EFFECTS OF RADIOTHERAPY ON BONE TISSUE*

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Abstract OBJECTIVE: To investigate the effects of radiotherapy on bone tissues and the accuracy of gray level measurements on radiographic images. MATERIALS AND METHODS: Four Wistar rats were submitted to external radiotherapy (single 3000 cGy dose) on an area of 2 cm × 2 cm of their right legs. The animals were sacrificed six weeks after radiotherapy, and both irradiated and contralateral (non-irradiated) legs were removed, dissected, evaluated for thickness, x-rayed in a standardized form and histologically processed (stained with hematoxylin-eosin and picrosirius red). The radiographs were digitalized and the gray level average was measured with the ImageTool® software. RESULTS: The femur thickness of non-irradiated legs was greater than that of the irradiated legs (p < 0.05). Radiographically, the findings indicated a higher bone density in the non-irradiated legs, although with no statistically significant difference (p > 0.05). Histological analysis of the irradiated legs demonstrated a decrease in the number of osteocytes and Haversian canals, although with no statistically significance (p > 0.05). On the other hand, a significant increase in adipocytes was observed, resulting in a reduction of medullary tissue in the irradiated legs (p < 0.05), besides a higher osteoblastic activity in the non-irradiated legs (p < 0.05). CONCLUSION: Radiotherapy within the above mentioned parameters determined a decrease in activity of bone remodeling, which could be radiographically detected in the majority of the evaluated specimens. Keywords: Radiotherapy; Ionizing radiation; Bone tissue.

INTRODUCTION

According to data from The World Health Organization (WHO)¹, more than 11 million people are diagnosed with cancer each year, with estimated 16 million new cases per year as from 2020. Besides, cancer annually causes seven million deaths, that is to say, 12.5% of deaths in the world. Amongst therapeutic modalities, radiotherapy represents a well established method for treatment of head and neck cancer. Approximately 50% of patients with cancer are submitted to radiotherapy at some phase of their treatment, either isolatedly or in association with other forms of oncologic therapy².

Although being frequently observed in the treatment of malignant tumors, the use of high radiation doses may generate undesirable side effects, since ionizing radiation cannot differentiate tumor cells from healthy...
As with other tissues, bones also are affected by the radiation effects, resulting in a significant change of the bone regeneration capacity when it is injured. One of these alterations would be a disorder in the balance between the osteoblastic and osteoclastic activities, leading to a bone destructive process. Also, a decrease in the number of osteocytes and osteoblasts may be observed following the tissue irradiation. Significant post-radiation alterations of the bone matrix develop slowly, the initial changes being a result from an injury to the bone remodeling system, i.e., osteoblasts, osteocytes and osteoclasts. Osteoblasts tend to be more sensitive than osteoclasts, osteocytes and osteoclasts. Osteoblastic activities, leading to bone formation, are halted, while osteoclastic activities, leading to bone resorption, proceed at a greater rate. Thus, there is a decrease in the mineralization process, which may lead to a spontaneous bone fractures and osteoradionecrosis. Endothelial cells also are heavily affected, and the vascular fibrosis results in a decrease in the vascularization, affecting the bone and medullary cells vitality, so the area remains susceptible to infection and necrosis, even after a small trauma. For this reason, dental extractions are contraindicated during an one-year period following radiotherapy.

The severity of the tissue lesion will depend on the total radiotherapy dose, the effective biological dose, the size of the radiation field, the number of radiotherapy sessions and the interval between them, the dose fractioning, and the surgical and/or traumatic aggression to the irradiated tissue. Severe cases of tissue destruction usually are associated with doses > 7,000 cGy, although 6,000 cGy may result in mandible osteoradionecrosis.

The present study was aimed at evaluating the radiotherapy effects on bone tissues, and the accuracy of the measurement of gray levels on radiographic images as a predictor of histological alterations of bone tissues in animal models.

**MATERIALS AND METHODS**

The present study utilized four male, adult, clinically healthy rats *Rattus norvegicus albinus, Rodentia, Mammalia*, of the lineage Wistar, weighing about 210–260 g. These animals were kept in individual cages measuring 20 cm \( \times \) 30 cm \( \times \) 13 cm, at 22°C room temperature, luminosity (12-hour day/12-hour night cycle), 50% relative humidity, and received commercial rodents diet (Nuvilab® CR 1) and water ad libitum.

All of the animals were submitted to general anesthesia by intraperitoneally injected sodic thiopental in the dosage 0.2 ml/100 g. Then the rats underwent trichotomy on their back legs coxofemoral region, and were immobilized during the irradiation procedure by means of an especially developed acrylic device based on an experiment performed by Machado. The animals were submitted to a single session of ionizing radiation from a cobalt-60 source, with a total of 3,000 cGy delivered to a 2 cm \( \times \) 2 cm area of their right legs, utilizing only one irradiation field from the top to the bottom. The rest of the animal body was protected by lead blocks inserted into the radiotherapy device. The animals were sacrificed six weeks after radiotherapy, and both irradiated and non-irradiated legs were dissected.

The animals' legs were placed with their ventral surfaces directly onto an imaging plate for cephalometric radiography of a DenOptix® (300 dpi, pixel de 85 μm) digital radiographic system. A five-step aluminium wedge with 1 mm increment was added to the apparatus. The radiographic device (Tixmex® – 70 kV e 7 mA) was set for 0.06 s exposure time, 1.20 cm focal distance and perpendicular beam.

After the radiographic exposure, the plate was read by the DenOptix® system, allowing the generation of the corresponding digital images which were exported and stored as bitmap files. These files are opened in the Photoshop® application for brightness correction with basis on the penetrometer and saved again. After that, the images were opened with the Image Tool® software, and a polygon was traced on the irradiated region of each rat. The mean gray level was measured by the “histogram” tool. So the mean gray levels measured for each specimen were compared (Student-\( t \) test).

Then the specimens were longitudinally incised to expose the whole femur whose thickness was measured with a digital Starrett 727 series pachymeter and a Zeiss® magnifying glass.

The femurs were sent for histological processing and hematoxylin-eosin and picrosirius red staining. The following parameters were analyzed on the histological slides: presence of collagen fibers, level of periosteal osteoblastic activity, degree of bone reabsorption near the medulla, and amount of adipous tissue. The latest three items were evaluated as for their intensity degree as follows: mild intensity, grade 1; moderate intensity, grade 2; severe intensity, grade 3. These results were evaluated by means of the non-parametric chi-square test (error probability, 5%). Additionally, a counting of the number of osteocytes and Haversian canal was performed in 10 fields. Such results were analyzed by means of the non-parametric Kruskal-Wallis test (error probability, 5%).

**RESULTS**

After the femurs thickness measurement, a lower thickness was observed on the irradiated legs (4.20 mm \( \times \) 2.84 mm) as compared with the non-irradiated legs (4.67 mm \( \times \) 3.11 mm). This value was statistically significant (\( p < 0.05 \)).

Regarding the digital radiographic analysis, it was possible to observe that, in the irradiated legs, the bone tissue amount was smaller (mean gray levels, 118) as compared with the non-irradiated legs (mean gray level, 123.5), but this difference was not statistically significant (\( p > 0.05 \)) (Figure 1).

Histologically, the irradiated legs presented a high amount of adipous tissue and, consequently, a decrease in the amount of medullary tissue (Figure 2). A low index of osteoblastic activity has occurred, this activity being formed by periosteal osteoblasts. Also, areas of moderate bone reabsorption were observed. This osteoclastic activity was characterized by the presence of lacunae of cortical bone reabsorption near the medullary portion. Collagen fibers were found in 50% of specimens. A counting demonstrated a marked scarcity of osteocytes. The same has occurred with the Haversian canals. Also on the contralateral, non-irradiated legs, a low number of osteocytes were found, but the difference be-
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The same has occurred with the counting of Haversian canals (\( p > 0.05 \)). However, as regards the adipous/medullary tissues ratio, a mild presence of fat cells, and a moderate presence of medullary tissue were observed (Figure 3). This ratio was statistically significant as compared with the irradiated legs (\( p < 0.05 \)). As regards the osteoblastic activity, the non-irradiated leg presented moderate to intense activity (Figure 4), and this difference was statistically significant (\( p < 0.05 \)). The presence of collagen fibers has not been observed, but this difference has not been statistically significant as compared with the irradiated leg (\( p > 0.05 \)). Mild to moderate bone reabsorption has been observed, although with no statistically significant difference (\( p > 0.05 \)).

DISCUSSION

The animal model chosen for the present study was the rat, since it is the most utilized for evaluating secondary effects of radiotherapy in studies developed by several authors (4,9,14,15).

Because of difficulties inherent to animal model studies, the rats were exposed to a single radiation dose enough to cause trabecular changes; besides, this single dose was based on protocols reported by previous studies (4,9,14–16), ranging between 25 Gy and 35 Gy. The cobalt-60 apparatus was chosen because of its higher accessibility and for knowingly causing more adverse effects than other radiation sources (17).

Based on the results of digital radiographic evaluation, it is possible to infer that the method has presented a good sensitivity because, even in the absence of a statistically significant mean gray level between irradiated and contralateral legs, these results are similar to those of the histological analysis where significant differences also have not been found among some structures evaluated. The sensitivity of the evaluation of gray levels on digital radiographic images of bone tissues has already been analyzed both in other in vivo studies (18) and animal model studies (19).

The literature has demonstrated that osseous alterations observed on irradiated bone are visible and are directly related to cellular scarcity in the bone structure (15). However, this assertion has been just partially evidenced with the present study, since although the irradiated bone has presented less cellularized, it cannot be affirmed that this has occurred as a result of radiotherapy, since the contralateral leg has not demonstrated statistically significant difference as regards the number of osteocytes.

The same has occurred with regard to the number of Haversian canals. On the irradiated leg, a little number of Haversian canals was observed, but this number was not statistically significant in comparison with the contralateral leg. Studies developed by Morales et al. (20) have found a decreased local vascularization after exposure of rabbits mandibles to ionizing radiation. However, the animals mandibles utilized by these researchers as a comparison media, had not been submitted to any type of radiotherapy, as a comparison media.

Besides these effects, a low osteoblastic activity could be observed in the irradiated leg. These findings are similar in studies developed by Matsumura et al. (21), Dare et al. (22) and Dudziak et al. (23), who have found a decrease in osteoblastic proliferation, leading to the idea that ionizing radiation implies the terminal differentiation between precursor bone cells and osteoblasts. The same fact has not occurred in the non-irradiated leg. The collagen production, however, was not affected, being ob-
served in 50% of the irradiated legs although this finding has not been statistically significant as compared with the contralateral leg which has not presented col-

Zones of bone resorption were visible, although not with the expected intensity. Once more, there was no significant difference between irradiated and non-irrad-

ated legs. These findings go against those of Kiyohara et al., who have found bone resorption in irradiated legs as from the fourth week after radiotherapy, with thin-

ning of bone trabeculae. However, the bone thickness observed in the irradiated leg was statistically smaller than in the non-irradiated leg, contradicting the histological find-

ings. This suggests that, in some moment, a possible bone reabsorption process has occurred. Also, an expressive increase in fat tissue was found, with the consequent re-

duction of bone marrow tissue. This fact had already been previously docu-

mented.

CONCLUSION

It may be concluded that radiotherapy in the dose utilized in the present study, has determined a decrease in the bone remodeling activity in the majority of specimens evaluated, which could be radiographically detected by measuring the gray levels of the bone tissue on digital radiographic images.

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