw-bone disease in all of the studied specimens. It is well known that bone homeostasis depends on the maintenance of a balanced activity between osteoblasts and osteoclasts; when bone resorption is decreased through inhibition of osteoclasts, the bone matrix is not degraded and nonvital bone accumulates. Therefore, the presence of lamellar bone with empty lacunae seen within the osteomyelitic pattern may be explained by the accumulation of nonvital bone due to dysfunction of osteoclasts.

Blood vessel abnormalities involve their number, type, and structure; they are confined to the unexposed areas, because in the exposed ones vasculature is almost absent whereas the resection margins showed normal bone and vessels. Whether these vessel abnormalities are related to inflammation or impaired angiogenesis and vascularization remains to be determined. In addition, in the present series patients preoperatively underwent HBO therapy; it is well known that hyperbaric oxygen possesses significant angiogenic potential and therefore may have con-
tributed to the hypervascularization seen in the osteomyelitic part of the resected jawbones. On the other hand, hypervascularization may seem not to fit with bisphosphonates’ antiangiogenetic effect, but discontinuation of bisphosphonates in the present patients may have had a role in minimizing this effect.

Vascularization has been indicated among etiopathogenetic factors of bisphosphonate-associated bone disease; it has been postulated that the mandible may be especially susceptible to it because of its terminal vascularization. In the present study, in agreement with others, most bone lesions associated with bisphosphonate therapy were localized in the mandible (73% of cases); however, no histopathologic differences in vessels appearance were found between mandible and maxilla.

CONCLUSIONS
In conclusion, histopathologic examination correlated well with CT and MRI, which are the choice for the evaluation of bisphosphonate-associated jawbone osteonecrosis; in particular, bone alterations exhibit great variation between exposed and unexposed areas. The former express the features of an osteonecrotic process, whereas the latter resemble in many aspects an osteomyelitic one; if there is a continuous progression from the osteomyelitic to the osteonecrotic pattern, as observed in the avascular osteonecrosis of epiphyseal bone, it cannot be determined with the present data, probably because bone resection was decided when the bone exposure was sufficiently advanced and the necrotic bone had already separated from its osteomyelitic bed. MRI may have a special value in assessing and characterizing these two different patterns and probably in defining the real extent of disease.

REFERENCES

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