Jaw Bone Changes on Panoramic Imaging
after Head and Neck Radiotherapy

by

King Chong Chan

A thesis submitted in conformity with the requirements
for the degree of Master of Science in Oral and Maxillofacial Radiology

Discipline of Oral and Maxillofacial Radiology, Faculty of Dentistry
University of Toronto

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Abstract

Gnathic changes after head and neck radiotherapy have not been thoroughly characterized radiographically. The objectives of this study are to characterize changes to the teeth and jaws on panoramic images following intensity modulated radiotherapy (IMRT), and to determine whether subject comorbidities and radiation dose affect these changes. This retrospective analysis reviews the charts and panoramic images of 126 head and neck cancer patients who received IMRT at Princess Margaret Hospital between January 1, 2005 and December 31, 2008. Of the 126 subjects, 75 (60%) showed changes on panoramic radiographs; 66 (88%) of which consisted of widened periodontal ligament spaces (WPLS). The median time to WPLS was 29 months after IMRT. Female sex and dose correlated with decreased time to WPLS. These results suggest that WPLS is a common radiographic sign following IMRT that may not require endodontic intervention as post-IMRT WPLS is unrelated to the pulpal status of the tooth.
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Chapter 1

1. Introduction

1.1. Literature Review

1.1.1. Ionizing Radiation for Cancer Treatment

Radiation is the transmission of energy through space and matter (1). Ionizing radiation, such as x rays, has sufficient energy to eject an electron from an atom. The atom becomes electrically charged or ionized. In biologic matter, the dislodged electrons from ionized atoms can interact with water molecules in the cell to generate highly-reactive free radicals that damage cellular components (1,2). This indirect effect constitutes the main biologic interaction between x rays and matter.

The deoxyribonucleic acid (DNA) within the chromosomes in the nucleus of a cell is most sensitive to the biologic effects of ionizing radiation (1).

Structural integrity of chromosomal DNA is crucial for a cell to complete division and propagate. DNA is a double-stranded helical molecule. The sequence of bases within the two DNA strands contains the genetic code for the growth, development and function of all cells in an organism. Ionizing radiation can modify the arrangement of the DNA molecule by inducing base damage that may lead to errors in repair, by cross-linking the strands, by disrupting the chemical bonds between the strands, or by causing single or double-stranded breaks. Base damage, cross-links and single-stranded DNA breaks are not lethal to the cell. Double-stranded DNA breaks, however, have three potential outcomes for the cell. If the broken fragments rejoin to their original configuration, cell viability is preserved. More likely though, the broken fragments fail to rejoin, or they re-assort and rejoin to different broken fragments. The latter two outcomes produce deletions and structural abnormalities in the chromosomes that may preclude cellular division and cause cell death (1,2). Although all types of radiation-induced damage can be repaired by cellular enzymes, cancer cells have a lower chance of recovery than healthy cells (1-3). Cancer cells have lost the ability to regulate cellular division and cannot respond to
signals that would normally prompt healthy cells to repair damaged DNA before replication. In other words, cancer cells cannot repair double-stranded DNA breaks to avoid cell death because the molecular mechanisms that reconstitute broken strands cannot be activated (1,2). A balance favouring healthy cell recovery and cancer cell death is achieved at low radiation doses of \( \leq 2 \) Gray (Gy) (1-3). Consequently, excessive damage to normal tissues can be minimized and cancer damage can be maximized if the total dose of therapeutic ionizing radiation, often 60 to 70 Gy for head and neck cancers, is fractionated into a series of smaller doses that are delivered daily over several weeks (1,2).

The therapeutic application of ionizing radiation for cancer was first documented in January 1896, two months after the discovery of x rays by Wilhelm Conrad Roentgen (4). Emil Grubbe, a medical scientist, treated a patient with recurrent breast cancer using x rays generated from a Crookes tube (4,5). Radiotherapy is currently delivered by an advanced type of external beam radiation therapy known as intensity modulated radiation therapy (IMRT). Since the mid-1990s, IMRT has become a key modality in the control and eradication of malignant disease (6-11). IMRT differs from earlier modalities of external beam radiation therapy by being able to more tightly conform treatment to the three-dimensional shape of the tumour by controlling the intensity of the dose delivered by each radiation beam. Consequently, the tumour receives the highest doses, while the adjacent healthy tissues receive lower doses (7,10,11). IMRT aims to minimize damage of healthy tissues, but ionizing radiation still passes through normal anatomic structures in order to reach the tumour. Importantly, the mandible is affected by therapeutic radiation of head and neck cancer because this osseous structure is in close proximity to the tumour target (12). Depending on the radiation dose absorbed, irradiated mature bone may be clinically and radiographically unremarkable, may show incidental radiographic findings, or may be symptomatic, necrotic and fractured (6,8,9,12-16). To understand the factors involved in the varied imaging manifestations of irradiated mature bone, an overview of bone metabolism and a review of recent research in the mechanisms of radiation effects on mature bone are presented.

1.1.2. Radiation Effects on Mature Bone at the Cellular Level

1.1.2.1. Overview of Bone Metabolism
Bone is a complex tissue consisting of various cell types in a calcified matrix of collagen. The mineralized and rigid structure of bone provides mechanical support for the human body. Throughout life, bone adapts to mechanical stresses continuously by remodeling itself. The dynamic process of bone remodeling is vital in the regulation of bone structure and function, and is maintained by a balance between bone resorption and bone formation (15,17-19). The main cellular participants of bone remodeling are osteoclasts, osteoblasts, osteocytes, and bone lining cells (17,18). Osteoclasts are capable of bone resorption. They are multinucleated, giant cells derived from fusion of osteoclast precursors, which are mononuclear hematopoietic marrow stem cells of the monocyte/macrophage family. Osteoblasts are bone forming, mononuclear cells derived from mesenchymal stem cells in marrow. Osteoblasts terminally differentiate into osteocytes if entrapped in bone matrix, or bone lining cells if found along bone surfaces (17,18). Osteocytes have long, slender, dendritic processes within bone that detect mechanical stress to initiate bone remodeling. Bone lining cells mediate interaction between osteoblasts and osteoclast precursors at sites of remodeling (17,18). Together, these cells form the basic multicellular unit (BMU) of bone remodeling (17,18). Although the exact mechanism of bone remodeling remains to be resolved, the process is known to involve an intricate signaling network among cells of the BMU.

Cross-talk between cells of the BMU regulates bone remodeling. Mechanical stress to bone is sensed by osteocytes, and induces expression of monocyte-colony stimulation factor (M-CSF) and receptor activator of nuclear factor-kappa B ligand (RANKL) on bone lining cells. M-CSF and RANKL are key factors of osteoclastogenesis. M-CSF stimulates proliferation of osteoclast precursors and activates receptor activator of nuclear factor-kappa B (RANK) on the osteoclast precursors to promote differentiation and maturation. Fusion of the osteoclast precursors into mature, multinucleated osteoclasts involves both M-CSF and RANKL. As mature osteoclasts attach to the bone surface for resorption, a canopy of bone lining cells overlies the osteoclasts to form the bone remodeling complex (BRC). The BRC is a contained, richly vascular microenvironment of bone at sites of active remodeling. Blood delivers oxygen and nutrients to cells of the BRC for energy production and cellular function, and removes calcium and waste products from the resorbed bone cavity (17,18). Osteoclastic bone resorption also releases factors such as bone morphogenetic proteins (BMPs), insulin-like growth factors (IGFs) and transforming growth factor beta (TGFβ) from the bone matrix (17,18). These factors are
believed to be deposited within the bone matrix by osteoblasts during previous bone formation (17,18). BMPs, IGFs and TGF-β activate a transcription factor, RUNX2, in mesenchymal stem cells to commit to the osteoblast lineage. Multiple complex signaling pathways, such as the BMP/TGF-β and Wnt/β-catenin pathways, are involved in osteoblast differentiation and bone formation. Mature osteoblasts secrete a decoy receptor of RANKL, osteoprotegerin (OPG), to inhibit osteoclastogenesis. Bone formation begins as mature osteoblasts deposit collagen in the form of osteoid to fill the resorbed bone cavity. Some osteoblasts become encased in osteoid and develop into osteocysts. Once the osteoid matrix is calcified, bone remodeling stops at the BRC (17,18).

Recent in vitro studies suggest a role of the immune system in bone remodeling. Pro-inflammatory cytokines, namely IL-1, TNF-α and IL-6, have been observed to stimulate osteoclast differentiation and function. Activated T-lymphocytes have been shown to promote osteoclastogenesis by secretion of RANKL. Precursor B lymphocytes, too, have been found to differentiate into osteoclasts (17,18). The preliminary findings suggest that the immune system tips the balance of bone remodeling towards resorption. However, resting T lymphocytes have been shown to suppress osteoclastogenesis (17,18). The mechanisms of immune cell-induced osteoclast differentiation remain to be elucidated. An interaction between immune cells and osteoclast precursors in bone remodeling is possible because both cell types originate from hematopoietic marrow (17,18).

In summary, bone remodeling is regulated by a complex cellular network supported by a rich vascular supply. Osteoblasts, osteoclasts and capillaries work in concert to maintain structural and functional integrity of bone. Disruption of a single element of the BMU affects the function of neighboring components. The effect of ionizing radiation on mature bone is therefore a complicated, multifactorial process.

1.1.2.2. Radiation Effects on Osteoblasts

Recent in vitro experiments on the effects of ionizing radiation on osteoblasts have shown that x rays can affect mature bone by two possible mechanisms: altering the production of collagenous bone matrix by osteoblasts and promoting osteoblast depletion (7,20-24).
In 2000, Gal et al. irradiated MC3T3-E1 osteoblast cells derived from mouse calvarium at 0, 2, 4, and 6 Gy (21). The study found a significant decrease in collagen production at 4 Gy, and an increase in transforming growth factor \( \beta 1 \) (TGF-\( \beta 1 \)) receptors in the osteoblasts over seven days post-irradiation (21). TGF-\( \beta 1 \) is a key stimulator of collagen synthesis (21,22), but the investigators did not provide a plausible explanation to link the increase in TGF-\( \beta 1 \) receptor number with the decrease in collagen production. In 2007, a mechanism of decreased collagen production by irradiated osteoblasts was proposed. Sakurai et al. found that radiation decreased type I collagen mRNA expression 6 hours after exposure (23). However, the investigators used C2C12 cells, which are a different mouse osteoblast cell line from that previously used by Gal et al.; therefore, the proposed mechanism of decreased collagen production may not be identical in the MC3T3-E1 osteoblast. In 2010, Lau et al. found a dose-dependent increase in TGF-\( \beta 1 \) gene expression that peaked at 24 hours after irradiation of mouse OTC-1 osteoblasts at 2, 4, and 7 Gy (24). The significance of upregulated TGF-\( \beta 1 \) gene expression was unknown because the levels returned to control values a week after irradiation regardless of dose (24). Furthermore, Lau et al. did not measure collagen production in the study to allow for correlation with the observed transient increase in TGF-\( \beta 1 \) gene expression. However, osteoblastic activity in collagen synthesis can be speculated to increase as a result of upregulated TGF-\( \beta 1 \) expression. Other factors are likely involved in the effect of radiation on collagen production because collagen synthesis is a complex molecular process involving numerous genes and regulatory stages (22). Future experiments involving additional components of collagen synthesis and the same osteoblastic cell lines may reconcile the currently truncated findings of radiation-induced effects on osteoblastic collagen production.

Osteoblast depletion is currently believed to result from cell cycle arrest and the subsequent lack of cellular proliferation. In addition to reduced collagen production, Gal et al. showed that radiation decreased osteoblast proliferation over seven days after exposure (21). However, no explanation was proposed for this observation. In 2004, Szymczyk et al. exposed mouse MC3T3-E1 osteoblast cells to 0, 15, 30, and 60 Gy of radiation. A day after irradiation, a dose-dependent increase in the accumulation of osteoblasts arrested at the premitotic phase of the cell cycle was found (25). The irradiated osteoblasts did not undergo mitosis, and the rate of cellular proliferation decreased (25). The study also showed that irradiated osteoblasts became hypersensitive to agents that induced apoptosis via the mitochondria-pathway (25). Ca\(^{2+}\) ion
pair, an apoptotic agent in the bone matrix, was found to initiate apoptosis in the irradiated osteoblasts (25). In 2010, Lau et al. found a dose-dependent effect in cell cycle arrest and mitotic death of irradiated mouse OTC-1 osteoblasts (24). These observations of dose-dependent, radiation-induced, mitotic death in osteoblasts support the established mechanisms of the biologic effects of ionizing radiation on cells (1). However, the decrease in osteoblast numbers may not necessarily correlate with a decrease in osteoblast activity. The recent in vitro finding of a dose-dependent increase in TGF-β1 gene expression in OTC-1 osteoblasts (24) suggests that ionizing radiation may stimulate osteoblastic collagen synthesis up to a certain dose, above which osteoblast activity may decrease. Additional in vitro research correlating the effects of various doses of radiation on osteoblastic collagen synthesis with osteoblastic cell survival may provide more insight into the response of osteoblasts to radiation.

1.1.2.3. Radiation Effects on Osteoclasts

Ionizing radiation has been found to increase the number and function of osteoclasts in post-pubertal animals (20,26,27). In 2003, Sawajiri et al. examined the effects of radiation on osteoclast number by irradiating the hind-leg of post-pubertal Wistar rats at 15, 22.5, and 30 Gy (26). At 15 and 22.5 Gy, osteoclast numbers increased transiently until five days post-irradiation (26). At 30 Gy, the osteoclast population dropped sharply a day after exposure, suggesting that a high radiation dose may have directly killed the cells (26). In 2008, Willey et al. exposed post-pubertal C57BL/6 mice to a whole-body radiation dose of 2 Gy (20). The study found increased serum TRAP levels, and increased osteoclast number and resorption surfaces along bone trabeculae three days after radiation (20). However, the study was limited to observing the radiation effects at a single time point; therefore, any change in osteoclast population and activity thereafter could not be determined.

In 2009, Kondo et al. replicated Willey’s study but introduced an additional dose and observational time point (27). In addition to confirming the findings of the previous experiment, Kondo’s study demonstrated a dose-dependent and time-dependent decrease in tibial cancellous bone volume (27). At 2 Gy, a 20% and 43% decrease in bone volume was observed three and ten days after radiation exposure, respectively (27). On the other hand, a 28% decrease in bone volume was evident ten days after exposure at 1 Gy (27). The study also examined radiation
effects on the murine lumbar vertebrae, and found bone volume decreased one month post-irradiation (27). In summary, Kondo et al. showed that radiation compromised osseous architecture in both the appendicular and axial mature skeleton by an increase in osteoclast number and decrease in bone volume (27).

Despite different experimental designs and animal models, the findings of an early increase in osteoclast population and decrease in bone volume after low-dose radiation to the mature skeleton is consistent. However, bone metabolism involves an intricate interplay between osteoclasts and osteoblasts. The osteoclast-based studies did not investigate the effects of radiation on osteoblasts. Although radiation has been shown to decrease osteoblast number as presented previously, the effect on osteoblast activity remains unclear (21,24,25). During bone remodeling, osteoclastic resorption releases osteoblastic stimulatory factors from the bone matrix, causing an increase in osteoblast differentiation and bone formation. An increase in bone volume is possible if the osteoclast-based studies had measured osteoblast activity and included longer time periods of observation. Consequently, the observed radiation-induced decrease in bone volume may be counterbalanced by a later increase in bone volume.

1.1.2.4. Radiation Effects on the Vascular Components of Bone

Radiation injury to the vasculature of mature bone was first described by James Ewing, an American pathologist, in 1926 (7,15,28). The loss of blood supply in bone after irradiation was attributed to obliterative endarteritis and periarteritis. The Haversian canals were narrowed as the cytoplasm of irradiated endothelial cells became swollen and vacuolated. Walls of small blood vessels became necrotic. With time, subintimal fibrosis and hyaline thickening of the media developed in the vessel walls, further compromising the vascular lumen. Currently, these changes are accepted to be characteristic of radiation-induced vascular injury, and have been confirmed by various animal experimental studies (7,28). One study, performed by Takahashi et al. in 1994, detailed the vascular and accompanying cellular changes in mature bone after radiation exposure (29).

Radiation is known to damage both the cells and vasculature of mature bone. Takahashi et al. characterized acute and chronic histomorphological changes in the Haversian system of rabbit knees after a single dose of radiation at 25, 50, or 100 Gy over a period of 52 weeks (29). At
four weeks post-irradiation, injury to the blood vessels included dilated capillaries in the Haversian canals, increased vessel wall permeability, tissue edema, degenerative changes in the endothelial cells lining the capillaries, and subsequently, occlusion of the Haversian canals. The perivascular bone matrix was also resorbed by osteoclasts. Between 12 and 24 weeks post-irradiation, the Haversian systems became markedly hypovascular and hypocellular; all cell types, including endothelial cells, osteoblasts, osteocytes and osteoclasts, decreased in number. At 52 weeks post-irradiation, the vascular damage was irreversible. These radiation-induced bone changes were present irrespective of the experimental radiation dose, with higher doses producing more severe damage (29). A dose and time-dependent increase in bone porosity due to suppressed bone formation and unopposed bone resorption was observed at four weeks post-irradiation with minimal increase thereafter (29). According to the investigators, their findings suggested that bone damage in the early-to-intermediate phase was dominated by inflammatory stromal changes, similar to those seen in acute inflammation of other connective tissue types (29). In the intermediate-to-late phase, radiation-induced bone injury was dominated by mitotic cell death and additional vascular damage (29).

Various *in vitro* and *in vivo* experiments have demonstrated the dose-dependent, detrimental effects of radiation on the cells and vasculature of mature bone. Although recent *in vitro* experiments suggest an early decrease in the number and function of osteoblasts, the findings of cell line based studies do not reflect the *in situ* environment of mature bone, a highly vascularized tissue. Furthermore, *in vivo* experiments using extragnathic bones may not directly reflect the reaction of the mandibular bone to radiation. The mandible is anatomically unique from the axial and appendicular skeleton because it contains teeth. Teeth are attached to the mandible via periodontal ligaments, comprised of a thin but densely organized group of fibrous connective tissues. Also, the mandible can be regarded as an end-organ because it has no terminal collateral anastomoses in its main blood supply via the inferior alveolar artery. Theoretically, if the lumen of the inferior alveolar artery becomes obliterated by the effects of radiation, segments of the mandible will become ischemic. If the obliteration progresses, necrosis develops, leading to the formation of sequestra, which are dead bone fragments detached from vital bone. The presence of sequestra defines osteoradionecrosis, the most severe clinical and radiographic manifestation of irradiated bone. In practice, however, the incidence of mandibular osteoradionecrosis is low (13,14,30-33). Furthermore, radiotherapy is not
administered in a single-dose, but fractionated in multiple, smaller, daily doses over several weeks to allow healthy tissues to repair sublethal damage. Radiation damage to the tissues therefore may not be as severe clinically as when they are produced experimentally. Consequently, the results of the aforementioned *in vitro* and *in vivo* experiments may not represent the response of the mandible in patients undergoing radiotherapy. Animal studies using fractionated doses and focusing on the reaction of the mature mandibular bone to radiation may mimic the clinical scenario in humans better.

**1.1.3. Radiation Effects on Mature Mandibular Bone in Animal Studies**

Clinical, radiographic and histologic findings of the radiation effects on the mature mandible were most thoroughly characterized in animal studies of the 1950s (34-38). Recent research in the histologic changes of the irradiated mature mandible in rat studies only validated the findings of earlier studies. More recent descriptions are not as detailed as those presented in the 1950s (37,38).

In 1954, Medak et al. exposed the right cheeks of six dentulous, adult, *Macaca rhesus* monkeys to 600 Roentgen (R) of x rays daily for a total dose ranging from 4200 R to 6000 R (34). The monkeys were clinically observed for changes to the oral soft tissues for five months, at which time, the mandibles were also radiographically examined. Of the six monkeys, two (5000 R and 6000 R) had intact gingival and alveolar mucosa after irradiation, and radiographically visible widened periodontal ligament spaces along the root surfaces of teeth on the irradiated side as compared to the non-irradiated side (34). The clinical manifestations of the other four monkeys were characteristic of osteoradionecrosis, and included necrosis of the gingiva, alveolar mucosa and alveolar process on the irradiated side, as well as radiographically visible bone sequestra (34). The drawback of the study was that radiographs of the mandibles were made only once. Widened periodontal ligament spaces could have been present at earlier time points in the monkeys that developed osteoradionecrosis, but earlier radiographs were not available to validate or refute the speculation. Likewise, widened periodontal ligament spaces in the monkeys might not have progressed to osteoradionecrosis but later radiographs were also unavailable for analysis. Nevertheless, the monkeys developed the two types of radiation-induced injury within the same time period of five months (34). Furthermore, the monkey irradiated at the lowest dose
of 4200 R developed osteoradionecrosis (34). Consequently, widened periodontal ligament spaces and osteoradionecrosis might represent two manifestations in a spectrum of radiation-induced damage of the mature mandible, possibly modified by additional factors that were not addressed in the study. Histologic analysis was not performed in the study.

In 1958, Chambers et al. exposed seven dentulous mandibles of adult mongrel dogs to 3000 R to 8000 R of x rays (35). Within two weeks after irradiation, the gingival and alveolar mucosa overlying the mandibles became inflamed. Healing of the soft tissues occurred in mandibles irradiated at ≤ 4000 R (35). Mucosal necrosis and mandibular bone exposure developed in mandibles irradiated at ≥ 5000 R, but the time of clinical osteoradionecrosis was not presented (35). Two months after irradiation, radiographic changes of bone resorption and widened periodontal ligament spaces were noted in mandibles that received ≥ 5000 R. Histologic correlation showed an acute inflammatory infiltrate within the medullary spaces in bone, and an inflammatory dissolution of the normally thin and densely packed periodontal ligament fibers (35). The irradiated periodontal ligament fibers were loosely arranged, edematous and widened. Effects on osteoblasts, osteoclasts and capillaries in the irradiated bone were not documented. Although radiographs were made at specified intervals of the study, Chambers et al. did not document whether the bone resorption and widened periodontal ligament spaces stabilized or progressed (35). The time of the radiographic findings was also not correlated to the time of clinical bone exposure. Similar to the study by Medak et al., a temporal relationship between bone resorption, widened periodontal ligament space and osteoradionecrosis was not explored in the study by Chambers et al. The only reliable finding was that the clinical and radiographic features developed in the animals after exposure to ionizing radiation; therefore, a plausible explanation could be that the manifestations represented different types of radiation effects on the mature mandible.

The two previous studies recorded the dose in Roentgen, a unit of radiation exposure for the ionization of air by x rays. Gray (Gy) is the current unit of radiation dose used in radiotherapy because it represents tissue absorbed dose. Consequently, the effects of dose from the two studies in the 1950s cannot be directly compared with the effects of dose used in contemporary radiotherapy. In 1979, Rohrer et al. irradiated the mandibles of eight dentulous, adult, *Macaca mulatta* monkeys to a total dose of 45 Gy, delivered in ten fractions over 12 days (36). This dose
regimen was calculated to represent the human equivalent of 70 Gy in 35 fractions over seven weeks, a realistic protocol for irradiation of head and neck cancer (36). The monkeys were euthanized at six months post-irradiation, and the mandibular specimens were analyzed histologically for radiation-induced injury (36). Histological changes were noted in the osteocytes, periosteum, periodontal ligaments, hematopoietic marrow, and the Haversian canals. Loss of osteocytes from lacunae was limited to the cortical bone in the direct path of the radiation beam. The periosteum became hypocellular and hypovascular, and lost adherence to bone. The periodontal ligaments became hypovascular and densely collagenized with loss of fiber orientation (36). Unlike the study by Chambers et al., Rohrer et al. did not report histologic widening or inflammatory changes of the irradiated periodontal ligaments. The marrow showed fibrosis and deposition of new bone. Obliterative endarteritis was also detected in the marrow and the Haversian canals (36). None of the mandibular specimens had histologic evidence of sequestra (36). In addition, none of the monkeys developed any clinical signs of osteoradionecrosis at euthanization (36). The findings of the study may be similar to the condition of the irradiated mandible without osteoradionecrosis in cancer survivors, as Rohrer et al. strived to simulate human radiotherapeutic parameters in their experiment. Although Rohrer et al. did not evaluate the mandibular specimens for radiographic changes, the deposition of excess collagen within the periodontal ligaments could have presented radiographically as widened periodontal ligament spaces, which is also seen in systemic sclerosis (39). Furthermore, deposition of new bone in the marrow could increase the amount of bone per unit area of the mandible and present radiographically as bone sclerosis. Consequently, the densely collagenized periodontal ligaments and the deposition of new bone in the marrow seen by Rohrer et al in the irradiated mandible might have radiographically manifested as widened periodontal ligament spaces and bone sclerosis, respectively. The histologic features of hypocellularity and hypovascularity of the irradiated mandible might theoretically suggest impaired bone metabolism. However, the study evaluated the specimens at only one time point; therefore, the possibility of recovery, stabilization or progression of the radiation-induced cellular and vascular changes could not be determined.

Given the findings of the three animal studies presented above, the radiation effects on the mature mandible likely exist in a spectrum of clinical, radiographic and histologic presentations. Although no pathophysiological mechanism was proposed in the three studies, the histologic
findings of hypocellularity and hypovascularity in the irradiated monkey mandibles paralleled findings of the previously reviewed in vitro study of irradiated rabbit knees. Furthermore, the presence of inflammatory changes to the irradiated widened periodontal ligaments suggests a role of the immune system in the response of radiation-induced changes. To date, research has failed to produce a unified theory of the many different in vitro and in vivo findings of irradiated bone.

1.1.4. Radiation Effects on Mature Bone in Cancer Survivors after Radiotherapy

Radiation-induced changes in the irradiated mature bone have been described in the radiographic and histopathologic literature by various terms. Three common examples of these terms are post-radiation atrophy, radiation osteitis, and osteoradionecrosis (15,16,20,21,23,25,28,29). However, none of these terms accurately describes the pathophysiologic mechanism behind radiation-induced bone injury, or encompasses the wide spectrum of radiation-induced bone changes. The term of post-radiation atrophy refers only to a decreased number of bone cells and vasculature, and does not describe the inflammatory reaction that accompanies radiation-induced bone changes (15,16,20,21,23,25,28,29). Conversely, the term of radiation osteitis refers only to the inflammatory process in irradiated bone, and does not include the radiation-induced hypocellular and hypovascular effects on bone. The third term, osteoradionecrosis, is defined radiographically and histologically as a type of radiation-induced bone change with bone death (13,40,41), and does not account for the less severe radiation-induced bone changes without sequestra, such as widened periodontal ligament spaces in the irradiated mandible as mentioned previously. In the medical radiologic literature, the term of radiation-induced bone changes has been used to describe the osteopenic and osteosclerotic changes in the irradiated axial and appendicular skeleton of cancer patients (6,8,9,42-44). Most of these changes stabilize without sequestra, and are detected incidentally in asymptomatic, otherwise healthy, cancer survivors. Consequently, the term of radiation-induced bone changes may be most appropriate to describe the radiographic features of irradiated bone in the absence of sequestra, and osteoradionecrosis be reserved for those changes with sequestra.
Radiographic follow-up of patients who received radiotherapy for malignancies often reveals incidental findings of bone changes (6,8,9,42-44). A history of radiotherapy is crucial for a diagnosis of post-radiotherapy adult bone changes. Skeletal changes after radiotherapy have a temporal pattern of development (6,8,9,42-44). During the first 12 months after radiotherapy, radiographic changes in bone are rarely detected (6,8,9,43). Demineralization and osteopenia of the irradiated axial and appendicular skeleton are radiographically evident after a year post-irradiation (6,8,9,43). This latent period between radiotherapy and detection of bone changes is likely due to the slow metabolic turnover rate of the mature skeleton, and the low sensitivity of conventional radiographs for osteopenia (6). This pattern of bone loss may represent the only evidence of radiation bone injury unless infection or trauma occurs (6,9,42,43). Bone repair is attempted in regions of radiation-induced bone changes, and bone is deposited onto unresorbed trabeculae (6,8,9). Consequently, radiographs of bone in the irradiation field two to three years after radiotherapy show mottled areas of resorption, coarse trabeculation and focal areas of sclerosis (6,8,9). The constellation of these radiographic findings is most appropriately termed radiation-induced bone changes, as discussed previously. Effects of radiation on the mature skeleton primarily depend on irradiation dose, field and site (6,8,9,42,43). The following sections review the published, radiographically evident changes in the extragnathic and gnathic bones after radiotherapy for malignancy.

1.1.4.1. Extragnathic Bones

Post-radiotherapy changes in the ribs, clavicle, scapula, and humerus are common in patients who were irradiated for breast carcinoma and Hodgkin’s lymphoma (6,8,9). Osteopenia, disorganized and coarse trabecular pattern, and cortical irregularity are radiographically evident after a minimum radiation dose of 45 Gy (6,8,9). Complications include fractures, which often affect the third, fourth and fifth ribs, and are asymptomatic and multiple (6,9). The earliest radiographic sign of rib fracture is an abrupt change in contour with an inconspicuous fracture plane (6). Clavicular fractures frequently accompany rib fractures (6). Fractures may show resorption of their margins, or heal with sclerosis (9). Post-radiotherapy bone changes in the shoulder are similar to those of a neuropathic joint (8,9).
Radiation-induced bone changes of the pelvis are often seen in patients who were irradiated for prostatic and gynecologic malignancies (6,8,9). A characteristic pattern is a mixture of osteopenia and patchy sclerosis in the sacrum. The sacroiliac joints exhibit irregular joint widening with sclerosis on the ilieal aspects, resembling radiographic changes of osteitis condensans ilii (6,8,9). Similar changes, mimicking osteitis pubis, are also present in the pubic bones (6,8,9). Post-radiotherapy pelvic bone changes predispose affected patients to sacral insufficiency fractures, especially in postmenopausal women with osteoporosis (6,8,9). Fractures of the pubic bone and femoral neck are additional complications of pelvic bone irradiation (6,9). Risk factors of post-radiotherapy fractures include high radiation dose, high beam energies, and underlying osteoporosis (8). Acetabular protrusion and avascular necrosis of the femoral head are rare effects of pelvic bone radiotherapy (6,9).

Post-radiotherapy osteopenia of the long bones is often asymptomatic and appears at least one year after irradiation (6,8,9). Cortical thinning, bone remodeling and osteonecrosis are late effects of radiotherapy (6,8,9). Periosteal new bone formation is not present in radiation-induced bone changes of the long bones. Muscle atrophy and dystrophic soft tissue calcification may be associated with these osseous changes (6).

1.1.4.2. Gnathic Bones

The effects of radiotherapy on the jaw bones have not been thoroughly characterized in the medical and dental radiology literature. To date, most radiographic findings of post-radiotherapy jaw bone changes have been classified as osteoradionecrosis (6,8,9,14,45-47). In a review of published reports, however, the radiographic descriptions of osteoradionecrosis do not consistently include the defining feature of sequestra (6,9,13,14,47). As discussed previously, the radiographic presence of bone sequestrum is required for a radiographic diagnosis of osteoradionecrosis because necrotic bone cannot be assumed to be histologically present when it is not radiographically visible. The one exception is the presence of a pathologic fracture without sequestrum in imaging because fragments of dead bone are invariably present at the histologic level. Consequently, some of the published data may be radiographically describing radiation-induced jaw bone changes. Differentiating these changes from osteoradionecrosis is important because of different clinical presentation, management and prognosis. As in the
aforementioned animal studies, radiation-induced jaw bone changes are clinically asymptomatic and incidental radiographic findings (35,36). The current literature has two case reports that document similar findings in human subjects (45,46).

In 1986, Fujita et al. presented a case report of a 57 year old human female who developed widening of the periodontal ligament space and periodontal disease-like bone loss after radiotherapy for a basal cell carcinoma of the gingiva (45). These two radiation-induced jaw bone changes were localized to the site of irradiation and were not accompanied by clinical or radiographic signs of osteoradionecrosis (45). The patient received a total radiation dose of 60 Gy (45). Periapical radiographs showed visible widening of the periodontal ligament space at six months post-irradiation. Periodontal ligament spaces were not widened before radiotherapy nor at 16 days and three months after radiotherapy. Two years after radiotherapy, the teeth with the radiographically widened periodontal ligament space became clinically mobile and bone loss was radiographically apparent in the surrounding alveolar process (45). The authors of the case report suggested that the observed radiation-induced jaw bone changes were due to radiation effects on the vascular supply of the periodontal ligament, which resulted in an edematous increase to the volume of the periodontal ligament. Fujita et al. also recommended periodic periapical imaging to monitor the progress and extent of the radiation-induced periodontal bone changes, and conservative management in terms of oral hygiene to preserve the integrity of the irradiated bone (45).

A second case report of similar radiation-induced jaw bone changes was presented in 1993 (46). A 40 year old female developed widened periodontal ligament space and periodontal disease-like bone loss localized to the posterior maxillary teeth after radiotherapy for nasopharyngeal carcinoma at a total dose of 60 Gy (46). Panoramic radiographs before radiotherapy and at three, four and six years after radiotherapy monitored the condition of the irradiated jaws (46). Widening of the periodontal ligament space of the posterior maxillary teeth was visible three years post-irradiation, with progression and loss of the periodontal bone thereafter. The affected teeth exfoliated due to the periodontal disease-like bone loss by the sixth year after radiotherapy (46). Vigorous oral hygiene measures were prescribed to minimize the risk of infection and extraction (46). As in the previous case, the patient presented by Yusof and Bakri did not develop osteoradionecrosis (46).
The two previously presented 1986 and 1993 case reports are currently the most recent publications on radiation-induced jaw bone changes. As mentioned by Yusof and Bakri, the medical and dental radiology literature is replete with reports of osteoradionecrosis and lacks large case series on the comparatively more benign post-radiotherapy jaw bone changes (46). Because these changes mimic common inflammatory dental disease, clinicians unfamiliar with radiation-induced widened periodontal ligament space and periodontal disease-like bone loss may extract the affected teeth and increase the risk of osteoradionecrosis for patients (40,45,46). Alternatively, if clinicians are aware of post-radiotherapy jaw bone changes, conservative measures such as periodic radiographic monitoring and vigorous oral hygiene may be prescribed to avoid dental infection that may compromise the bone. Consequently, characterizing radiation-induced jaw bone changes may allow interventions to be developed that minimize the risk of osteoradionecrosis.

1.1.4.2.1. Jaw Osteoradionecrosis after Radiotherapy

A brief discussion of jaw osteoradionecrosis is warranted in order to appreciate the seriousness of this post-radiotherapy complication for affected patients. This in turn emphasizes the need to distinguish osteoradionecrosis from the aforementioned incidental radiographic bone changes such as widened periodontal ligament space. As discussed previously, the presence of bone sequestrum defines a radiographic diagnosis of jaw osteoradionecrosis in patients with a history of head and neck radiotherapy. Clinically, jaw osteoradionecrosis is defined by the exposure of devitalized bone in such patients (13,40). In a recent review of jaw osteoradionecrosis, Chrcanovic et al. noted that the published clinical definitions differ on the timing of bone exposure (13,40). Some authors do not specify a time period of bone exposure, while others have set a two-, three-, or six-month duration (13). Chrcanovic et al. proposed that bone should be clinically exposed for a minimum of three months before diagnosis of jaw osteoradionecrosis. The reason is that tissue healing after most surgeries requires up to one month. Also, the long six-month time point is not clinically practical because of patient morbidity (13,40). Furthermore, most cases of jaw osteoradionecrosis develop between three months and three years after radiotherapy (13,48,49). Despite the variable defining time periods for osteoradionecrosis, Chrcanovic et al. found that most studies published to date agree on the following parameters for a clinical diagnosis: that the site of osteoradionecrosis is in the path of
primary radiation beam in therapy; that the site is free of recurrent malignancy; that bone 
exposure at the site is due to mucosal breakdown and/or failure to heal; that the exposed bone is 
non-vital due to hypoxic necrosis; and that the minimal duration of bone exposure for a diagnosis 
of osteoradionecrosis is three months (13).

The incidence of jaw osteoradionecrosis fluctuates widely from 0% to 37.5% in the literature 
(13,14,30). Reasons for this include different definitions of osteoradionecrosis, different sample 
sizes, variations in dental disease prevalence in the study sample and different types and total 
doses of radiation therapy. The largest study on osteoradionecrosis to date, in which the disease 
is defined as “a pathologic process that developed following irradiation of the bone and that was 
diagnosed by histologic study”, found an incidence of 8.2% in 830 head and neck cancer patients 
who received radiation therapy between 1969 and 1999 (30). This reported incidence of 
osteoradionecrosis has declined to less than 1% in recent years with the advent of IMRT (13,31-
33).

Studer et al. identified one case of osteoradionecrosis, defined as bone exposure with necrosis at 
the superficial cortical bone, in their case series of 123 head and neck cancer patients who 
received IMRT (31). Similarly, Ben-David et al. found no cases of osteoradionecrosis, defined 
as bone exposure persisting more than six weeks, in their case series of 176 patients after IMRT 
for head and neck malignancies. They attributed this finding to compliant patients who followed 
a stringent regimen of dental care before, during and after irradiation (32). A recent publication 
on the benefits of IMRT in head and neck cancer patients noted that most of Studer et al. and 
Ben-David et al. cases were oropharyngeal cancers, and therefore, used an oral cavity and 
mandibular dose constraint during IMRT (33). The mandible remains at risk for 
osteoradionecrosis after IMRT for oral cavity cancers because mandibular bone sparing is not 
easily achieved or recommended for disease eradication when the site of cancer is close to the 
jaws (33,50). Furthermore, some patients may be more predisposed to osteoradionecrosis than 
others because they may have additional risk factors for the condition (13,31-33,50,51).

Certain factors predispose the development of jaw osteoradionecrosis after radiotherapy. 
Radiation dose is the most well-established risk factor of jaw osteoradionecrosis; the higher the 
radiation dose, the greater the risk of disease (13,30,40,49,52-57). A total radiation dose greater
than 65 Gy is associated with an increased risk of jaw osteoradionecrosis (30,40,49,56). The disease is rare if the total radiation dose is less than 50 Gy (30,40). However, the risk of jaw osteoradionecrosis increases if a total radiation dose of 60 Gy is used in brachytherapy, a type of radiotherapy in which the radiation source is implanted in the tumour target (40,54,58).

Recently, studies have found that lower radiation dose rates, smaller fraction sizes and smaller radiotherapy field sizes are associated with a lower incidence of jaw osteoradionecrosis (40,56,58,59). Glanzmann et al. demonstrated a significant decrease in the incidence of jaw osteoradionecrosis in patients treated with two fractions of 1.2 Gy per day up to a total dose of 82 Gy, compared to patients treated with one fraction of 2.00 to 2.22 Gy per day up to a total dose of 80 Gy (56). Similarly, disease incidence is less common if radiotherapy is hyperfractionated up to 80 Gy, or when moderately accelerated fractionated radiotherapy is used with a boost of up to 72 Gy (40). Hyperfractionation differs from conventional fractionation in that a greater number of reduced dose fractions are delivered over a conventional treatment time to give a higher total dose. In accelerated fractionation, conventional dose fractions are delivered over a shortened treatment time. Both hyperfractionated and accelerated fractionated radiotherapy aim to minimize repair of radiation-induced sublethal damage in rapidly dividing cancer cells, and to spare tissues of low radiosensitivity such as mature bone (1). Furthermore, the incidence of jaw osteoradionecrosis is exceedingly low in patients treated with IMRT because only the tumour receives the highest radiation dose in the treatment field (31,32). In summary, treatment parameters of radiotherapy, namely total dose, dose rate, fraction and field sizes, are important risk factors of jaw osteoradionecrosis.

Surgical resection and concurrent chemotherapy for malignancy have been implicated as potential risk factors of jaw osteoradionecrosis. Celik et al. found that the disease develops earlier after marginal resections without microvascular free tissue transfer, when compared with segmental resections with microvascular free tissue transfer (60). The authors attributed their finding to the improved vascularity of the surgical site from the free tissue transfer (60). Conversely, Reuther et al. found that patients who received segmental resections developed jaw osteoradionecrosis earlier than those who received marginal resections (30); this study did not specify whether microvascular free tissue transfer was performed in either type of resections. Consequently, the development of osteoradionecrosis may not necessarily be due to the type of surgical resection, but the presence or absence of a microvascular free tissue transfer. To clarify
these uncertainties, marginal resections with and without free tissue transfer, and segmental resections with and without free tissue transfer should be compared. Similarly, concurrent chemotherapy has not been firmly established as a risk factor of jaw osteoradionecrosis. Although Glanzmann et al. were unable to show an association between concurrent chemotherapy and osteoradionecrosis (56), Reuther et al. found that patients with combined pre-surgical radio- and chemotherapy developed osteoradionecrosis earlier than those treated with pre-surgical radiotherapy alone (30). It is highly possible that these differences are related to the different study sample characteristics, such as size and chemotherapeutic agent administered.

Trauma to the irradiated area has been well established as a main risk factor of jaw osteoradionecrosis (40,49,52,55,59,61,62). Most cases of jaw osteoradionecrosis develop after post-radiotherapy dental extraction or denture irritation (40,49,61,62). Wound healing is impaired because radiation structurally alters the vasculature, thereby reducing blood flow, promoting secondary infection and precipitating osteoradionecrosis (61-63). This proposed disease mechanism of damaged vasculature may also explain spontaneous cases of jaw osteoradionecrosis because hypovascularization and endarteritis obliterans are common histologic features in these cases (48).

Caries and periodontitis are associated with jaw osteoradionecrosis. Dental disease is the well-recognized risk factor that led to the prescription and practice of stringent oral health care and extraction of unrestorable teeth prior to radiotherapy (30,52,62-64). Recently, Katsura et al. not only confirmed this finding, but also found oral health status after radiotherapy to be equally important (52). Poor periodontal status post-irradiation, namely greater than five mm periodontal pocket depth, greater than 40% dental plaque score, greater than 60% bone loss level, and widening of the periodontal ligament space with disappearance of the lamina dura radiographically, significantly increased risk of osteoradionecrosis (52). This study also found a significantly greater number of smokers in the osteoradionecrosis group than the disease-free group (52). Smoking may potentiate poor oral hygiene, and enhance hypovascularity in irradiated tissues by causing vasoconstriction, thereby increasing risk of mucosal breakdown, bone exposure and necrosis. In addition, smoking is a recognized risk factor of periodontal disease. A similar contributor to poor oral hygiene is alcohol abuse. Consequently, both
smoking and alcohol abuse have been recognized as additional risk factors of jaw osteoradionecrosis (13,30,48,49,52).

Patients who received head and neck radiotherapy have a risk of jaw osteoradionecrosis (13,30). Although the current disease incidence is low, the possibility exists that some patients may develop jaw osteoradionecrosis in the future. In addition, these patients may now be harboring some resorptive and sclerotic post-radiotherapy changes in the jaws in the absence of any clinical findings, similar to those seen in the extragnathic skeleton. The jaws are different from bones of the appendicular and axial skeleton because they house dentition. The teeth are connected to the alveolar processes of the maxillae and mandible by periodontal ligaments, which form fibrous joints called gomphoses. If radiotherapy is known to induce widening of the sacroiliac joints, also a fibrous joint, radiation may have a similar effect on the gomphoses of the jaws.

Furthermore, widening of the periodontal ligament space on radiographs has been reported in animal studies and two case reports, as previously mentioned (35,36,45,46). In cases where the irradiated mandible develops these changes, the radiographic appearance may mimic inflammatory dental disease, just as post-radiotherapy bone changes can resemble other pathologies in extragnathic bones. For instance, if radiation-induced bone resorption affects the alveolar process of the mandible or widens the periodontal ligament space, the radiographic appearance may mimic periodontal bone loss and periapical inflammatory disease, respectively. Consequently, dentists unfamiliar with these radiation-induced jaw bone changes may initiate unnecessary treatment such as endodontic therapy and tooth extraction, the latter of which predisposes patients to osteoradionecrosis. To date, the medical and dental radiology literature has a paucity of case reports on radiation-induced jaw bone changes (45,46).
1.2. Statement of the Problem

Widened periodontal ligament space and periodontal disease-like bone loss are two radiographic features of radiation-induced jaw bone changes that have been reported in the absence of osteoradionecrosis (45,46). Bone sclerosis and resorption with and without sequestra have been radiographically characterized as osteoradionecrosis in the literature. However, the presence of bone sequestra is the only reliable radiographic criterion for a diagnosis of osteoradionecrosis. Bone sclerosis and resorption without sequestra could be considered as two additional radiation-induced jaw bone changes that are not definitive for bone necrosis. To date, these four types of post-radiotherapy jaw bone changes have not been documented nor analyzed in a large patient sample by multiple radiologists as observers. The time after radiation exposure at which these changes become visible on radiographs is unknown. Likewise, whether these changes recover, stabilize or inevitably lead to osteoradionecrosis remains to be determined. Moreover, patients who received head and neck radiotherapy may not all develop these changes. Dose and the field of radiotherapy may impact on the development of radiation-induced jaw bone changes because in vitro experiments have shown a dose-dependent effect of radiation on osteoblasts and osteoclasts. The dose may be higher at sites with radiographic changes than those without radiographic changes because 45 Gy is the threshold dose for radiation-induced changes in the extragnathic skeleton on plain film imaging. Patient comorbidities that affect blood flow and bone remodeling, such as diabetes, surgery and corticosteroid use, may also affect the development of post-radiotherapy jaw bone changes. However, the published literature currently lacks a large scale study that details these changes and examines them in relation to patient comorbidities and radiation dose. Without establishing what these changes represent, their presence in the clinical setting may lead to misdiagnosis and inappropriate management. Thus, characterizing radiation-induced jaw bone changes serves to educate clinicians to avoid unnecessary endodontic and surgical treatment.
1.3. Aim

The existing radiographic reports of head and neck cancer patients who received IMRT at Princess Margaret Hospital document four post-radiotherapy subclinical jaw bone imaging changes. These are widened periodontal ligament space, bone sclerosis, periodontal disease-like bone loss and bone resorption. The purpose of this research is to document the frequency of these changes on panoramic imaging, and the length of time between IMRT and the detection of these changes in a patient population that has had a common application of radiotherapy in the form of IMRT. Also, the effect of patient clinical and dose data on these changes will be investigated.
1.4. Objectives

- To characterize all visible jaw bone changes on panoramic imaging after IMRT for head and neck malignancy.

- To determine whether subject comorbidities have an effect on widened periodontal ligament space changes.

- To determine whether mean mandibular body doses $\geq 45$ Gy have an effect on widened periodontal ligament space changes.
1.5. Hypotheses

1.5.1. Alternate Hypotheses

- Changes to the appearance of the jaw bones are detectable on panoramic imaging after IMRT.

- Widened periodontal ligament space changes are affected by one or more of the following subject comorbidities: age, sex, diabetes, corticosteroid use, bisphosphonate use, smoking history, alcohol history, surgery before IMRT and chemotherapy concurrent with IMRT.

- Widened periodontal ligament space changes are affected by mean mandibular body doses ≥ 45 Gy.

1.5.2. Null Hypotheses

- Changes to the appearance of the jaw bones are not detectable on panoramic imaging after IMRT.

- Widened periodontal ligament space changes are not affected by one or more of the following subject comorbidities: age, sex, diabetes, corticosteroid use, bisphosphonate use, smoking history, alcohol history, surgery before IMRT and chemotherapy concurrent with IMRT.

- Widened periodontal ligament space changes are not affected by mean mandibular body doses ≥ 45 Gy.
2. Materials and Methods

This study was approved by the Health Sciences Research Ethics Board of the University of Toronto and the Research Ethics Board of the University Health Network. Patient identifiers were kept confidential and were removed from the data and thesis.

2.1. Research Design

This study was a single-institution, retrospective review of patient charts and panoramic images from the Princess Margaret Hospital (PMH) Department of Dental Oncology.

The study sample consisted of head and neck cancer patients who received pre-radiotherapy dental care at the PMH Department of Dental Oncology from January 1, 2005 to December 31, 2008. The patient database (FileMaker Pro 9.0 version 1; FileMaker Inc., Santa Clara, CA) at the PMH Department of Dental Oncology was used to identify subjects fulfilling the inclusion and exclusion criteria of the study. To be eligible for the study, subjects needed to have received IMRT at the PMH Department of Radiation Oncology within this time interval, and have medical charts, dental charts, and at least two panoramic images, one of which had to predate radiotherapy. The first panoramic image, referred to as the pre-IMRT baseline image, was compared to subsequent post-IMRT images. Subjects diagnosed with ORN as defined by the criteria of the PMH Department of Dental Oncology (Appendix 1) were excluded from the study. Subjects were also excluded if they were retreated with IMRT because dose data were indeterminable.

A total of 1859 head and neck cancer subjects were identified to have received pre-radiotherapy dental care and IMRT at the PMH Departments of Dental Oncology and Radiation Oncology, respectively, between January 1, 2005 and December 31, 2008. However many of these patients were discharged from the PMH Department of Dental Oncology after IMRT and returned to their family dentists for follow-up. Only 151 of the 1859 subjects in the database had the minimum of two panoramic images available for review. Of the 151 subjects, 25 were excluded because 23
subjects had ORN and two subjects were retreated with IMRT. The final study sample consisted of 126 subjects, all of whom had electronic medical and paper or electronic dental charts available for review.

2.2. Data Collection

Data collection for the study consisted of two parts: radiographic data collection and chart review.

2.2.1. Radiographic Data Collection

The primary investigator (KCC) collected all panoramic images available for the 126 subjects in the study sample. Panoramic images consisted of 204 film-based (Sirona ORTHOPHOS® 3; Sirona Dental Systems GmbH, Bensheim, Germany) and 88 digital images (Kodak 9000; Kodak Dental Systems, Carestream Health Inc., Rochester, NY). Film-based images of each subject were placed into an envelope labeled with the subject's medical record number, name, and the number of film-based and digital images available for the subject. Digital images were accessed via a DICOM viewer (eFilm version 2.1.2; Merge Healthcare, Milwaukee, WI) on a 20.1-inch flat panel liquid crystal display (Dell™ UltraSharp™ 2007FP; Dell, Inc., Austin, TX) with a high-definition graphics processing unit (ATI Radeon HD 2400 Series; Advanced Micro Devices Inc., Sunnyvale, CA). The envelopes containing the panoramic images of the 126 subjects were randomly arranged for analysis.

The study had three observers, consisting of an oral radiology resident (KCC) and two certified specialists in oral and maxillofacial radiology (MJP, SEP). Prior to radiographic data collection, a calibration study was conducted to allow the three observers to discuss and agree on the definitions of the four subclinical jaw bone changes, namely widened periodontal ligament space, bone sclerosis, periodontal disease-like bone loss, and bone resorption. Examples of the changes used for the calibration study were derived from the panoramic images of five of the 126 subjects. These five subjects were selected based on four specific findings in the radiographic reports from the patient database. Data from the calibration study were used only to help define the radiographic appearance of the four specific findings, and were not collected nor analyzed for
the study proper. The agreed upon set of definitions of the four radiographic findings are illustrated in Figures 1 to 3.

**Figure 1.** **Widened periodontal ligament space.** Widened periodontal ligament space is defined as an increased width of the periodontal ligament space greater than 0.5 mm along the entire length of the tooth root without an epicenter. This type of widening differs from that associated with periodontal and periapical inflammatory diseases, in which an epicenter exists at the alveolar crest and root apex of the tooth, respectively. As compared to the cropped panoramic image of the posterior right mandible before IMRT (A), the image after IMRT (B) shows the presence of widened periodontal ligament space involving the mandibular right first and second premolars, and second and third molars.
Figure 2. Bone sclerosis and periodontal disease-like bone loss. Bone sclerosis is defined as a region of increased bone density due to an increased number of bone trabeculae. Periodontal disease-like bone loss is defined as vertical bone loss in the alveolar process as measured from the level of the cementoenamel junction of the adjacent tooth. As compared to the cropped panoramic image of the posterior right mandible before IMRT (A), the image after IMRT (B) shows the presence of bone sclerosis localized to the mandibular right second premolar and first molar region, and periodontal disease-like bone loss of the mandibular right second molar.
Figure 3. Bone resorption. Bone resorption is defined as a region of decreased bone density due to a decreased number of bone trabeculae. As compared to the cropped panoramic image of the posterior right mandible before IMRT (A), the image after IMRT (B) shows the presence of bone resorption localized to the right retromolar region.
To minimize bias amongst the three observers for the study proper, the panoramic images of each of these five subjects were randomly presented to the observers after approximately eight months. The detailed instructions generated from this exercise are listed in Appendix 2.

The three observers independently evaluated a total of 292 panoramic images (126 pre-IMRT baseline; 166 post-IMRT) over a period of nine months. None of the three observers had access to the radiographic reports of the 126 subjects in the patient database at the time of the review. For each subject, the medical record number, observer’s initials, date of panoramic image, and the presence or absence of change were recorded onto the data collection instrument. The instrument was a diagrammatic outline of osseous and dental structures visible on a panoramic image (Appendix 3). When detected on the image, each observer recorded the four types of radiographic findings by drawing the finding at the specific anatomic site on the data collection instrument using a red or blue marker and symbols (Appendix 4). If similar findings were noted on the pre-IMRT baseline panoramic images, these findings were recorded to distinguish them from those detected after IMRT. If the observer was unsure on whether a finding was already present on the baseline film or a preceding film, the observer was permitted to view and compare the preceding image to the one being analyzed. The presence or absence of a finding was determined when at least two of the three observers recorded the finding. The primary investigator superimposed the data collection instruments from the three observers to produce a copy of the finalized observations. The diagrammatic data were transferred to a spreadsheet (Microsoft Office Excel 2007; Microsoft Corporation®, Redmond, WA; Appendix 5A) for statistical analysis. Each row on the spreadsheet corresponded to a particular date of a panoramic image. Data from the baseline image were recorded in the first row, and those from follow-up images in subsequent rows. Each column corresponded to a particular sextant of the maxillae and mandible on the diagram. For the widened periodontal ligament space change, affected teeth in a particular sextant were recorded by the Universal tooth numbering system under the column for that sextant. Sextants with unaffected teeth were marked as "0". For bone sclerosis, periodontal disease-like bone loss and bone resorption, the presence or absence of these changes in a particular sextant was marked as "1" or "0", respectively, under the corresponding column on the spreadsheet. In preparation for the statistical tests required for this project, the spreadsheet data for the widened periodontal ligament space change were rearranged to a per tooth basis (Appendix 5B). Each row on the per tooth spreadsheet was labeled to
represent a specific tooth in the jaws using the Universal tooth numbering system. The presence or absence of a tooth and whether it had widened periodontal ligament space were marked as "Yes" or "No", respectively, under the appropriate columns. Patient status of "Alive" or "Dead" was also recorded because it affected the risk of developing the change. Dead patients who did not have widened periodontal ligament space detected within the study time frame were no longer at risk of the change and thus, important to be accounted for in the statistical analysis.

2.2.2. Chart Review

The primary investigator extracted subject demographical and clinical data from the medical and dental charts of the 126 subjects. Demographical data included subject age and sex. Clinical data included the following possible subject comorbidities: diabetes, corticosteroid use, bisphosphonate use, smoking history, alcohol history, cancer surgery before IMRT, and concurrent chemotherapy. Medical charts were electronically accessed via clinical desktop software (Electronic Patient Record Citrix MetaFrame; QuadraMed® CPR, Reston, VA). Dental charts were in paper or electronic formats. Electronic dental charts were accessed via clinical desktop software (ABELDent version 9.2.10.744; ABELDent Inc., Burlington, ON). All subject demographical and clinical data were tabulated in a spreadsheet (Microsoft Office Excel 2007; Microsoft Corporation®, Redmond, WA; Appendix 6) for statistical analysis.

2.2.3. Dose Data

Dose data of the 126 subjects were obtained from their IMRT treatment plans in the Pinnacle³ treatment planning system (TPS) (Pinnacle³ versions 8.0h and 9.0; Philips Medical Systems, Madison, WI). Computed tomographic (CT) studies of the subjects were imported into the TPS for IMRT planning purposes. The tumour (target volume) and adjacent normal anatomic structures (organs at risk) were contoured on axial CT slices of each subject at the time of treatment planning. For this study, the primary investigator contoured the right and left sides of the mandibular body for dose determination. The minimum, maximum and mean doses for the right and left sides of the mandibular body were calculated by the TPS, and exported to the spreadsheet used for demographical and clinical data (Microsoft Office Excel 2007; Microsoft Corporation®, Redmond, WA; Appendix 6).
2.3. Statistical Analysis

Statistical analyses were performed by SAS software and user's guide (SAS system version 9.2; SAS Institute Inc., Cary, NC). SAS software (SAS system version 9.2; SAS Institute Inc., Cary, NC) merged the data from the three spreadsheets (Appendices 5A, 5B, 6) to facilitate statistical analysis. Descriptive statistics were used to describe subject demographics and radiographic, demographical, clinical and dose data in the study sample. Categorical variables were expressed as counts and proportions, whereas continuous variables were expressed as mean±standard deviation. Kappa statistics were used for inter-observer variability among the three observers in the detection of the four subclinical jaw bone changes. For widened periodontal ligament space, agreement was based on the identification of the changes by tooth. For bone sclerosis, periodontal disease-like bone loss and bone resorption, agreement was based on the identification of the changes by sextant. The outcome variable of interest was time to detection of widened periodontal ligament space on panoramic imaging after IMRT. The cumulative incidence rate was calculated using the competing risk analysis method of Pepe and Mori (65). Univariate and multivariate Fine and Gray hazards regression model with competing risk (66) was used to determine the effects of subject demographical, clinical and dose data on the outcome variable. All P-values were two-sided and considered significant if less than 0.05.
Chapter 3

3. Results

3.1. Radiographic Findings on Panoramic Imaging after IMRT

3.1.1. Frequency of Radiographic Findings

Of the 126 subjects in the study sample, 75 (60%) had bone changes detected on panoramic imaging after IMRT, whereas 51 (40%) did not have changes detected. The 75 subjects with radiographic bone changes did not have similar changes detected on the baseline panoramic images before IMRT. Of the 75 subjects with changes detected, 66 (88%) had widened periodontal ligament space, 24 (32%) had bone sclerosis, 8 (11%) had periodontal disease-like bone loss and 8 (11%) had bone resorption. The level of inter-observer agreement among the three observers on identifying the four types of changes at their sites of occurrence is presented in Table 1. There was substantial agreement only on the detection of the most common change of widened periodontal ligament space (WPLS). Considering this high rate of agreement and the high frequency of detection of WPLS changes, the study was focused on analysis of the WPLS change. Bone sclerosis, periodontal disease-like bone loss and bone resorption were not further analyzed because of the high variability among the three observers in detecting and outlining regions of these bone changes. This variability was likely due to random image distortion from one panoramic image to the next and also in the difficulty for the observer to try to transfer observations from these variable panoramic images to a standard diagram.
Table 1. Results of Kappa analysis for inter-observer variability among the three observers in detecting the four types of bone changes at their sites of occurrence, using the Landis and Koch interpretation (67)

<table>
<thead>
<tr>
<th>Type of Jaw Bone Changes</th>
<th>Kappa Statistic</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Widened periodontal ligament space</td>
<td>0.61</td>
<td>Substantial agreement</td>
</tr>
<tr>
<td>Bone sclerosis</td>
<td>0.22</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>Periodontal disease-like bone loss</td>
<td>0.38</td>
<td>Fair agreement</td>
</tr>
<tr>
<td>Bone resorption</td>
<td>0.33</td>
<td>Fair agreement</td>
</tr>
</tbody>
</table>
3.1.2. Cumulative Incidence Rate of WPLS

The imaging demographics of the 66 subjects with WPLS change and the 51 subjects with no change detected are summarized in Table 2. A time-to-event analysis adjusting for competing risks was performed to estimate the cumulative incidence rate of WPLS detection in the study sample for three reasons. First, all 117 subjects had different numbers of follow-up panoramic images that were taken at different time periods after IMRT. Second, although the 51 subjects in the No Change subgroup did not have WPLS detected within their follow-up time periods in the study, the possibility exists that these subjects may develop WPLS beyond the study time frame. Therefore, these data offered partial information on the incidence of WPLS, and 41 subjects were censored from the time-to-event analysis after no change was detected on their last panoramic image available within the study time frame. The remaining 10 subjects of the 51 with no change detected were competing risks. Competing risks are conditions that preclude the possibility of detecting WPLS, and were identified in this study as complete edentulism and subject death before WPLS detection.
Table 2. Imaging demographics of subjects with WPLS Change and No Change

<table>
<thead>
<tr>
<th>Number of panoramic images available after IMRT</th>
<th>WPLS Change n = 66 (%)</th>
<th>No Change n = 51 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46 (70%)</td>
<td>40 (78%)</td>
</tr>
<tr>
<td>2</td>
<td>15 (23%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>3</td>
<td>4 (6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>4</td>
<td>1 (1%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Follow-up time periods of panoramic images after IMRT</th>
<th>WPLS Change</th>
<th>No Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 - 12 months</td>
<td>14 (21%)</td>
<td>8 (16%)</td>
</tr>
<tr>
<td>13 - 24 months</td>
<td>23 (35%)</td>
<td>16 (31%)</td>
</tr>
<tr>
<td>25 - 36 months</td>
<td>19 (29%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>37 - 48 months</td>
<td>6 (9%)</td>
<td>14 (27%)</td>
</tr>
<tr>
<td>49 - 60 months</td>
<td>4 (6%)</td>
<td>4 (8%)</td>
</tr>
</tbody>
</table>
Figure 4 shows the cumulative incidence rate of WPLS as determined by the time-to-event analysis adjusting for competing risks. The 1-, 2- and 3-year cumulative incidence rates of WPLS detection were 12.2% [95% confidence interval (CI): 5.8% - 18.6%], 34.2% (95% CI: 24.5% - 44.0%) and 55.4% (95% CI: 45.3% - 65.6%), respectively. In other words, by the first, second and third year after IMRT, 12.2%, 34.2% and 55.4% of the subjects in the study sample had WPLS detected on panoramic imaging, respectively. The time of the median cumulative incidence rate of WPLS was estimated to be 29 months.
Figure 4. Cumulative incidence rate of WPLS. Time-to-event analysis adjusted for competing risks on the cumulative incidence rate of WPLS detection on panoramic imaging after IMRT. Dotted lines represent the 95% confidence interval for WPLS detection.
3.2. Effect of Subject Demographical and Clinical Characteristics on WPLS

Table 3 summarizes the subject characteristics of the WPLS Change and No Change subgroups. Chi-squared, Fisher's exact and t-tests found no statistical significant differences in the subject characteristics between the two subgroups (P>0.05). However, these statistical analyses assume that all subjects were monitored for the presence or absence of WPLS over a specific time span, and do not account for the different follow-up time periods. Moreover, the Chi-squared, Fisher's exact and t-tests do not adjust for competing risks. Thus, the correct statistical analysis to determine whether the subject demographical and clinical characteristics affect WPLS is the time-to-event per subject analysis using the Fine and Gray hazards regression model for competing risks. Two subject characteristics, namely history of corticosteroid and bisphosphonate use, were omitted from the regression model because the small number of subjects with these characteristics precludes a meaningful statistical analysis.
Table 3. Subject characteristics of the WPLS Change and No Change subgroups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>WPLS Change n = 66 (%)</th>
<th>No Change n = 51 (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>54 ± 11</td>
<td>57 ± 11</td>
<td>0.14</td>
</tr>
<tr>
<td>Range</td>
<td>30 - 81</td>
<td>32 - 79</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td>0.65</td>
</tr>
<tr>
<td>Male</td>
<td>40 (61%)</td>
<td>33 (65%)</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>26 (39%)</td>
<td>18 (35%)</td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td>0.28</td>
</tr>
<tr>
<td>Yes</td>
<td>5 (8%)</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>61 (92%)</td>
<td>44 (86%)</td>
<td></td>
</tr>
<tr>
<td>Corticosteroid use#</td>
<td></td>
<td></td>
<td>0.44</td>
</tr>
<tr>
<td>Yes</td>
<td>0 (0%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>66 (100%)</td>
<td>50 (98%)</td>
<td></td>
</tr>
<tr>
<td>Bisphosphonate use#</td>
<td></td>
<td></td>
<td>0.50</td>
</tr>
<tr>
<td>Yes</td>
<td>2 (3%)</td>
<td>0 (0%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>64 (97%)</td>
<td>51 (100%)</td>
<td></td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
<td>0.12</td>
</tr>
<tr>
<td>Never</td>
<td>28 (42%)</td>
<td>14 (27%)</td>
<td></td>
</tr>
<tr>
<td>Former</td>
<td>24 (36%)</td>
<td>28 (55%)</td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>14 (22%)</td>
<td>9 (18%)</td>
<td></td>
</tr>
<tr>
<td>Alcohol history</td>
<td></td>
<td></td>
<td>0.29</td>
</tr>
<tr>
<td>Yes</td>
<td>39 (59%)</td>
<td>35 (69%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>27 (41%)</td>
<td>16 (31%)</td>
<td></td>
</tr>
<tr>
<td>Cancer surgery before IMRT</td>
<td></td>
<td></td>
<td>0.45</td>
</tr>
<tr>
<td>Yes</td>
<td>19 (29%)</td>
<td>18 (35%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>47 (71%)</td>
<td>33 (65%)</td>
<td></td>
</tr>
<tr>
<td>Concurrent chemotherapy</td>
<td></td>
<td></td>
<td>0.37</td>
</tr>
<tr>
<td>Yes</td>
<td>34 (52%)</td>
<td>22 (43%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>32 (48%)</td>
<td>29 (57%)</td>
<td></td>
</tr>
</tbody>
</table>

*Two-tailed t-tests were performed for the continuous variable of age.

#Two-tailed Fisher’s exact tests were performed for the discrete variables of corticosteroid use and bisphosphonate use.

Chi-squared tests compared the two subgroups for all other variables.
Table 4 summarizes the results of the univariate analysis using the Fine and Gray method. None of the subject characteristics examined had statistically significant effects on the time to WPLS detection per subject. However, the univariate analysis does not adjust for other subject characteristics that may affect the time to WPLS detection. Thus, a multivariate analysis was required, results of which are presented after the univariate analysis for the dose variable.
Table 4. Univariate analysis using Fine and Gray model for competing risks to determine the effect of subject characteristics on the time to WPLS detection per subject on panoramic imaging after IMRT

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.986</td>
<td>0.968 - 1.004</td>
<td>0.137</td>
</tr>
<tr>
<td>Female sex</td>
<td>1.133</td>
<td>0.705 - 1.821</td>
<td>0.606</td>
</tr>
<tr>
<td>Diabetes</td>
<td>0.594</td>
<td>0.238 - 1.483</td>
<td>0.265</td>
</tr>
<tr>
<td>Current smoker</td>
<td>1.688</td>
<td>0.877 - 3.248</td>
<td>0.117</td>
</tr>
<tr>
<td>Alcohol history</td>
<td>0.876</td>
<td>0.558 - 1.374</td>
<td>0.563</td>
</tr>
<tr>
<td>Cancer surgery</td>
<td>1.067</td>
<td>0.620 - 1.835</td>
<td>0.815</td>
</tr>
<tr>
<td>Concurrent chemotherapy</td>
<td>1.233</td>
<td>0.774 - 1.966</td>
<td>0.378</td>
</tr>
</tbody>
</table>
3.3. Effect of Mean Mandibular Body Dose on WPLS

A time to WPLS detection per tooth analysis was performed to account for the variation in the number of teeth present in each subject of the study sample. Dose data were available for the mandibular body. The total number of mandibular teeth for the dose analysis was 1275, 151 of which had WPLS detected, 1083 of which were censored, and 41 of which were competing risks. The teeth within the right and left sides of the mandibular body were matched to the mean dose delivered to the right and left sides of the mandibular body, respectively. The side-specific mean dose of the mandibular body was designated as mean mandibular body dose in the analysis.

In this study, the mean mandibular body dose had a statistically significant effect on the time to WPLS detection (Hazard ratio = 1.061, 95% CI: 1.042 - 1.079, P<0.001). Previous reports in the appendicular and axial skeleton had demonstrated that 45 Gy was the threshold for radiation-induced extragnathic bone changes visible on plain film imaging (6,8,9). To investigate whether higher mean mandibular body doses might have a different effect than lower doses on the time to WPLS in this study, the doses were stratified at 45 Gy. Teeth in mandibular bodies that received mean doses ≥ 45 Gy were found to have WPLS detected earlier than teeth in mandibular bodies that received mean doses < 45 Gy (Hazard ratio = 2.443, 95% CI: 1.617 - 3.693, P<0.001; Figure 5).
Figure 5. Effect of mean mandibular body dose on WPLS per tooth. Difference in the time to WPLS detection on panoramic imaging after IMRT as stratified by a mean mandibular body dose of 45 Gy.
3.4. Multivariate Fine and Gray Regression Model for Competing Risks

The variables of subject age, sex, diabetes, smoking history, alcohol history, cancer surgery, concurrent chemotherapy and mean mandibular body dose, both as a continuous variable and as a categorical variable stratified at 45 Gy, were incorporated into a multivariate Fine and Gray regression model for competing risks. Table 5 summarizes the results of the final multivariate analysis. Only the variables of subject sex, mean mandibular body dose and mean mandibular body doses ≥ 45 Gy had statistically significant effects on the time to WPLS detection.
Table 5. Multivariate analysis using Fine and Gray model for competing risks

<table>
<thead>
<tr>
<th>Predictors of WPLS</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>1.493</td>
<td>1.096 - 2.034</td>
<td>0.011</td>
</tr>
<tr>
<td>Mean mandibular body dose</td>
<td>1.059</td>
<td>1.040 - 1.077</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean mandibular body doses ≥ 45 Gy</td>
<td>2.377</td>
<td>1.569 - 3.599</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Chapter 4

4. Discussion

This research is the first large case series to document and characterize subclinical jaw bone changes that occur on panoramic imaging after head and neck radiotherapy. This study found that widened periodontal ligament space (WPLS) was the most common change followed by bone sclerosis, periodontal disease-like bone loss and bone resorption. A time-to-event analysis estimated the median time at which WPLS changes became radiographically visible to be approximately two and a half years after radiotherapy. WPLS was found to appear earlier in female subjects and mandibular bodies that had received mean doses ≥ 45 Gy.

4.1. Jaw bone changes were visible on panoramic imaging after IMRT

Three observers independently detected four post-IMRT jaw bone changes on panoramic imaging in 60% of the study sample. Substantial agreement was found for the most frequent change of WPLS. Bone sclerosis, periodontal disease-like bone loss and bone resorption had fair agreement. A possible reason for these findings is that anatomic ghost shadows inherent in panoramic images might have blurred regions of bone sclerosis and resorption. Thus, the observers might not have appreciated slight increases in radiopacity and decreases in radiolucency on the radiographs. However, the radiolucent periodontal ligament space has high contrast between the adjacent radiopaque tooth root and alveolar process. This in turn might have allowed for WPLS to be more readily visualized than bone sclerosis and resorption. Periodontal disease-like bone loss might have been underestimated in the study because panoramic imaging is not the modality of choice to assess the height of the alveolar process. The x-ray beam angulation in panoramic imaging is not aligned perpendicular to the long axis of the teeth and distorts the view of the height of the alveolar process in relation to the cementoenamel junctions and roots of the teeth. The resolution of panoramic imaging is also inadequate to detect subtle changes in the height of the alveolar process. Thus, due to the high rate of observer
agreement and high frequency of detection, WPLS was determined to be the most reliable radiographic indicator of post-IMRT change on panoramic imaging in this study.

This study found that 12.2%, 34.2% and 55.4% of subjects had WPLS detected on panoramic imaging by the first, second and third year after IMRT, respectively. The median time to WPLS detection on panoramic imaging was 29 months after IMRT. No other reports have documented the rate of WPLS as only two single case reports are available for comparison (45,46). Fujita et al. first reported WPLS on periapical radiographs of one patient taken six months after radiotherapy (45). In contrast, Yusof and Bakri detected WPLS on a panoramic radiograph of another patient taken three years after radiotherapy (46). However, the likelihood that WPLS was visible before this three year mark could not be excluded because Yusof and Bakri did not have radiographs at earlier time points available for review (46). Similarly, the estimated cumulative incidence rate of WPLS in the first year after IMRT in this study might be low because approximately 75% of the subjects had only one panoramic image after IMRT, and approximately 80% of these images were dated more than one year after IMRT. Thus, the incidence rate of post-IMRT WPLS as determined by this study might underestimate the true incidence and timing because the numbers and time periods of the follow-up panoramic images available were highly variable. As the literature currently does not have a large case series for comparison, further investigation of the incidence rate of post-IMRT WPLS using a large study sample and panoramic images made at set time points with narrower follow-up intervals is warranted.

The mechanism of WPLS changes is currently unknown. Two animal studies had independently demonstrated histologic features of inflammation within the periodontal ligament fibers of teeth that had radiographic WPLS changes (35,36). At two months post-irradiation, the thin, densely packed and organized periodontal ligament fibers became infiltrated by acute inflammatory cells. The fibers lost their orientation, and the periodontal ligament became loose, edematous and widened (35). At six months post-irradiation, another animal study showed that the periodontal ligament was replaced by extensive fibrosis along the entire length of the tooth root (36). Fibrosis is a characteristic outcome of chronic inflammation and represents replacement of injured tissue by collagen (22). Although these radiographic-histologic correlations were derived from different studies, their findings suggest that acute and chronic inflammation of the
irradiated periodontal ligament fibers might be the disease mechanism of radiographic WPLS changes. Inflammation of the entire periodontal ligament shown histologically could be correlated to the lack of an epicenter of WPLS. Although the state of the irradiated lamina dura was not reported in either animal study (35,36), the inflammatory infiltrate might have increased the volume of the periodontal ligament, and caused pressure resorption of the adjacent lamina dura to expand the periodontal ligament space. As the inflammatory response subsided with time, the periodontal ligament and resorbed bone were replaced by fibrosis, and bone remodeling reestablished the lamina dura. Although this speculation is derived from animal studies, human patients with systemic sclerosis have similar widening of the radiographic periodontal ligament space due to extensive collagen deposition within the periodontal ligament (39). Therefore, radiation-induced fibrosis of the periodontal ligament may still be a plausible mechanism of WPLS changes in this study.

The exfoliation of teeth with WPLS and breakdown of adjacent periodontal bone, as documented in the two published case reports (45,46), might indirectly support the proposed inflammatory mechanism for radiographic WPLS changes. Fibrosis of the irradiated periodontal ligament might compromise the shock-absorbing function of the fibers, such that occlusal forces might no longer be resisted as effectively. Consequently, heavy occlusion on teeth with WPLS might not be cushioned by the fibrotic periodontal ligament fibers. The possibility of damage to the alveolar process with time increases, particularly because the ability of irradiated bone to remodel is compromised. This effect might eventually lead to periodontal bone loss, increased tooth mobility and exfoliation. However, tooth loss and alveolar process destruction might not be the only sequelae of WPLS because these outcomes were derived from two case reports (45,46). WPLS could stabilize with time because theoretically, the fibrosed periodontal ligament is no longer inflamed. Future long-term studies that monitor subjects with WPLS changes may provide additional insight in the prognosis of affected teeth. The effect of occlusion on teeth with WPLS can also be examined as a predisposing factor of potential tooth loss. In summary, radiation-induced fibrotic changes of the periodontal ligament fibers might account for the radiographically visible widening of the periodontal ligament space.

The focus of this study was primarily on WPLS changes after IMRT. Although bone sclerosis, resorption and periodontal disease-like bone loss were also apparent on the panoramic images,
further statistical investigation was not performed due to the small number of cases identified and the low rate of observer agreement. However, ionizing radiation causes damage to all components of mature bone (7,20-29), and may contribute to the development of these three changes. The early, radiation-induced increase in osteoclastic function after low-dose radiation to the mature skeleton might account for the bone resorptive changes (20,26,27). Bone sclerosis might follow radiation-induced resorption because osteoclastic resorption releases osteoblastic stimulatory factors from the bone matrix to increase osteoblast differentiation and bone formation (17,18). Periodontal disease-like bone loss might be secondary to occlusal trauma of teeth with WPLS changes, as discussed previously. Furthermore, the alveolar process adjacent to WPLS changes likely received similar doses during IMRT and thus, bone remodeling induced by occlusal stress might be compromised. Nevertheless, the validity of these speculations is unknown because the complex pathophysiologic mechanism of irradiated mature bone remains to be elucidated.

In this study, WPLS was the major radiographic finding in subjects who were treated with IMRT for head and neck cancer. The clinical significance of this finding is that WPLS represents a radiographic sequela of head and neck radiotherapy. WPLS is likely due to radiation-induced fibrosis of the entire periodontal ligament, and does not have an epicenter as seen in periodontitis and periapical inflammatory disease. To avoid misdiagnosis and unnecessary endodontic therapy, flap surgery or other periodontal disease interventions, post-radiotherapy WPLS and its radiographic differentiating feature need to be incorporated into continuing educational courses on dental management of the cancer survivor after radiotherapy. Furthermore, extraction of teeth with WPLS needs to be avoided because dental surgery in the irradiated jaw increases the risk of osteoradionecrosis (40,45,46,49,52,55,59,61,62). Therefore, dental intervention is not required for WPLS because this radiation-induced change is not a sign of common dental disease. Clinical vitality testing of teeth with WPLS would determine their pulpal status to substantiate a non-endodontic, non-surgical approach to WPLS. This study did not have long-term, follow-up panoramic images available to determine the prognosis of teeth with WPLS. However, annual radiographic follow-up may be prescribed to monitor whether WPLS changes regress or stabilize with time, or lead to tooth mobility and exfoliation, as reported previously (45,46). In the event that affected teeth do become loose, as documented in two case reports (45,46), the teeth can be
splinted to minimize mobility but not extracted. Thus, WPLS signals a history of head and neck radiotherapy and watchful waiting appears to be the most appropriate management at present.

4.2. Of the subject comorbidities examined, subject sex had an effect on WPLS

This study found that subjects of the female sex had WPLS detected earlier than subjects of the male sex. Males and females have immunological differences (68). Females tend to be more prone to autoimmune diseases, suggesting that their immune system may be more active than males (68). Radiographic widening of the periodontal ligament space was histologically shown in animal studies to represent inflammatory dissolution of the ligament fibers and eventual fibrosis (35,36). Thus, the inflammatory reaction of the irradiated fibers might have been more pronounced in female subjects, such that they had WPLS detected earlier than male subjects. However, the immunological differences between the sexes remain an active topic of research and may not entirely explain the biological significance of this finding. Moreover, the earlier time to WPLS detection might be an artifact because female subjects in this study had on average their first post-IMRT follow-up panoramic image earlier than male subjects.

Subject age and comorbidities of diabetes, smoking and alcohol history, cancer surgery and chemotherapy concurrent with IMRT did not have statistically significant effects on the time to WPLS detection in this study. The age distribution was not statistically different between subjects who had WPLS detected and those who did not have any bone change identified. The number of subjects who had diabetes was small, and the statistical power to detect a true difference in the time to WPLS detection for this comorbidity was low. Likewise, bisphosphonate use and corticosteroid use were two subject comorbidities that were omitted from the regression analysis because there were only two bisphosphonate users and one corticosteroid user in the study sample. Further investigation with more subjects who are diabetic and use bisphosphonates and corticosteroids would be of interest.

Smoking and alcohol history were not found to affect the time to WPLS detection. Smoking causes vasoconstriction (52). WPLS was histologically shown to represent inflamed periodontal ligament fibers and eventual fibrosis (35,36). Blood vessels are dilated in the inflammatory
response for leukocytes to arrive at the site of injury (22). Thus, the periodontal ligament fibers might not have been inflamed in smokers because vasoconstriction reduces the diameter of the blood vessels and hinders delivery of leukocytes. Alcohol use is known to predispose subjects to osteopenia and osteoporosis (69). However, the WPLS changes were often isolated and identified in the absence of adjacent bone resorptive changes. Thus, alcohol might not have a significant biologic effect on WPLS.

Cancer surgery may involve removal of the soft tissue malignancy or resection of part of the mandible or maxillae in the presence of bone invasion (30,60). Excision of cancer limited to oral mucosa does not affect adjacent osseous structures. Similarly, mandibular resection followed by surgical grafting removes the teeth and alveolar process such that changes to the periodontal ligament space are no longer possible. Thus, cancer surgery was not expected to be associated with WPLS.

Chemotherapy concurrent with IMRT had no effect on the time to WPLS detection in this study. Most chemotherapeutic agents target cells that have a high mitotic rate (22). Cells of the periodontal ligament, consisting mostly of undifferentiated mesenchymal cells and fibroblasts, are not actively dividing (70). Thus, chemotherapy likely did not have an effect on the periodontal ligament.

4.3. Mean mandibular body dose had an effect on WPLS

Mean mandibular body dose was found to have a statistically significant association with WPLS over time, with doses ≥ 45 Gy related to earlier change after IMRT as compared with doses < 45 Gy. These findings provide statistical evidence to strengthen the suggestion that post-radiotherapy bone changes seen in the axial and appendicular skeleton are radiation-induced (6,8,9). This study did not have the data to estimate a potential threshold dose for WPLS changes because tooth-specific doses would be required for such an analysis. In the jaws, a threshold dose for these changes has not been reported. The published literature has two case reports that showed WPLS in jaws that were irradiated at a total dose of 60 Gy (45,46). The total dose might not be the same as the dose at the site of WPLS, and cannot be regarded as the threshold dose for this change. Similarly, the side-specific, mean mandibular body doses
available for this study might not be as accurate as tooth-specific doses. However, the side-specific nature of the doses did allow for a more representative correlation between dose and WPLS than whole mandibular body doses. Thus, tooth-specific doses are required to estimate the threshold dose for WPLS.

4.4. Future Directions

A long-term, prospective study may determine the prognosis of teeth with post-IMRT WPLS to help establish appropriate follow-up intervals and management. The treatment and sequela of WPLS need to be determined and cannot be deduced from two case reports. Dentists are not encouraged to treat teeth with WPLS for fear of potentiating the development of osteoradionecrosis. Such a prospective study should also include imaging of subjects at narrow, set time intervals. This would allow for a better estimation of the incidence rate of WPLS after radiotherapy. Moreover, enrollment of more subjects with comorbidities such as diabetes, bisphosphonate and corticosteroid use would permit a more meaningful statistical analysis.

The effect of different occlusal forces should be investigated as an additional, potential predictor of WPLS. Subjects who are bruxers may be more likely than non-bruxers to develop WPLS. A primary function of the healthy periodontal ligament fibers is to resist occlusal forces. Heavy occlusal forces may further compromise the irradiated periodontal ligament and aggravate the inflammatory response. A study that compares the likelihood of post-IMRT WPLS changes in bruxers and non-bruxers may determine whether heavy occlusal forces predispose subjects to WPLS.

WPLS and osteoradionecrosis are two effects of ionizing radiation on bone. However, it is not known whether WPLS will eventually evolve into osteoradionecrosis. Given that ionizing radiation induces both conditions, WPLS may precede osteoradionecrosis at sites of change. Following subjects clinically and radiographically for WPLS and osteoradionecrosis long-term may determine whether such a relationship exists. The significance of this finding will enable clinicians to monitor areas with WPLS more closely for development of osteoradionecrosis. Conversely, if WPLS is found to appear on imaging independently of osteoradionecrosis, subject
comorbidities and radiation dose of the two groups can be compared to determine the set of potential predictors that favour development of WPLS in lieu of osteoradionecrosis.

Development of a panoramic-like outline showing the dose distribution of the maxillae and mandible from IMRT may serve as a research and clinical tool to map areas of bone changes. This visual tool facilitates correlation of high and low dose areas to the presence and absence of changes, respectively. Furthermore, tooth-specific doses may allow for a more accurate threshold dose to be determined for WPLS. From a clinical perspective, such panoramic dose maps may help direct dentists to monitor high-dose regions selectively and serve to educate patients. Radiation oncologists may also use these data to devise treatment plans that further limit the dose received by the jaws to minimize the risk of WPLS.
Chapter 5

5. Conclusions

This study is the first case series to show that WPLS is a common radiographic finding after head and neck radiotherapy. Radiation exposure was found to be associated with WPLS over time, with doses \( \geq 45 \) Gy having the change visible on panoramic imaging earlier than doses \( < 45 \). Post-IMRT WPLS should be recognized and differentiated from the type of widening of the periodontal ligament space secondary to odontogenic inflammatory disease so that needless therapy is avoided. Furthermore, extraction of teeth with WPLS is contraindicated as surgery may precipitate the development of osteoradionecrosis. The finding that female subjects were correlated to a decreased time to WPLS warrants further investigation. Nevertheless, this study has laid the groundwork for future research on post-radiotherapy WPLS changes.
References


(52) Katsura K, Sasai K, Sato K, Saito M, Hoshina H, Hayashi T. Relationship between oral health status and development of osteoradionecrosis of the mandible: a retrospective


Appendix 1

Diagnostic Criteria for Osteoradionecrosis

Department of Dental Oncology, Princess Margaret Hospital

1. Bone exposure greater than one month’s duration greater than 1 cm in size; or

2. Bone exposure greater than one month’s duration with two or more contiguous sites, each measuring greater than 5 mm in size; or

3. Bone exposure greater than 3 months' duration between 5 mm and 1 cm in size; or

4. No bone exposure but oral/skin fistula and radiographic evidence of sequestra formation and/or inflammatory periosteal bone formation; or

5. Pathologic fracture with radiographic changes consistent with sequestra formation with or without periosteal bone formation; or

6. No soft tissue changes but obvious radiographic evidence of sequestra formation and/or inflammatory periosteal bone formation
Appendix 2

Instructions to Observers for Radiographic Data Collection

Important Points:

- The materials which are provided to you for data collection include:
  - This instruction sheet;
  - A set of brown envelopes, each of which contains the panoramic radiographs belonging to a subject of the study;
  - A set of diagrams of a panoramic outline to record the jaw bone changes (Appendix 3);
  - A sheet outlining the standardized set of symbols to be used to record the changes (Appendix 4);
  - A blue marker and a red marker to record the changes;
  - A stapler;
  - Two light boxes for viewing the panoramic radiographs; and
  - A computer with access to a DICOM viewer (eFilm version 2.1.2; Merge Healthcare, Milwaukee, WI) for the digital panoramic images

- On the front of each brown envelope are the subject’s medical record number (MRN), the subject’s last and first names, and the total number of panoramic images available. The number of film-based and digital images are also indicated.

- Collect data case by case. Only move onto the next subject after all images for the present subject have been examined, their findings recorded and the radiographs returned to the brown envelope corresponding to that subject.

- The steps for data collection are as follows:-
1. To begin data collection for a subject, turn off the ceiling lights. Turn on the 2 light boxes.

2. Take all panoramic radiographs out of the brown envelope.

3. Organize the images chronologically, from oldest (pre-IMRT baseline image, to which subsequent images are compared) to most recent. If the case has digital images, use the DICOM viewer.

4. On a new diagram of panoramic outline, record the MRN of the subject, your initials, and the date of the corresponding panoramic image. One diagram corresponds to one image.

5. Put the baseline image onto a light box. Look at the baseline image to determine if there are areas of widened periodontal ligament space, bone sclerosis, periodontal disease bone loss, and bone resorption. If none, check the box beside “No Changes” and move onto step 6. If there are one or more of these 4 bone changes, check the box beside “Changes”, record them using the standardized set of symbols onto the diagram before moving to step 6.

6. Set the first diagram aside. Put the next (second) panoramic image onto the second light box. Repeat step 4. Compare this image to the previous image to determine if there are any of the 4 bone changes and record as in step 5.

7. Set the second diagram aside. Replace the previous image on the light box with the next (third in this case) image in the series. Compare the baseline and third images for bone changes and continue to repeat steps 4 through 6. If changes are detected on the third image, compare it to the second image as well to determine if these changes are present on the second image. Record only new changes onto the subsequent diagrams.

8. Repeat steps 4 through 7 until all panoramic images of a subject have been examined and their findings recorded.
9. Staple all diagrams belonging to a subject together and set them aside. Return all images to the brown envelope and close digital images on the computer.

10. Repeat steps 2 through 9 for the next cases.
Appendix 3

Radiographic Data Collection Instrument

MRN _______________________

Observer Initials ______________

Date of Panoramic Image (Month/Year): __________/__________

___No Changes

___Changes: Please draw changes onto the diagram of a panoramic outline below using the standardized set of symbols.
Appendix 4

Standardized Set of Coloured Symbols of Subclinical Jaw Bone Changes

- **Widened periodontal ligament space** – outline the affected teeth with the blue marker

- **Periodontal disease-like bone changes** – outline the level of bone loss with the blue marker

- **Bone sclerosis** – outline the area of bone sclerosis with the red marker

- **Bone resorption** – outline the area of bone resorption with the blue marker
Appendix 5

A. Section of Spreadsheet used for Radiographic Data Collection

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# Appendix 6

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