Histopathologic and Histomorphometric Analysis of Irradiation Injury in Bone and the Surrounding Soft Tissues of the Jaws

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Purpose: Surgery of irradiated tissue has an increased complication rate because of the development of hypovascular, hypocellular, and hypoxic tissue. This study was undertaken to perform histopathologic and histomorphometric analyses of irradiation tissue injury in bone and the surrounding soft tissues.

Material and Methods: The histopathologic findings of 40 human mandibular bones and the surrounding soft tissue specimens obtained from different patients who underwent surgical procedures for treatment of osteoradionecrosis of the jaws were reviewed.

Results: Histopathologic examination showed 7 processes in the following order of appearance: hyperemia, endarteritis, thrombosis, cell loss, hypovascularity, increase of fat in the bone marrow cavity, and fibrosis. Histomorphometric analysis showed significant hypocellularity (P = .007), hypovascularity (P < .001), and fibrosis (P < .001) in irradiated specimens compared with control specimens.

Conclusion: These results showed that radiation injuries affect the bone and surrounding soft tissues. However, the irradiation-induced injuries, such as cellular loss (hypocellularity) and fibrosis, were more expressive in bone tissue than in the surrounding soft tissues.

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Surgery and radiation have been the standard treatment for advanced cancers of the head and neck. Osteoradionecrosis (ORN) of the jaws is one of the most severe late complications of radiation therapy.[1-4] It is defined as exposed irradiated bone tissue that fails to heal over a period of 3 months without signs of primary tumor, recurrence, or metastatic disease.[5,6] Marx[5] found that ORN was characterized by progressive obliterative endarteritis and the development of hypovascular, hypocellular, and hypoxic tissues ("3-H concept"), in which there is an imbalance between cell death and collagen breakdown exceeding the normal homeostasis of cell repair and collagen synthesis, which can undergo necrosis spontaneously or in response to trauma. Micro-organisms play only a contaminating role in ORN.[7]

A new theory was proposed by Delanian and Lefaix[8] based on the radiation-induced fibro-atrophic process to explain the damage to normal tissues, including bone and soft tissues, constituting a local and unavoidable sequela to high-dose radiotherapy. They described 3 distinct phases. An initial prefibrotic phase is characterized mainly by changes in endothelial cells. Cytokines, released in response to injury,

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attract leukocytes to the site of injury that trigger an acute inflammatory response characterized by increased vascular permeability with local edema formation, destruction of endothelial cells, and an association with vascular thrombosis. This can lead to necrosis of microvessels, local ischemia, and tissue loss. Currently, it is not fully understood how fibroblasts are activated to become myofibroblasts. The second constitutive organized phase is characterized by the radiation-induced fibro-atrophic tissue composed of fibroblasts and the extracellular matrix. The reactive oxygen species–mediated release of cytokines results in a high density of active fibroblasts (myofibroblasts) characterized by high rates of proliferation and secretion of abnormal products in a disorganized extracellular matrix with a decreased capacity to degrade these components. The third phase, late fibroatrophy, can last for decades after radiotherapy and consists of poorly vascularized and cellular tissue with few fibroblasts and a dense extracellular matrix. An attempted tissue remodeling occurs with the formation of fragile healed tissues with a high inherent risk of reactivated inflammation in the event of local injury.[8]

On histopathologic evaluation, hyperemia, inflammation (endarteritis), thrombosis, cellular damage, hypovascularity, and fibrosis are observed after radiation exposure.[9] In general, hyperemia, endarteritis, cellular damage, and vascular thrombosis begin soon after radiation exposure and is maintained for an additional 6 months. Hypo-vascularization and fibrosis occur 6 to 12 months after radiotherapy and represent the end stage of radiation tissue injury.[9]

Although specific treatments for ORN have not been well defined, surgical approaches are necessary in most cases, from conservative intervention to invasive surgery.[1,10] ORN requires treatment when there is pain, impaired function, or active infection. There are limited options for treatment of refractory and persistent cases of ORN other than complete bone surgical resection or multimodal therapy in which hyperbaric oxygen therapy (HBO) is combined with surgical resection of necrotic bone. However, the exact physiologic mechanisms of HBO are not completely understood. No consensus exists regarding its prophylactic use, its mechanism of action, and its effectiveness in the prevention and treatment of ORN.[11-22] There is some evidence that HBO improves outcome to prevent the development of ORN after dental extraction in patients irradiated for head and neck tumors.[23] There also is some evidence that HBO might improve outcome in irradiated patients who need resection and reconstruction surgery.[24] Two recent randomized controlled trials concluded that HBO was of little or no benefit for the treatment of ORN, especially in patients who underwent free tissue transfer.[25,26] A recent Cochrane meta-analysis suggested that for patients with late radiation tissue injury, HBO therapy is associated with an improved outcome.[19] In summary, the efficacy of HBO in the management of irradiated patients has been contradictory and there is a lack of randomized controlled double-blinded trials and further studies are needed to evaluate the indication of this therapy.[15-22] Based on the new theory of radiation-induced fibro-atrophic tissue in which the main event in the progression of ORN is the activation and dysregulation of fibroblastic activity that leads to atrophic tissue within a previously irradiated tissue, some clinical studies have suggested the use of pentoxifylline combined with tocopherol (vitamin E) to prevent and treat ORN.[8,27]

Despite the better understanding of the pathogenesis of ORN and advances in its treatment modalities, some important clinical dilemmas remain. Marx and Johnson[9] mentioned the uncertainty about the risk of ORN as a function of time since radiation and as a result of oral and maxillofacial surgical procedures. The data from their tissue biopsy study showed that after 6 months, the greater extent radiation tissue injuries indicated a higher risk for ORN. They found evidence of progressive vascular damage causing endarteritis, thrombosis, and fibrosis in animal and human histologic studies.[9] In addition, they believed that vascular damage is responsible for many of the late effects observed in ORN tissues.[9] However, this tissue biopsy study was based on qualitative histology. Few studies thus far have been undertaken to elucidate the importance of the relation between radiation tissue injury and the risk for ORN and to create clinical guidelines for irradiated patients. Studies in this field could improve the management of patients with head and neck cancer before and after irradiation.

The purpose of this study was to perform histopathologic and histomorphometric analyses of the effects of irradiation injury on mandibular bone and the surrounding soft tissues.

Material and Methods

This study was approved by the Cancer Hospital Center institutional review board and all participants signed an informed consent agreement. The study subjects consisted of 40 irradiated specimens obtained from different patients who were treated for ORN. Fifteen nonirradiated mandibular bone and surrounding soft tissue samples obtained from different patients who were treated for head and neck tumors were used as control specimens. Irradiated and control (nonirradiated) specimens were similar for age, gender, and race, and there were no major differences between them. The diagnostic criteria for ORN was a slow-healing radiation-induced necrosis of bone with

associated soft tissue necrosis for at least 3 months with the absence of local primary tumor necrosis, recurrence, or metastatic disease.

Radiation therapy consisted of external beam radiation in all irradiated specimens. The specimens received megavoltage delivery (linear accelerator, 4 MeV). The average total dose of radiation for these specimens was 5,942 cGy (range, 5,040 to 7,040 cGy). The average dose rate per day for these specimens was 174 cGy (range, 109 to 200 cGy). The average time of specimen evaluation after completion of radiation treatment was 22.1 months (range, 2 to 108 months).

Individual cases were excluded from the study if they had a history of local or systemic disorders related to arterial changes, such as diabetes mellitus, rheumatic fever, arterial vasculitis, or collagen diseases. Furthermore, patients who had local diseases of the mandible, such as tumors, tumor-like lesions, and cysts, were excluded by histopathologic examination. Patient records and clinical follow-up provided the following information: age, gender, race, initial tumor diagnosis and staging, treatment sequence, total radiation exposure, period of radiation therapy, and date of specimen evaluations.

PREPARATION OF SPECIMENS AND HISTOPATHOLOGIC EXAMINATION

All bone and soft tissues specimens had been fixed in formalin and embedded in paraffin. Bone specimens were decalcified in a solution of 10% buffered formalin with 10% ethylenediaminetetra-acetic acid. Five-micrometer-thick sections were obtained from the original paraffin blocks and then stained with hematoxylin and eosin for light microscopic histopathologic and histomorphometric observations. The histopathologic and histomorphometric examinations were performed in a blinded manner by 2 pathologists. The amount of hyperemia, endarteritis (inflammation), thrombosis, and fat in the bone marrow cavity were categorized as abundant, moderate, rare, or none for each specimen. Serial sections were used for immunohistochemical evaluation.

HISTOMORPHOMETRIC MEASUREMENTS

Histomorphometric measurements were performed with a computer-supported histometric system consisting of a microcomputer and software program (IMAGELAB, São Paulo, Brazil) for processing the microscopic appearance. Measurements were performed using an objective of ×10 and a zoom of 2.5. Histomorphometry of cellularity and fibrosis was measured and calculated for 3 areas with greater vascular density. The mean percentages for the mentioned parameters were calculated for each area in the irradiated specimens and compared with the mean value for the control specimens. Areas of inflammation and necrosis were excluded.

IMMUNOHISTOCHEMISTRY PROTOCOL FOR CD34

Endothelial vascular immunostaining was performed using the streptavidin, biotin, and immunoperoxidase (DAKO, Hamburg, Germany) method. Deparaffinized and dehydrated 5-μm-thick sections were incubated with 3% H_2O_2 in distilled water for 15 minutes to block endogenous peroxide activity. Antigen retrieval was performed with a pressure-cooking method in sodium citrate buffer 0.1 mol/L (pH 6.0).[28] After pretreatment, sections were blocked with 10% normal goat serum and Tris-buffered saline (TBS) for 15 minutes and then incubated with monoclonal antibody QBEnd10 to CD34 (DAKO) at a 1:50 dilution for 1 hour at room temperature. The secondary biotinylated antibody and the streptavidin-and-peroxidase conjugate were applied according to the manufacturer's instructions (Super Sensitive System, BioGenex, San Ramon, CA).[28] A chromogenic precipitate was obtained by immersing the sections for 10 minutes in a ready-to-use AEC substrate solution (DAKO). After counterstaining with Mayer hematoxylin, the sections were cover-slipped with glycerol gelatin. All incubations were performed at room temperature and all washings between incubations were performed in TBS.

EVALUATION OF ENDOTHELIAL MARKER CD34 IMMUNOREACTIVITY

Vessels were counted according to a standard in which 3 areas with dense vascularity were identified and selected in each tissue section under low-power magnification (×100). In each of these "hot spots," microvessels (capillaries and small venules) were counted at ×400 magnification. Any brown-staining endothelial cell or endothelial cell cluster that was clearly separate from adjacent microvessels and other connective tissue elements was considered a single countable microvessel.[29] Vessel lumens, although usually present, were not necessary for a structure to be defined as a microvessel, and red blood cells were not used to define a vessel lumen.[30] Areas of inflammation and necrosis were excluded. The ratio of vascular area per bone or soft tissue area was estimated by point vessel counting using an integrating 25-point eyepiece (integration plate, ×10; Carl Zeiss GmbH, Jena, Germany) on a ×400 field (×40 lens and ×10 eyepiece, 0.616 mm^2 per field area). Vessels coincident with the grid intersection points were counted. Three fields of higher vascular density previously identified in a ×100 magnification image were analyzed in each section, making up a total of 100 points. The

percentage of vascular area was calculated as: ([number of coincidences/total of counted points] × 100).

STATISTICAL ANALYSIS

All statistical analyses were performed with SPSS 10.0 for Windows (SPSS, Inc, Chicago, IL). Ninety-five percent confidence intervals were calculated for specimen groups. Statistical differences between mean percentages of cellularity, fibrosis, and vascular density were determined by the Student *t* test for unpaired observations. The Pearson correlation test was used to evaluate linear correlation between the mentioned parameters and time from radiotherapy in irradiated specimens with and without ORN. In all tests, α errors equal to or less than to 5% were considered significant.

Results

The histopathologic examination showed 7 processes: hyperemia, endarteritis, thrombosis, hypocellularity, loss of vascular content (hypovascularity), increase of fat in the bone marrow cavity, and fibrosis. In general, hyperemia and endarteritis were early effects of radiation and observed for up to 6 months after radiotherapy (Fig 1A, B). Thrombosis was seen only years after radiation and thrombi were densely fibrous (Fig 1C). Cell loss occurred rapidly after radiation and remained progressive through the years. Loss of vascular content, increase of fat in the bone marrow cavity, and fibrosis showed a linear relation with time after radiation and were considered the end stage of radiation tissue injury (Fig 1D-F).

There were significant differences between the mean cellularity values of irradiated specimens and control specimens (P = .007). The mean cellularity of irradiated specimen was 1.9% (range, 0.3 to 5.2%). The mean cellularity of control specimens was 7.8% (range, 1.7 to 30.5%; Fig 2). Cell loss was greater in bone than in soft tissues. When only bone tissue was evaluated, there was a significant decrease in mean cellularity in the irradiated specimens (1.6%) compared with the control specimens (8.1%; P = .003). When evaluating only the soft tissues, there was a decrease in cell loss in irradiated specimens (2.4%) compared with control soft tissues specimens (7.6%), but this was not statistically significant (P = .168).

There were significant differences between the mean fibrosis values of irradiated specimens and control specimens (P = .027). The mean fibrosis of irradiated specimens was 72.7% (range, 57.4 to 83.7%). The mean fibrosis of control specimens was 65.7% (range, 42.0 to 88.0%; Fig 3). Similarly, fibrosis was more evident in bone than in the soft tissues. When evaluating only the bone tissue, there was a significant increase in fibrosis in the irradiated specimens (74.9%) compared with the control specimens (62.4%; P = .007). No significant difference in soft tissues was found between irradiated and control specimens (P = .796).

For time-dependent irradiation tissue injury, irradiated specimens observed at 6 months after radiotherapy exhibited significant cell loss compared with irradiated specimens in the first 6 months after irradiation (P = .049). Similarly, examination of irradiated specimens at 6 months after irradiation showed a significant decrease in mean vascular density compared with irradiated specimens in the first 6 months after irradiation (P < .001). There was a trend toward statistical significance of an increase in fibrosis in irradiated specimens at 6 months after irradiation compared with irradiated specimens in the first 6 months after irradiation (P = .072).

Mean vascular density values of irradiated specimens and control specimens showed significant differences (P < .001). The mean vascular density of irradiated specimen was 3.0% (range, 1.7 to 4.7%). The mean vascular density of control specimens was 15.1% (range, 10.3 to 24.0%; Fig 4). Six months after radiation, all specimens showed considerable hypovascularity. However, no meaningful differences in mean vascular density values were found between those in irradiated bone and those in surrounding soft tissues. A sample of CD34 immunohistochemical staining of irradiated specimen is shown in Figure 5.

Discussion

There is still uncertainty about the pathophysiology of irradiated tissue injury and the exact nature of this process is not fully understood. The basic pathophysiology of irradiation tissue injury is normal tissue cell death and sublethal normal tissue cell damage leading to a wound healing defect.[31]

In the literature, there is a scarcity of clinical studies on the evaluation of radiation tissue injury in humans. In the present study, hyperemia and endarteritis began early in the radiation sequence and persisted for up to 6 months after radiotherapy. Thrombosis appeared to be a late effect in the radiation sequence, and the thrombi became fibrous in later years compared with the early thrombi, which were basically blood clots. Hypovascularity and fibrosis began at approximately 6 months after radiation and progressively worsened over time. Similar histologic findings have been reported in other bone tissue locations.[9,32,33] In addition to these histopathologic processes, the present histologic examination identified an increase of fat in the bone marrow cavity in irradiated bone specimens. This phenomenon could be related to retardation of the normal process of bone turnover,

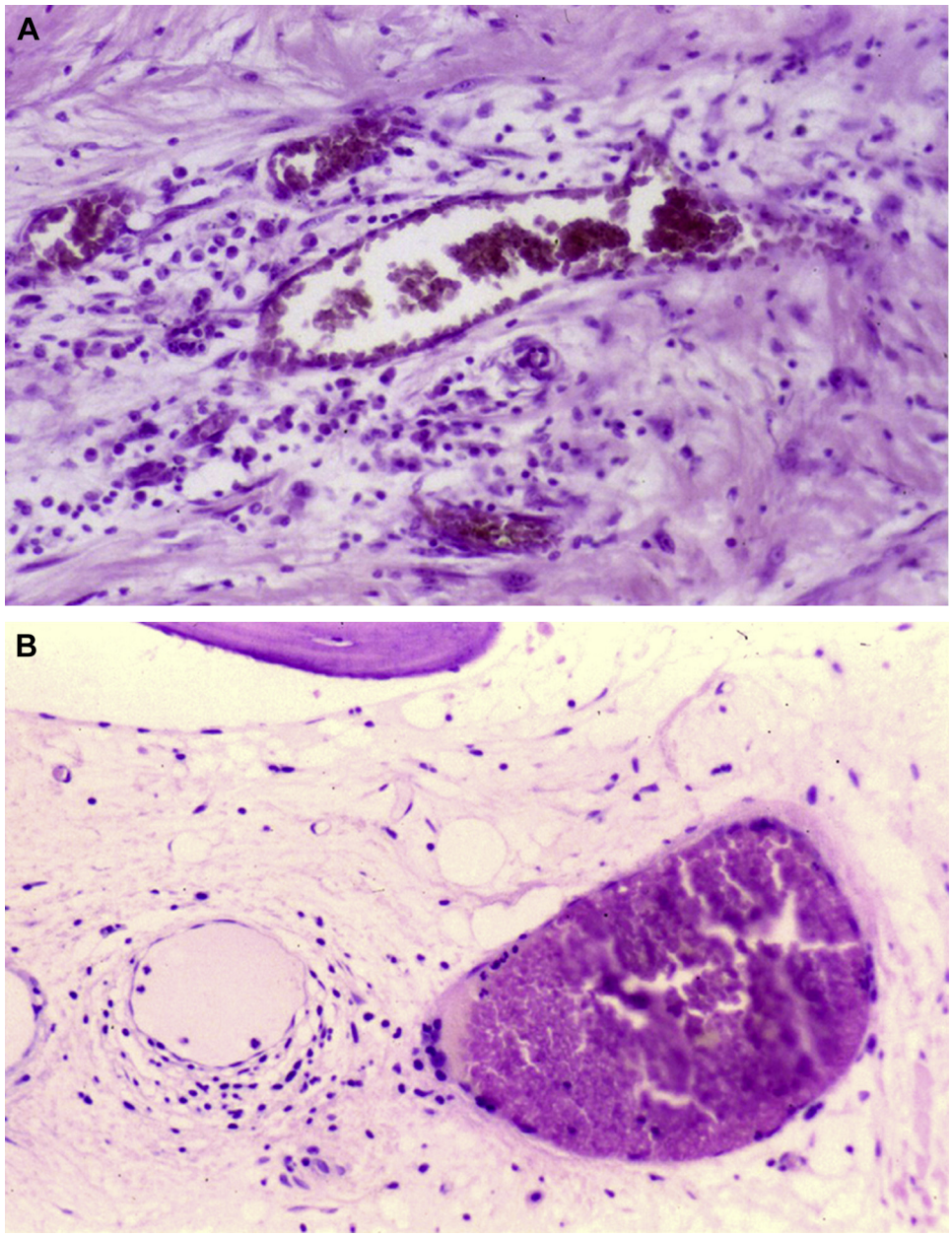

FIGURE 1. Photomicrographs of irradiated specimens stained with hematoxylin and eosin showing radiation tissue injuries. *A*, Early histologic effects of radiation exhibiting hyperemia and endarteritis (magnification, ×200). *B*, Early histologic effects of radiation exhibiting hyperemia, endarteritis, and cellular thrombosis formation. Note inflammatory cells inside the vessel lumens (magnification, ×200). **(Fig 1 continued on next page.)**

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because the mandible would be considered skeletally mature in this period. Although there is a tendency to recover from this event, bone formation appears to take a longer time to do so than does bone resorption. This large fatty bone marrow cavity would be considered an end stage of radiation tissue injury. Similar findings have been reported in studies of radiation in skeletally mature animals.[34]

Marx and Johnson[9] proposed the concept of hypovascular, hypocellular, and hypoxic tissue formation as the major problem related to irradiated wounds. The present results of measurements of irradiation tissue effects confirm the findings of these previous studies. In the present study, the measurement of cell loss (hypocellularity) in irradiated specimens indicated a significant decrease compared with the control specimens ($P = .007$). It seems that cell loss begins to a small degree just after radiation therapy and then progressively worsens throughout the years.[9] This phenomenon was observed in the present study, which

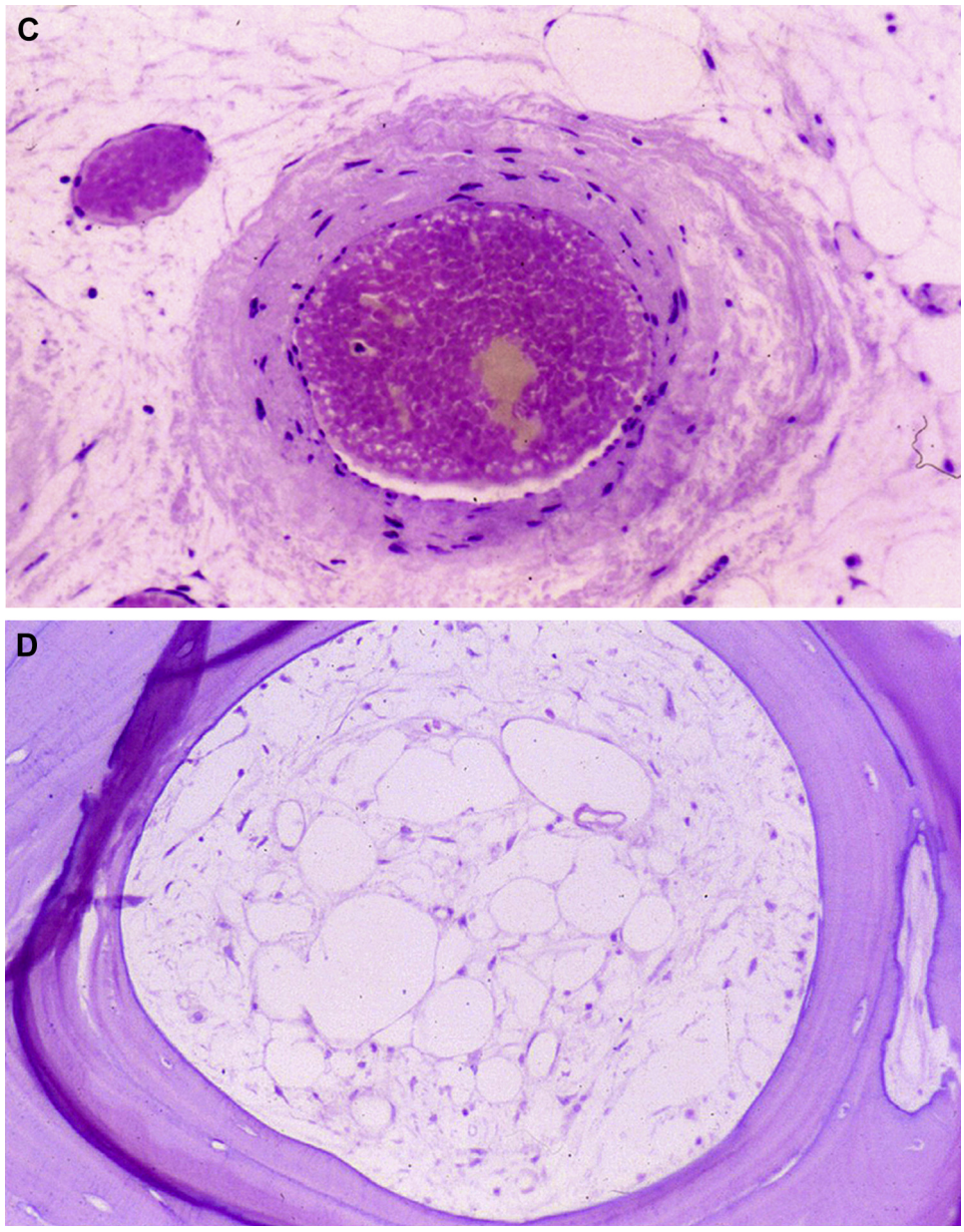

FIGURE 1 (cont'd). *C*, Early histologic effects of radiation exhibiting hyperemia, endarteritis, and cellular thrombosis formation. Note inflammatory cells inside the vessel lumens (magnification, ×200). *D*, Late histologic effect of radiation. Note the large and fatty bone marrow cavity (magnification, ×200). **(Fig 1 continued on next page.)**

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showed an increase in cell loss in irradiated specimens at 6 months after radiation (1.8%) compared with irradiated specimens in the first 6 months after radiation (2.4%), and this was statistically significant (P = .049). There has been a debate about whether the effect of irradiation is mainly on the bone or on the soft tissues. Regarding cell loss, bone specimens showed greater hypocellularity than soft tissue specimens. When considering the radiosensitivity of bone tissue, the particular physical condition with respect to the absorption of ionizing radiation must be considered. Because of its high calcium content, bone can absorb 30 to 40% more radiation than the surrounding soft tissues, and this factor could account for an increase in secondary radiation produced in bone under certain conditions.[9] Thus, the actual absorption of a given dose of irradiation is considerably greater in bone than in the overlying mucous membrane.[31] Therefore, bone cells would receive primary radiation and the maximum effect of the secondary rays.[31] Another point related to this fact is the importance of recognizing the differences in the quality of

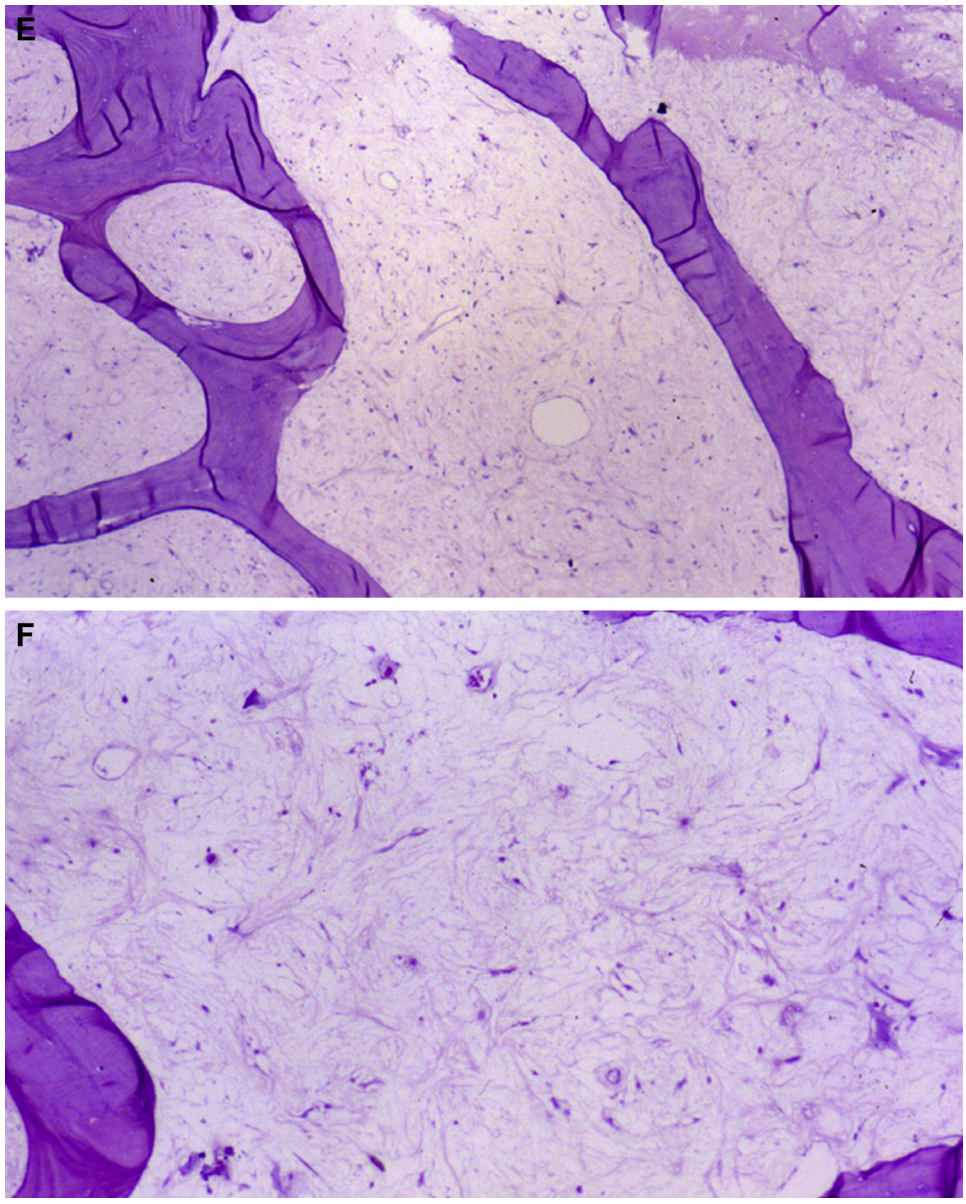

FIGURE 1 (cont'd). *E, F,* Late histologic effects of radiation. Note the characteristic hypovascular and hypocellular fibroses in the bone specimens (magnification, ×40 and ×100, respectively).

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radiation (kilovolt vs megavolt irradiation), because of the difference in absorption of radiation energy between bone and soft tissues. When using high-energy photons, the absorbed dose is approximately the same in bone and soft tissues.[27]

Hypocellularity is frequently present in irradiated tissue and is often associated with a disproportionate accumulation of collagen.[9,35] Marx and Tursun[35] compared the histopathologic features of suppurative osteomyelitis of the jaws, bisphosphonate-induced osteonecrosis of the jaws, and ORN of the jaws, and all 3 conditions evidenced the common finding of necrotic bone with empty osteocytic lacunae and Haversian and Volkmann canals, but each showed a distinctive histopathologic pattern indicating a different disease mechanism. ORN exhibited considerable marrow fibrosis, a shortage of cells, and the ghosts of old blood vessels. A recent theory for the pathogenesis of ORN has suggested that damage to bone is caused by radiation-induced fibrosis.[8] Thus, when ORN occurs in the jaw bone, there is a decrease in the bone matrix and its replacement with fibrous tissue.[8,36] The present results of the measurement of fibrosis in irradiated specimens indicated a significant increase compared with the control specimens (P = .027). Similarly, fibrosis also has been noted to appear at

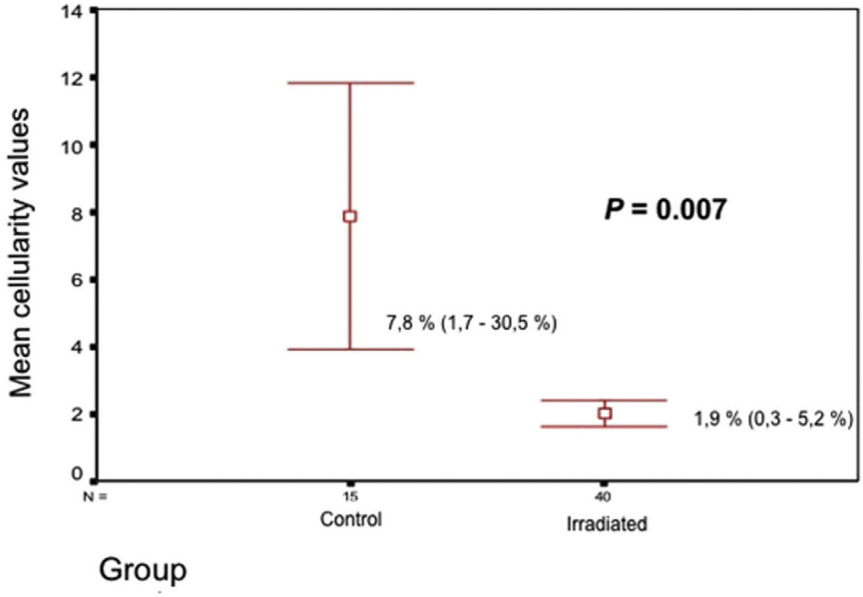

FIGURE 2. Mean cellularity values of irradiated specimens and control specimens.

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approximately 6 months and then progressively worsen, being an end stage of radiation tissue injury.[9] In the present study, fibrosis showed a great tendency to statistical significance in irradiated specimens at 6 months after radiation therapy (67.3%) compared with irradiated specimens in the first 6 months after radiotherapy (60.1%; $P = .072$). Fibrosis also was more evident in bone than in the surrounding soft tissues. As described earlier, bone might receive more radiation because of secondary rays, and recovery from this process appears to take a much longer time than for soft tissues.

The CD34 antigen is a sensitive marker of vascular endothelium and angiogenesis. The examination of CD34 has been reported in several physiologic and pathologic events.[37-39] The present study found radiation-induced hypovascularity using antihuman CD34 monoclonal antibody. These results for the measurement of vascular density in irradiated specimens indicated a significantly decreased vascular density

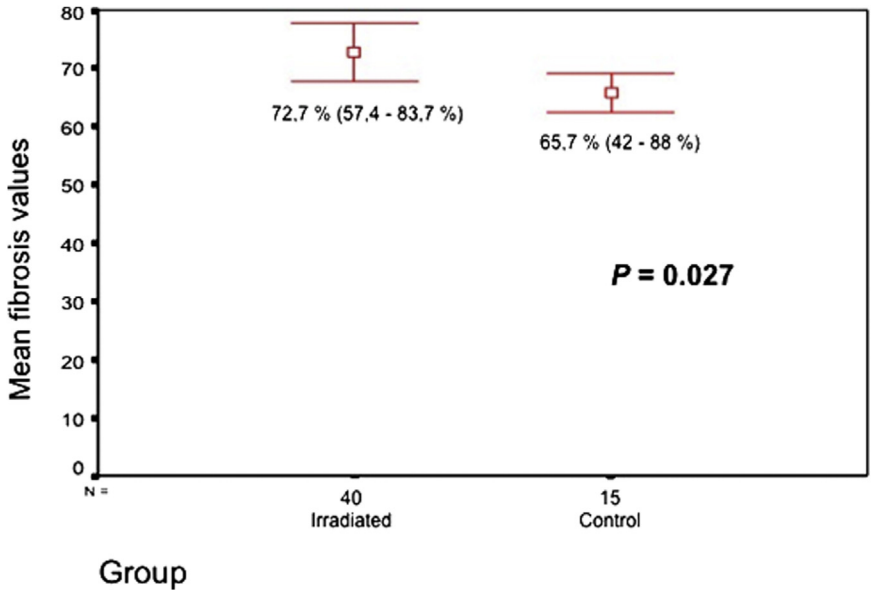

FIGURE 3. Mean fibrosis values of irradiated specimens and control specimens.

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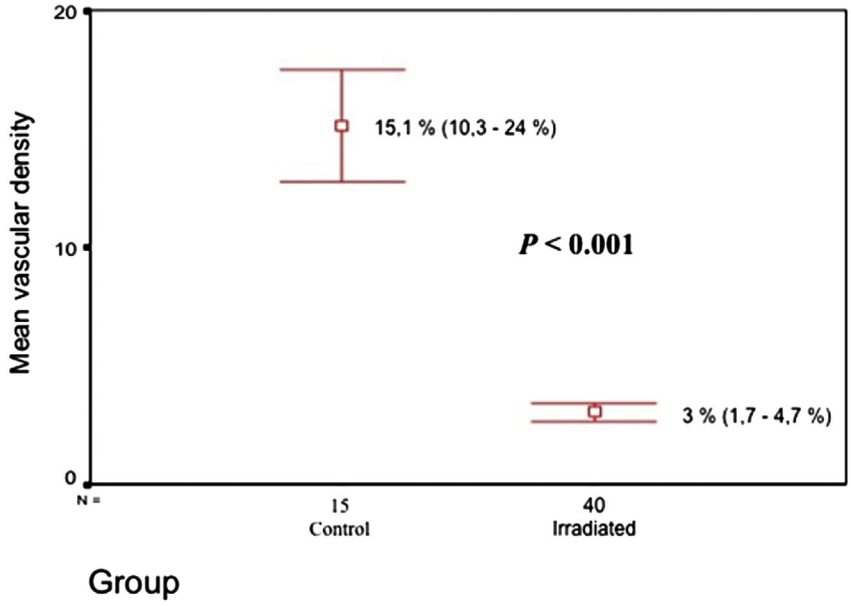

FIGURE 4. Mean vascular density of irradiated specimens and control specimens.

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compared with the control specimens ($P < .001$). A previous study has shown the inverse index of vascular density in the irradiated tissue and a direct risk for ORN.[9] The same study showed a linear relation with time indicating loss of vascular component and, therefore, loss of oxygen tension perfusion with time. In the present study, there was no evidence of spontaneous revascularization with time.

The present findings support previous findings that irradiation affects bone and the surrounding soft tissues. Although there has been a debate about whether the effect of irradiation is mainly on the bone or on the soft tissues, the present findings showed that irradiation-induced injuries, such as cellular loss (hypocellularity) and fibrosis, were more evident in bone than in the surrounding soft tissues. There also has been a discussion about whether the effect of irradiation is mainly on the cells or on the fine vasculature in irradiated tissue. The present results showed that the 2 mechanisms are evident and play an important role in the pathologic process of irradiation tissue injury.

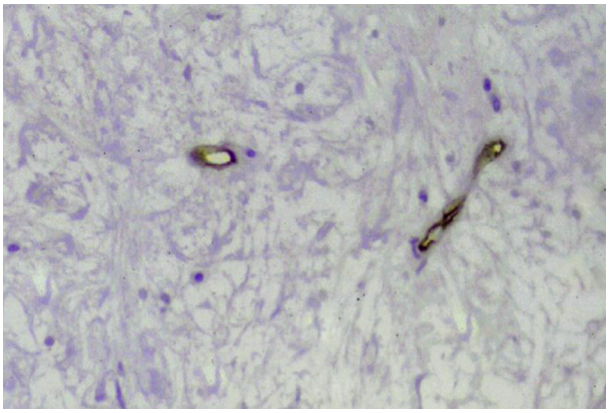

FIGURE 5. Photomicrograph of irradiated specimen. CD34 expression is scarce in the walls of blood capillaries in the bone tissue (immunohistochemical staining; magnification, ×400).

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References

1. Curi MM, Dib LL: Osteoradionecrosis of the jaws: A retrospective study of the background factors and treatment in 104 cases. J Oral Maxillofac Surg 55:540, 1997
2. Koka VN, Lusinchi A, Roland J, et al: Osteoradionecrosis of the mandible: Study of 104 cases treated by hemimandibulectomy. J Laryngolol Otol 104:305, 1990
3. Murray CG, Herson J, Daly TE, et al: Radiation necrosis of the mandible: A 10 year study. Part I. Factors influencing the onset of necrosis. Int J Radiat Oncol Biol Phys 6:543, 1980
4. Thorn JJ, Hansen HS, Specht L, et al: Osteoradionecrosis of the jaws: Clinical characteristics and relation to the field of radiation. J Oral Maxillofac Surg 58:1088, 2000
5. Marx RE: Osteoradionecrosis. A new concept of its pathophysiology. J Oral Maxillofac Surg 41:283, 1983
6. Rahman S, Maillou P, Barker D, et al: Radiotherapy and the oral environment the effects of radiotherapy on the hard and soft tissues of the mouth and its management. Eur J Prosthodont Restor Dent 21:80, 2013
7. Jegoux F, Malard O, Goyenvalle E, et al: Radiation effects on bone healing and reconstruction: Interpretation of the literature. Oral Surg Oral Med Oral Pathol 73:119, 2009
8. Delanian S, Lefaix JL: The radiation-induced fibroatrophic process: Therapeutic perspective via the antioxidant pathway. Radiother Oncol 73:119, 2004
9. Marx RE, Johnson RP: Studies in the radiobiology of osteoradionecrosis and their clinical significance. Oral Surg Oral Med Oral Pathol 64:379, 1987
10. Wong JK, Wood RE, Mclean M: Conservative management of osteoradionecrosis. Oral Surg Oral Med Oral Pathol 84:16, 1997

11. Aitasalo K, Niinikoski J, Grénman R, et al: A modified protocol for early treatment of osteomyelitis and osteoradionecrosis of the mandible. Head Neck 20:411, 1998
12. Curi MM, Dib LL, Kowalski LP, et al: Opportunistic actinomycosis in osteoradionecrosis of the jaws in patients affected by head and neck cancer: Incidence and clinical significance. Oral Oncol 36:294, 2000
13. London SD, Park SS, Gampper TJ, et al: Hyperbaric oxygen for the management of radionecrosis of bone and cartilage. Laryngoscope 108:1291, 1998
14. Curi MM, Dib LL, Kowalski LP: Management of refractory osteoradionecrosis of the jaws with surgery and adjunctive hyperbaric oxygen therapy. Int J Oral Maxillofac Surg 29:430, 2000
15. Svalestad J, Hellem S, Thorsen E, et al: Effect of hyperbaric oxygen treatment on irradiated oral mucosa: Microvessel density. Int J Oral Maxillofac Surg 44:301, 2015
16. Heyboer M III, Wojcik SM, McCabe JB, et al: Hyperbaric oxygen and dental extractions in irradiated patients: Short- and long-term outcomes. Undersea Hyperb Med 403:283, 2013
17. Feldmeier JJ: Hyperbaric oxygen therapy and delayed radiation injuries (soft tissue and bony necrosis): 2012 Update. Undersea Hyperb Med 39:1121, 2012
18. Chrcanovic BR, Albrektsson T, Wennerberg A: Dental implants in irradiated versus nonirradiated patients: A meta-analysis. Head Neck, 2015, http://dx.doi.org/10.1002/hed.23875, in press.
19. Bennett MH, Feldmeier J, Hampson N, et al: Hyperbaric oxygen therapy for late radiation tissue injury. Cochrane Database Syst Rev 5:CD005005, 2012
20. Hampson NB, Holm JR, Wreford-Brown CE, et al: Prospective assessment of outcomes in 411 patients treated with hyperbaric oxygen for chronic radiation tissue injury. Cancer 118:3860, 2012
21. Harding SA, Hodder SC, Courtney DJ, et al: Impact of perioperative hyperbaric oxygen therapy on the quality of life of maxillofacial patients who undergo surgery in irradiated fields. Int J Oral Maxillofac Surg 37:617, 2008
22. Kaur J, Hay KD, Macdonald H, et al: Retrospective audit of the use of the Marx protocol for prophylactic hyperbaric oxygen therapy in managing patients requiring dental extractions following radiotherapy to the head and neck. N Z Dent J 105: 47, 2009
23. Marx RE, Johnson RP, Kline SN: Prevention of osteoradionecrosis: A randomized prospective clinical trial of hyperbaric oxygen versus penicillin. J Am Dent Assoc 111:49, 1985
24. Bessereau J, Annane D: Treatment of osteoradionecrosis of the jaw: The case against the use of hyperbaric oxygen. J Oral Maxillofac Surg 68:1907, 2010
25. Pitak-Arnnop P, Sader R, Dhanuthai K, et al: Management of osteoradionecrosis of the jaws: An analysis of evidence. Eur J Surg Oncol 34:1123, 2008
26. Annane D, Depondt J, Aubert P, et al: Hyperbaric oxygen therapy for radionecrosis of the jaw: A randomized, placebo-controlled, double-blind trial from the ORN96 study group. J Clin Oncol 22: 4893, 2004
27. Delanian S, Chatel C, Porcher R, et al: Complete restoration of refractory mandibular osteoradionecrosis by prolonged treatment with a pentoxifylline-tocopherol-clodronate combination (PENTOCLO): A phase II trial. Int J Radiat Oncol Biol Phys 80: 832, 2011
28. Sezer O, Niemöller K, Eucker J, et al: Bone marrow microvessel density is a prognostic factor for survival in patients with multiple myeloma. Ann Hematol 79:574, 2000
29. Yoshino S, Kato M, Okada K: Prognostic significance of microvessel count in low stage renal cell carcinoma. Int J Urol 2:156, 1995
30. Weidner N, Semple JP, Welch WR, et al: Tumor angiogenesis and metastasis—Correlation in invasive breast carcinoma. N Engl J Med 324:1, 1991
31. Pappas GC: Bone changes in osteoradionecrosis. Oral Surg Oral Med Oral Pathol 27:622, 1969
32. El-Naggar AM, Hanna IRA, Chanana AD, et al: Bone marrow changes after localized acute and fractioned X irradiation. Radiat Res 84:46, 1980
33. Simmons DJ, Holtzman RB: Plugged Haversian canals and local dose in radium cases. Health Phys 44:179, 1983
34. King M, Casarett G, Weber D: A study of irradiated bone: I. Histopathologic and physiologic changes. J Nucl Med 20:1142, 1979
35. Marx RE, Tursun R: Suppurative osteomyelitis, bisphosphonate induced osteonecrosis, osteoradionecrosis: A blinded histopathologic comparison and its implications for the mechanism of each disease. Int J Oral Maxillofac Surg 41:283, 2012
36. Parker R, Berry H: Late effects of therapeutic irradiation on the skeleton and bone marrow. Cancer 37:1162, 1976
37. Makris A, Powles TJ, Kakolyris S, et al: Reduction in angiogenesis after neoadjuvant chemoendocrine therapy in patients with operable breast carcinoma. Cancer 85:1996, 1999
38. Semba I, Funakoshi K, Kitano M: Histomorphometric analysis of age changes in the human inferior alveolar artery. Arch Oral Biol 46:13, 2001
39. Yoshida H, Fujita S, Nishida M, et al: Angiogenesis in the human temporomandibular joint studied by immunohistochemistry for CD34 antigen. J Oral Pathol Med 28:289, 1999