

Tássio Edno Atanásio Pitorro

**EFEITOS DA RADIAÇÃO IONIZANTE PÓS-CIRÚRGICA NO
REPARO ÓSSEO E NA OSSEOINTEGRAÇÃO DE IMPLANTES:
ANÁLISES MICROTOMOGRÁFICA, HISTOMORFOMÉTRICA,
BIOMECÂNICA E DE SOBREVIDA.**

EFFECTS OF POST-SURGICAL IONIZING RADIATION ON BONE
REPAIR AND OSSEOINTEGRATION OF IMPLANTS:
MICROTOMOGRAPHIC, HISTOMORPHOMETRIC, BIOMECHANIC
AND SURVIVAL ANALYSIS.

Tese apresentada à Faculdade de
Odontologia da Universidade
Federal de Uberlândia, como
requisito parcial para obtenção do
Título de Doutor em Odontologia na
Área de Concentração de Clínica
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Uberlândia, 2023

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Uberlândia, 2023



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Programa de Pós-Graduação em:	Odontologia				
Defesa de:	Tese Doutorado, nº 93,. PPGODONTO				
Data:	Treze de Setembro de Dois Mil e Vinte e Três	Hora de início:	08:30	Hora de encerramento:	12:30
Matrícula do Discente:	11913ODO008				
Nome do Discente:	Tássio Edno Atanásio Pitorro				
Título do Trabalho:	Efeitos da radiação ionizante pós-cirúrgica no reparo ósseo e na osseointegração de implantes: análises microtomográfica, histomorfométrica, biomecânica e de sobrevida				
Área de concentração:	Clínica Odontológica Integrada				
Linha de pesquisa:	Processo de Reparo				
Projeto de Pesquisa de vinculação:	Processo de Reparo				

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P685 2023	Pitorro, Tássio Edno Atanásio, 1992- EFEITOS DA RADIAÇÃO IONIZANTE PÓS-CIRÚRGICA NO REPARO ÓSSEO E NA OSSEOINTEGRAÇÃO DE IMPLANTES: ANÁLISES MICROTOMOGRÁFICA, HISTOMORFOMÉTRICA, BIOMECÂNICA E DE SOBREVIDA. [recurso eletrônico] / Tássio Edno Atanásio Pitorro. - 2023. Orientadora: Priscilla Barbosa Ferreira Soares. Tese (Doutorado) - Universidade Federal de Uberlândia, Pós-graduação em Odontologia. Modo de acesso: Internet. Disponível em: http://doi.org/10.14393/ufu.te.2023.516 Inclui bibliografia. Inclui ilustrações. 1. Odontologia. I. Soares, Priscilla Barbosa Ferreira, 1974-, (Orient.). II. Universidade Federal de Uberlândia. Pós-graduação em Odontologia. III. Título. CDU: 616.314
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Bibliotecários responsáveis pela estrutura de acordo com o AACR2:
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DEDICATÓRIA

Dedico este trabalho à minha mãe (*Beatriz Manuel Rungo Pitorro*), que viveu até o dia 04 de Julho de 2021 e deixou muitas saudades.

AGRADECIMENTOS

O meu primeiro agradecimento vai para o Altíssimo, aquele que me ofereceu a vida e molda o meu destino com infinita sabedoria.

Agradeço aos meus anjos, Yolanda (Esposa), Giany (Filha) e Kiyan (Filho), que compartilham suas vidas comigo, alimentam minha alma e me doam alegria e coragem para suportar os desafios desta vida.

Aos meus Pais, Atanásio Pitorro e Beatriz Manuel Runo Pitorro, fontes da minha vida, espelhos do meu ser. Agradeço por me terem concebido, criado, educado, orientado, mimado, protegido, amado e por estarem constantemente presentes na minha vida, apesar da longa distância (sobretudo a minha mãe que vive agora espiritualmente). Escola foi sempre a principal ferramenta que me indicaram para moldar o meu destino.

Às minhas irmãs, Aura Coimbra e Vânia Pitorro. Agradeço pelas loucuras, risadas e fofocas que partilhamos, pela vossa existência, vossa companhia, amor, encorajamento e tudo que sequer caberia nesta página.

À minha orientadora, Prof^a Priscilla Soares, agradeço pela paciência, dedicação e presença. Abençoado sou por tê-la tido como orientadora. Agradeço imenso por não ter ouvido apenas as minhas questões académicas, mas também por ter demonstrado bastante afeto para comigo e com a minha família. Certamente que levarei suas atitudes como inspiração para minhas futuras orientações.

Ao Prof^o Carlos Soares, uma das primeiras pessoas como quem me comuniquei aquando da minha chegada em Uberlândia, pôs-se à disposição para me apoiar em qualquer situação e deu várias contribuições para a execução deste trabalho. Agradeço bastante.

Aos meus colegas da área de Periodontia e Implantodontia, Caroline Garcia, Clara Bites, Dhiancarlo Macedo, Elisangela Rodrigues, Guilherme Gonçalves, Jéssica Rodrigues, Juliana Borges, Milena Irie, Nayara Texeira, Nurys Prisinoto, Roberta Alves e Thaisa Carrera, que têm sido companheiros dignos do meu maior respeito, sempre prontos para me apoiar e ensinar. Não são apenas colegas, mas sobretudo amigos que têm morada garantida no meu coração. Têm o meu agradecimento.

À Juliana Borges, em especial, agradeço por me ter apoiado de forma incondicional, sobretudo no início do meu contato com a área de Periodontia e Implantodontia. Sua presença foi crucial para a execução deste trabalho.

Agradeço à Jéssica Afonso por ter apoiado na projeção e execução da parte de experimentação animal e por partilhar seus conhecimentos sem limitações.

Aos professores, colegas, técnicos e da área de Patologia e Diagnostico Bucal, Profº Paulo de Faria, Profº Adriano Loyola, Profº Sérgio Cardoso, Anaíra Ribeiro, Marcelo Garcia, Danielly Correia, Pedro Jayme, Débora Oliveira, Lúbia Cristina, Neide Moura, agradeço bastante por terem me acolhido e orientado, numa altura em que ainda não tinha norte e sabia quase nada de Patologia.

Aos profissionais do Departamento de Radiologia da Universidade Federal do Triangulo Mineiro, Francisco Américo Marcelino e Adriano Luiz Bianchini, agradeço bastante por nos terem acolhido e realizado a irradiação dos animais incluídos neste trabalho.

A todos professores e técnicos da faculdade, eu agradeço bastante pelo apoio disponível sempre que precisei.

À CAPES, ao CNPq e à FAPEMIG pela bolsa de doutorado e pelo apoio financeiro para execução das atividades de pesquisa, vai o meu agradecimento.

Agradeço ao Governo Brasileiro que, através de suas instituições permitiu a realização deste grande sonho de me tornar Doutor, sonho esse que até 5 anos atrás eu considerava uma verdadeira utopia, completamente impossível.

Agradeço igualmente ao Governo do meu País, Moçambique, pelo apoio prestado durante a minha formação.

KHANIMABO

(Obrigado)

EPÍGRAFE

"O que faz andar a estrada? É o sonho. Enquanto a gente sonhar a estrada permanecerá viva"
(Mia Couto)

SUMÁRIO

Resumo		08
Palavras-chave		08
Abstract		09
Keywords		09
1. Introdução e referencial teórico		10
1.1. Radioterapia		11
1.2. Osseointegração de implantes de titânio		12
1.3. Radioterapia e a osseointegração de implantes		12
1.4. Microtomografia computadorizada		13
2. Objetivos		15
2.1. Objetivos específico		15
3. Capítulos		16
3.1. Capítulo 1	Survival of Dental Implants Placed Pre-Radiotherapy Versus Post-Radiotherapy in Native Bone: A Systematic Review	16
3.2. Capítulo 2	Microtomographic analysis of surgically-created bone defects in rats irradiated after surgery: manual versus predefined region-of-interest definition methods	31
3. Capítulo 3	Biological and biomechanical evaluation of the effects caused by postoperative radiotherapy on the osseointegration of titanium implants in rats	45
4. Conclusões		67
Referências		68
Anexos		72

RESUMO

A Radioterapia (RT) é uma das estratégias terapêuticas utilizadas no tratamento do câncer, podendo estar associada à cirurgia e à quimioterapia. Apesar da sua eficiência, efeitos deletérios têm sido relatados na literatura. Parte desses efeitos interferem negativamente no metabolismo ósseo, podendo afetar o reparo de lesões, a osseointegração e a sobrevida de implantes dentários. Uma das metodologias utilizadas para avaliar esses efeitos é a microtomografia computadorizada (micro-CT) que, apesar de ter sido bastante estudada, algumas questões permanecem por esclarecer. Antes da realização da RT, pacientes com câncer de cabeça e pescoço passam por uma avaliação oral onde são detetados e eliminados focos de infecção, culminando geralmente com a extração de dentes, havendo necessidade de reabilitar esses pacientes, afim de restituir a função mastigatória e melhorar a qualidade de vida. Tradicionalmente, a instalação de implantes é realizada após a RT, alternativamente, pode ser realizada previamente. Neste contexto, 3 objetivos específicos foram definidos para o presente estudo: *objetivo específico 1*: Avaliar sistematicamente a taxa de sobrevida de implantes dentários instalados pré e pós-radioterapia. O estudo demonstrou que implantes instalados pré e pós-radioterapia tiveram altas taxas de sobrevida, semelhantes às de implantes instalados sem RT associada; *Objetivo específico 2*: avaliar a diferença entre os métodos manual e pré-definido para definição de região de interesse durante a análise de defeitos ósseos através da micro-TC e avaliar o processo de reparo do osso submetido à radioterapia após a cirurgia. Este estudo comprovou que, apesar do método manual ser mais demorado em comparação com o pré-definido, os resultados fornecidos por esse método refletiram um processo de reparo mais avançado, não tendo havido diferença significativa no reparo entre o osso irradiado e não irradiado. *Objetivo específico 3*: avaliar a influência da radiação ionizante na osseointegração de implantes de titânio instalados previamente à RT em ratos. Os resultados demonstraram que a influência da RT sobre a osseointegração dos implantes instalados previamente não foi significativa.

PALAVRAS-CHAVE: Implante dentário; Radioterapia; Osseointegração; Microtomografia por Raio-X

ABSTRACT

Radiotherapy (RT) is one of the strategies used for to treat cancer, which may be associated with surgery and chemotherapy. Despite its efficiency, deleterious effects have been reported in the literature. Part of these effects negatively interfere with the bone metabolism, which may affect bone repair, osseointegration and the survival of dental implants. One of the methodologies used to assess these effects is micro-computed tomography (micro-CT) which, despite having been extensively studied, some issues remain to be clarified. Before be submitted to RT, patients with head and neck cancer undergo an oral screening where oral foci are detected and eliminated, usually culminating in teeth extraction, with the need to rehabilitate these patients in order to restore masticatory function and improve their quality of life. Traditionally, implant placement is performed after RT, alternatively it can be performed previous. In this context, 3 specific objectives were defined for the present study: *Specific objective 1*: Systematically evaluate the survival rate of dental implants installed pre- and post-radiotherapy. The study demonstrated that implants installed pre- and post-radiotherapy had high survival rates, similar to those of implants installed without associated RT; *Specific objective 2*: to evaluate the difference between the manual and pre-defined methods for defining the region of interest during the analysis of bone defects using micro-CT and to evaluate the repair process of the bone submitted to radiotherapy after surgery. This study proved that, despite the manual method be time consuming compared to the predefined one, the results provided by this method reflected a more advanced repair process, with no significant difference in repair between irradiated and non-irradiated bone. *Specific objective 3*: to evaluate the influence of ionizing radiation on the osseointegration of titanium implants installed prior to radiotherapy in rats. The results showed that the influence of RT on the osseointegration of implants installed prior to radiotherapy was not significant.

KEYWORDS: Dental implant; Radiation therapy; Osseointegration; X-Ray Microtomography

1. INTRODUÇÃO E REFERENCIAL TEÓRICO

O câncer de cabeça e pescoço está entre os cânceres mais comuns a nível mundial (Gormley *et al.*, 2022; Barsouk *et al.*, 2023), com três quartos dos casos diagnosticados em países subdesenvolvidos (Di Carlo *et al.*, 2019). A Radioterapia é uma abordagem comumente utilizada para o seu tratamento, podendo ser associada à quimioterapia e à cirurgia (Dréno *et al.*, 2020). Como consequência das cirurgias ressectivas podem surgir problemas anatômicos e funcionais para os pacientes (Nobrega *et al.*, 2016), com potencial para perturbações significativas na fala, mastigação e deglutição, bem como na aparência dentária e facial, impactando negativamente na estética e na qualidade de vida (Moore *et al.*, 2019), podendo causar um desconforto psicológico considerável (Carini *et al.*, 2012).

A radiação ionizante causa alterações na composição e microarquitetura do tecido ósseo, uma vez que danifica os seus componentes orgânicos e minerais (Mendes *et al.*, 2020). Apesar dos equipamentos de radioterapia modernos possuírem tecnologia que aumenta a eficácia e precisão do tratamento, reduzindo os efeitos adversos e possibilitando o tratamento inclusive de cânceres de difícil acesso ou resistentes (Giraud *et al.*, 2022), alguns efeitos adversos continuam sendo reportados na literatura, nomeadamente, hipóxia, hipocelularidade e hipovascularidade (Nobrega *et al.*, 2016; Mendes *et al.*, 2020), ao nível dos tecidos, o que vai se traduzir na fraca resistência contra o trauma e infecções, para além da própria osteorradionecrose (Di Carlo *et al.*, 2019). Fatores como a dose total da radiação, volume do osso irradiado e, sobretudo o trauma cirúrgico são os principais fatores associados ao desenvolvimento da osteorradionecrose. Esses eram os principais motivos para a não instalação de implantes em pacientes irradiados, nos anos passados (Carini *et al.*, 2012).

Uma das metodologias mais utilizadas para avaliar as propriedades do tecido ósseo é a microtomografia (micro-CT) (Irie *et al.*, 2018), considerada como um exame de imagem de alta resolução e não destrutivo (Nascimento *et al.*, 2019), no qual as imagens são coletadas de múltiplos ângulos de visão ao redor da amostra e são reconstruídas tridimensionalmente em escala micrométrica, fornecendo informações sobre a geometria tridimensional e propriedades do tecido (Bouxsein *et al.*, 2010; Clark & Badea, 2014). Algumas variáveis associadas à obtenção, processamento e análise por micro-CT podem afetar os resultados morfológicos obtidos. Uma dessas variáveis é a definição da região de interesse.

Em pacientes que serão tratados com radiação ionizante, pode haver a necessidade de se realizarem extrações dentárias na área a ser irradiada para prevenir a osteorradionecrose e consequentemente, vários pacientes tornam-se edêntulos (Koudougou *et al.*, 2020; Printzell *et al.*, 2023) e esses pacientes demonstram necessidade e motivação para reabilitação dentária, no entanto, as mudanças anatômicas e fisiológicas causadas pela cirurgia, acabam dificultando a correta adaptação e funcionalidade de próteses removíveis (convencionais) (Ch'ng *et al.*, 2016; Zarzar *et al.*, 2023). Com a crescente taxa de sobrevida dos pacientes acometidos pelo câncer (Barsouk *et al.*, 2023), é indicada a instalação de implantes osseointegráveis, os quais serão responsáveis por reter e suportar a prótese, melhorando substancialmente a qualidade de vida e o conforto dos pacientes (Nobrega *et al.*, 2016). Por outro lado, os implantes osseointegrados contribuem para a retenção da altura óssea, enquanto as próteses convencionais estão associadas à perda óssea gradual (Ch'ng *et al.*, 2016). A reabilitação desses pacientes com recurso a implantes dentários, constitui um grande desafio que, em parte é amenizado pelo uso de implantes com superfície bioativas, as quais aceleram a osseointegração (Smeets *et al.*, 2016).

1.1. RADIOTERAPIA

Antes do advento da radioterapia, a cirurgia era o único tratamento curativo disponível para pacientes com câncer (Wicker *et al.*, 2022). Atualmente a radiação ionizante é utilizada como método terapêutico em 60-70% dos pacientes com câncer (Papi *et al.*, 2019; Giraud *et al.*, 2022). Ela induz a morte celular ao gerar quebras diretamente na fita de DNA ou através da indução de espécies reativas de oxigênio que causam danos substanciais ao DNA, proteínas, lipídios e RNA dentro da célula (Wicker *et al.*, 2022).

Os equipamentos de radioterapia produzidos atualmente, possuem tecnologia moderna que aumenta a eficácia e precisão do tratamento, reduzindo os efeitos adversos e possibilitando um maior poder terapêutico (Alfouzan, 2021; Giraud *et al.*, 2022). Os danos da radiação ionizante podem ser observados ao nível do osso, periósteo, mucosa e ao nível do endotélio vascular, podendo levar à hipóxia, hipocelularidade e hipovascularidade ao nível dos tecidos, o que vai se traduzir na fraca resistência contra o trauma e infecções, para além da própria osteorradionecrose, conduzindo à falha do implante (Di Carlo *et al.*, 2019; Alfouzan, 2021).

1.2. OSSEOINTEGRAÇÃO DE IMPLANTES DE TITÂNIO

O osso é um tecido vivo, dinâmico e sangrento. À semelhança de outros tecidos conjuntivos, ele possui células embebidas em uma abundante matriz extracelular mineralizada (Srivastava *et al.*, 2022). Fornece suporte e proteção locomotora e serve de reservatório dinâmico de minerais e proteínas, estando em remodelação constante, para além disso, o osso também desempenha, por meio do controle endócrino, um papel vital no equilíbrio de cálcio e fosfato dos fluidos corporais (Davies, 2007).

A osseointegração é definida como uma conexão estrutural e funcional direta entre osso vivo ordenado e a superfície de um implante de carga (Brånemark *et al.*, 1983). Representa a interação adequada do implante dentário com os tecidos circundantes, sendo por isso, um marcador de biocompatibilidade (Chauhan *et al.*, 2023), porém, para ser considerada osseointegração, não deve existir tecido mole interposto entre o osso e o implante (Yazdani *et al.*, 2018).

O primeiro passo na resposta do organismo à presença dos implantes envolve a adsorção de proteínas, lipídios, açúcar e íons específicos, estabelecendo um revestimento orgânico responsável por guiar o desempenho das células circundantes, levando à ativação de genes específicos (Bezerra *et al.*, 2017).

A osseointegração depende de parâmetros como o macro desenho do implante, tratamentos de superfície, técnicas cirúrgicas, tipo de osso e cuidados com o paciente (Chauhan *et al.*, 2023). O contato inicial do sangue com a superfície do implante inicia um processo dinâmico de atividade celular, começando pela ativação de plaquetas e leucócitos na região peri-implantar, ocorre igualmente a ligação de proteínas estruturais transitórias, como a fibrina, à superfície do implante (Davies, 2007). De seguida, ocorre a angiogênese e, eventualmente, osteogênese para alcançar a osseointegração (Insua *et al.*, 2017; Luke Yeo, 2020).

1.3. Radioterapia e a osseointegração de implantes

Com a vista a melhorar e acelerar a osseointegração de implantes sob condições adversas como as impostas pela radioterapia, inúmeras modificações na superfície do implante têm sido realizadas, uma vez que a superfície é a primeira parte do implante que interage com o organismo (Faeda *et al.*, 2009).

Após o período de 2 anos, há tendência de redução da falha dos implantes, mesmo assim, as falhas podem persistir até 20 anos após a instalação (Granström, 2005). A taxa de falha do implante tende a ser progressiva ao longo do tempo, aumentando de 1,4% nos

primeiros 12 meses para 3,9% antes dos 36 meses e para 5,1% em 60 meses (Ch'ng *et al.*, 2016). A radioterapia não interfere apenas no sucesso do implante, mas também pode contribuir para a redução da sua estabilidade primária (Carini *et al.*, 2012).

Existe ainda uma controvérsia em relação ao momento ideal para instalação de implante em pacientes a serem submetidos à radioterapia, porém, a vantagem de instalar o implante pré-radioterapia é que a osseointegração inicial ocorre antes do osso ser exposto à radiação, havendo por isso um risco reduzido de ocorrência de complicações como a osteorradionecrose (Di Carlo *et al.*, 2019), visto que os efeitos prejudiciais da radioterapia no osso começam até cerca de 6 semanas após o início da radioterapia (Ch'ng *et al.*, 2016). Associado a esta estratégia, existe um risco de posicionamento inadequado do implante (quando instalado durante a cirurgia ablativa), podendo inviabilizar a reabilitação subsequente e o risco de anulação de todo o processo de reabilitação devido a recorrência do tumor (Di Carlo *et al.*, 2019; Printzell *et al.*, 2019). Numa outra abordagem, recomendando-se instalar o implante após transcorrer o tempo médio para o diagnóstico de recorrência (em média 1 ano após o tratamento definitivo), particularmente em pacientes com doença mais avançada (Ch'ng *et al.*, 2016).

Uma preocupação clínica relacionada à instalação de implantes previamente à radioterapia é o efeito do retroespalhamento da radiação causada pela presença do titânio, podendo resultar em uma dose de radiação alterada nos tecidos ao redor, no entanto, evidências mostram que esse efeito não interfere no tratamento do câncer (Printzell *et al.*, 2023).

1.4. Microtomografia computadorizada

Esta metodologia possui inúmeras vantagens. Permite a análise da estrutura interna sem corte da amostra, reconstrução tridimensional da amostra e possibilidade de se utilizar a mesma amostra para outros testes (Tomaszewska *et al.*, 2017). Quantifica simultaneamente diferentes parâmetros, incluindo massa, densidade mineral, geometria e parâmetros trabeculares e corticais (Faot *et al.*, 2015). Escolher valores de *threshold* adequados e definir limites apropriados da região de interesse durante o processo de análise tridimensional é muito importante para promover a precisão dos resultados e reprodutibilidade do estudo (Chavez *et al.*, 2021). Porém, alguns estudos não fornecem detalhes sobre a metodologia utilizada para definição da região de interesse, dificultando a comparação de seus resultados quantitativos (Faot *et al.*, 2015).

Deve-se ter cuidado na padronização da região dos ossos longos (Bouxsein *et al.*, 2010). Se a região de interesse estiver localizada na diáfise, haverá diferenças em determinados parâmetros, como o a fração de volume ósseo (BV/TV) em relação a uma região de interesse que está contida na região metafisária (Buie *et al.*, 2007). Também é importante que toda a lesão se encaixe na região delimitada, evitando falso negativo ou falso positivo no caso de subestimação ou superestimação da lesão, respectivamente (Bouxsein *et al.*, 2010).

A definição da região de interesse pode ser realizada da mesma forma para osso trabecular e cortical (Buie *et al.*, 2007). O método manual fornece contornos mais precisos e confiáveis (Bouxsein *et al.*, 2010), no entanto, este método depende da experiência do operador e é demorado em comparação com métodos automáticos baseados em formas pré-definidas (circular, retangular, triangular e outros), uma vez que a região de interesse é desenhada em cada corte, embora a função de interpolação possa ser utilizada em um pequeno número de cortes onde as alterações no osso são mínimas (Buie *et al.*, 2007). Por outro lado, o método pré-definido reduz o número de contornos feitos à mão e otimiza tempo (Irie *et al.*, 2018). Uma das desvantagens da utilização de formatos predefinidos é que eles não conseguem envolver totalmente a região de interesse, sobretudo no caso de volumes com bordas irregulares (Buie *et al.*, 2007). Porém, em lesões com formatos regulares, como lesões circulares criadas por brocas, tanto em calvária quanto em osso longo, o método de definição da região de interesse utilizando formatos pré-definidos pode representar uma alternativa fácil e rápida (Bouxsein *et al.*, 2010).

Estudos sobre metodologias mostram-se bastante importantes, uma vez que proporcionam resultados que permitem a padronização e uma maior comparabilidade entre os estudos. Estudos que têm o objetivo final de procurar proporcionar conforto aos pacientes que já estão em sofrimento devido ao diagnóstico do câncer são de extrema importância.

2. OBJETIVOS

Investigar acerca da influência da radiação ionizante no reparo de lesões ósseas cirúrgicas, na osseointegração e sobrevida de implantes instalados previamente à radioterapia, bem como estabelecer a melhor metodologia para definição da região de interesse em lesões ósseas na avaliação por micro-CT.

2.1. Objetivos específicos

Objetivo específico 1: avaliar as taxas de sobrevida de implantes instalados pré e pós-radioterapia, comparando com a sobrevida de implantes instalados em sem radioterapia associada.

Objetivo específico 2: avaliar se existe diferença entre os métodos de definição manual e pré-definido para definição da região de interesse (ROI) durante a análise tridimensional de defeitos ósseos por micro-CT e avaliar o processo de reparo do osso submetido à radioterapia após a cirurgia.

Objetivo específico 3: avaliar a influência da radiação ionizante na osseointegração de implantes de titânio instalados previamente à radioterapia, em dois períodos de avaliação (14 ou 28 dias), utilizando duas superfícies de implantes diferentes (revestidas com hidroxiapatita nanoparticulada ou tratadas com duplo ataque ácido).

Survival of Dental Implants Placed Pre-Radiotherapy Versus Post-Radiotherapy in Native Bone: A Systematic Review

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Purpose: To systematically measure the survival rate of implants placed pre- and post-radiotherapy. **Materials and Methods:** After performing a systematic literature exploration of 10 databases, observational and quasi-experimental studies and case series estimating the survival of dental implants in patients with head and neck cancer placed before, after, and without radiotherapy were included, with no limit on language or year of publication. The Joanna Briggs Institute Critical Appraisal was used to assess the risk of bias in eligible studies. **Results:** During the first screening phase, 3,445 studies were found, among which 16 met the inclusion criteria. The median follow-up period was 60 months (range: 1 to 168 months). Seven (43.7%) articles had a moderate risk of bias, four (25%) had a high risk of bias, and five (31.3%) had a low risk of bias. The survival rate for post-radiotherapy, pre-radiotherapy, and without-radiotherapy implants was 80% to 100%, 89.4% to 97%, and 92.2% to 100%, respectively. **Conclusion:** Despite the alterations caused by ionizing radiation in peri-implant tissues, dental implants placed pre- and post-radiotherapy had high survival rates, similar to those placed without radiotherapy, which helps improve the condition of life of patients with head and neck cancer. *Int J Oral Maxillofac Implants* 2022;37:1100–1109. doi: 10.11607/jomi.9756

Keywords: dental implant, survival rate, head and neck cancer, radiotherapy, systematic review

Head and neck cancer (HNC) is the sixth most predominant cancer type.^{1,2} Most patients are diagnosed during advanced stages (III and IV) of the disease,^{3–5} resulting in radical treatment that can involve surgery, radiotherapy (RDT), and chemotherapy.^{6,7} Although the efficacy of RDT has been proven, it can have effects on oral health, such as reduced salivary flow rate, mucositis, radiation caries, progressive vascular fibrosis, trismus, modifications of bone morphology, and periodontal attachment loss.^{6–8} Progressive vascular fibrosis reduces bone vascularity and clinically manifests as osteoradionecrosis, which is one of the most serious complications of RDT.^{9,10} Osteoradionecrosis is characterized by exposed necrotic bone and pain and may present with pathologic fractures.^{11,12} These factors contribute to a reduction in bone repair capacity,

increasing the failure percentage of implants placed in irradiated tissues.^{3,5}

Ionizing radiation in the head and neck region can cause irreversible changes such as endarteritis, which leads to hypocellularity, hypoxia, and hypovascularity.⁶ These conditions seriously compromise bone healing and turnover, which interferes with the osseointegration of dental implants.⁵ The bone, vessel endothelium, periosteum, connective tissue of the mucosa, and teeth can be affected by radiotherapy.¹⁰ These conditions are favorable for the development of osteoradionecrosis and tissue dehiscence, leading to implant failure.¹²

Improved treatment for patients with HNC allows them to live for years, although with treatment sequelae, such as facial deformities, functional limitations, and the potential to develop depression.⁸ These sequelae have social implications for most patients, negatively influencing their quality of life.¹³ It is recommended that before initiating treatment with RDT, patients undergo screening of the oral cavity, so that oral foci, such as periodontal disease, deep carious cavities, and periapical infections, can be identified.¹⁴ Tooth extractions can be performed before RDT to prevent post-radiation sequelae in the oral cavity.¹⁵ Teeth loss during tumor resection surgery requires oral rehabilitation involving dental implants combined with fixed or removable prostheses.^{10,16} Implant-retained restorations can restore patients' function, self-esteem, and speech, contributing to

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Submitted October 26, 2021; accepted March 12, 2022.

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a better quality of life⁷ and resulting in a high degree of satisfaction.¹⁷ Dental implants are no longer contraindicated in patients treated with RDT,^{13,14} despite the challenging procedure due to the anatomical and functional changes that may be caused by surgery.⁷

Implant placement can be performed after RDT (post-RDT),¹⁵ although with a high risk of osteoradionecrosis.¹⁸ Alternatively, implant placement can be performed before RDT (pre-RDT); however, in this procedure, implants have to be placed during the correct time interval.¹⁹ There is an absence of studies providing an absolute indication about ionizing radiation effects on implant survival rates.^{13,19} Dental implants placed in irradiated patients have varying survival rates.^{20–22} Inconsistent results have been reported in the literature regarding the survival rates of dental implants placed in irradiated tissues compared with those placed in nonirradiated tissues. Thus, the most appropriate timing for implant placement is controversial and involves complex decision making. Therefore, this systematic review aimed to summarize and report the survival rates of implants in pre- and post-radiotherapy groups and compare the outcomes between the groups and to implants placed in patients without RDT.

MATERIALS AND METHODS

Study Registration

This systematic review was conducted in conformity with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement²³ and the Joanna Briggs Institute (JBI) Manual.²⁴ The systematic review protocol was registered a priori in PROSPERO (CRD42021242331; <https://www.crd.york.ac.uk/prospere/>). The only deviation from the original registered protocol was that in the last version of the review, it was decided to include case series.

Research Question and Eligibility Criteria

This systematic review was intended to answer the question, “What are the individual survival rates of implants placed pre- and post-RDT compared to those placed without RDT?”

The inclusion criteria were as follows: (1) quasi-experimental studies, (2) case series, (3) observational studies (longitudinal retrospective or prospective), and (4) randomized clinical trials that evaluated implant survival in patients with HNC who obtained dental implants in native bone from the maxilla and/or mandible with or without associated chemotherapy and RDT. The exclusion criteria were as follows: (1) studies that exclusively included patients with cancer types that were not HNC, (2) studies that exclusively focused on implants placed in extraoral regions or in bone graft, (3) studies that exclusively included patients with comorbidities

such as diabetes and osteoporosis, and (4) studies in which the sample was exclusively composed of patients with HNC treated without RDT and/or with hyperbaric oxygen therapy (HBOT). In addition, studies not associated with the study goal, letters to the editor/editorials, review articles, personal opinions, books/book chapters, textbooks, conference abstracts, in vitro studies, and patents were excluded. There were no limitations on language, year of publication, or type of dental implant placed.

Sources of Information and Search

The search was performed until June 2020, with search alerts as a self-updating tool, in the following databases: MEDLINE (via PubMed), Scopus, LILACS, SciELO, Embase, Cochrane Library, and Web of Science. OpenGrey, OpenThesis, and Google Scholar databases were used to fractionally obtain the “gray literature.” Medical Subject Headings, Health Sciences Descriptors, and Embase Subject Headings resources were used to elect search descriptors. Furthermore, synonyms and free terms were used to enlarge the search. The Boolean operators “AND” and “OR” were also used to enhance the research strategy through several combinations (Table 1). The search terms were fitting to each database. A manual search was also performed through a systematized analysis of the references of the eligible studies.

The results of studies obtained from the bibliographic search were exported to EndNote Web software (Thomson Reuters), on which duplicates were excluded. The remaining results were then exported to Microsoft Word 2019 (Microsoft), including results obtained from the “gray literature,” followed by manual removal of the remaining duplicate articles.

Study Selection

The study selection process was accomplished in three phases. The first phase focused on the analysis of article titles; those not connected to the topic were removed. In the second phase, abstracts were read, and the exclusion criteria were applied. Articles whose titles matched the study aims but did not have available abstracts were fully analyzed in this phase. In the last phase, the full texts of preliminary eligible studies were obtained and evaluated. Studies that did not meet the inclusion criteria were excluded. References of the studies included in this phase were carefully assessed for manual research.

Two reviewers (T.E.A.P. and N.T.A.R.) independently read the articles included in each phase, and doubts or disagreements between them were checked with a third reviewer (L.R.P.) to reach consensus and make a final decision. If an article could not be found online, other libraries were searched. The full texts of studies published in languages other than English or Portuguese were translated.

Table 1 Strategies for Database Search

Database	Search strategy (Jun 22, 2020)
PubMed http://www.ncbi.nlm.nih.gov/pubmed	((“Head and neck cancer” OR “Neoplasms, Head and Neck” OR “Head Neoplasms” OR “Cancer of Head” OR “Neck Neoplasms” OR “Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm” OR “Oral Neoplasms” OR “Cancer of Mouth” OR “Cancer of the Mouth” OR “Mouth Cancer” OR “Neoplasms” OR “Neoplasia” OR “Tumor” OR “Cancer” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Benign Neoplasm”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous” OR “Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”) NOT (“Animal Experimentation” OR “Animal Research” OR “Animal Experimental Use” OR “In Vitro Techniques” OR “In Vitro”))
Scopus http://www.scopus.com/	((“Head and neck cancer” OR “Neoplasms, Head and Neck” OR “Head Neoplasms” OR “Cancer of Head” OR “Neck Neoplasms”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm” OR “Oral Neoplasms” OR “Cancer of Mouth” OR “Cancer of the Mouth” OR “Mouth Cancer”) AND (“Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Neoplasms” OR “Neoplasia” OR “Tumor” OR “Cancer” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Benign Neoplasm”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”))
LILACS http://lilacs.bvsalud.org/	((“Head and neck cancer” OR “Neoplasms, Head and Neck” OR “Head Neoplasms” OR “Cancer of Head” AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Neck Neoplasms” OR “Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm” OR “Oral Neoplasms”) AND (“Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Cancer of Mouth” OR “Cancer of the Mouth” OR “Mouth Cancer” OR “Neoplasms” OR “Neoplasia”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Tumor” OR “Cancer” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Benign Neoplasm”) AND (“Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”))
SciELO http://www.scielo.org/	((“Head and neck cancer” OR “Neoplasms, Head and Neck”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Head Neoplasms” OR “Cancer of Head”) AND (“Dental Implantation, Endosseous” OR “Endosseous Dental Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis”)) ((“Oral Neoplasms” OR “Cancer of Mouth” OR “Cancer of the Mouth”) AND (“Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”)) ((“Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”))
Web Of Science http://apps.webofknowledge.com/	((“Head and neck cancer” OR “Neoplasms, Head and Neck” OR “Head Neoplasms” OR “Cancer of Head” OR “Neck Neoplasms” OR “Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm” OR “Oral Neoplasms” OR “Cancer of Mouth” OR “Cancer of the Mouth” OR “Mouth Cancer” OR “Neoplasms” OR “Neoplasia” OR “Tumor” OR “Cancer” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Benign Neoplasm”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous” OR “Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”) NOT (“Animal Experimentation” OR “Animal Research” OR “Animal Experimental Use” OR “In Vitro Techniques” OR “In Vitro”))
Embase https://www.elsevier.com/solutions/embase-biomedical-research	(‘head and neck cancer’/exp OR ‘head and neck cancer’ OR ‘neoplasms, head and neck’ OR ‘head neoplasms’/exp OR ‘head neoplasms’ OR ‘cancer of head’ OR ‘neck neoplasms’/exp OR ‘neck cancer’ OR ‘oral cancer’/exp OR ‘oral cancer’ OR ‘mouth cancer’/exp OR ‘mouth cancer’ OR ‘neoplasms’/exp OR ‘neoplasms’ OR ‘neoplasia’/exp OR ‘neoplasia’ OR ‘tumor’/exp OR ‘tumor’ OR ‘cancer’/exp OR ‘cancer’ OR ‘malignant neoplasm’/exp OR ‘malignant neoplasm’ OR ‘neoplasm, malignant’ OR ‘benign neoplasm’/exp OR ‘benign neoplasm’) AND (‘dental implants’/exp OR ‘dental implants’ OR ‘implant, dental’ OR ‘surgical dental prosthesis’ OR ‘dental implantation, endosseous’/exp OR ‘dental implantation, endosseous’ OR ‘endosseous dental implantation’ OR ‘osseointegrated dental implantation’ OR ‘dental implantation, osseointegrated’ OR ‘endosseous implantation’) AND (‘radiotherapy’/exp OR ‘radiotherapy’ OR ‘radiation therapy’/exp OR ‘radiation therapy’ OR ‘radiation treatment’/exp OR ‘radiation treatment’ OR ‘targeted radiotherapy’ OR ‘targeted radiation therapy’) NOT (‘animal experimentation’/exp OR ‘animal experimentation’ OR ‘animal research’/exp OR ‘animal research’ OR ‘animal experimental use’ OR ‘in vitro techniques’/exp OR ‘in vitro techniques’ OR ‘in vitro’/exp OR ‘in vitro’)
OpenGrey http://www.opengrey.eu/	(Head and neck cancer) AND (Dental implants) AND (Radiotherapy) (Neoplasms, Head and Neck) AND (Dental implants) AND (Radiotherapy) (Head and neck cancer) AND (Dental implants) (Oral cancer) AND (Implant, Dental)
OpenThesis http://www.openthesis.org/	(Head and neck cancer) AND (Dental implants) AND (Radiotherapy) (Neoplasms, Head and Neck) AND (Dental implants) AND (Radiotherapy) (Oral cancer) AND (Dental implants) AND (Radiotherapy)
Google Scholar https://scholar.google.com	(Head and neck cancer) AND (Dental implants) AND (Radiotherapy) (Neoplasms, Head and Neck) AND (Dental implants) AND (Radiotherapy) (Oral cancer) AND (Dental implants) AND (Radiotherapy) (Mouth Neoplasm) AND (Dental implants) AND (Radiotherapy) (Head and neck cancer) AND (Dental implants) AND (Radiation therapy)
Cochrane (Trails) https://www.cochranelibrary.com	((“Head and neck cancer” OR “Neoplasms, Head and Neck” OR “Head Neoplasms” OR “Cancer of Head” OR “Neck Neoplasms” OR “Neck Cancer” OR “Oral cancer” OR “Mouth Neoplasm” OR “Oral Neoplasms” OR “Cancer of Mouth” OR “Cancer of the Mouth” OR “Mouth Cancer” OR “Neoplasms” OR “Neoplasia” OR “Tumor” OR “Cancer” OR “Malignant Neoplasm” OR “Neoplasm, Malignant” OR “Benign Neoplasm”) AND (“Dental implants” OR “Implant, Dental” OR “Surgical Dental Prosthesis” OR “Dental Implantation, Endosseous” OR “Endosseous Dental Implantation” OR “Osseointegrated Dental Implantation” OR “Dental Implantation, Osseointegrated” OR “Endosseous Implantation”) AND (“Radiotherapy” OR “Radiation Therapy” OR “Radiation Treatment” OR “Targeted Radiotherapy” OR “Targeted Radiation Therapy”))

Data Collection

Data were collected from text, tables, and images. Before data extraction, to ensure consistency among reviewers, a calibration exercise consisting of simultaneous data extraction from one eligible study was performed. Any disagreement between the reviewers in this phase was also resolved through discussions, and when both reviewers disagreed, a third reviewer (L.R.P.) was consulted to make a final decision. The ethical criteria involved in the studies as well as the checklist used were collected.

Data on the following were extracted from the articles: (1) identification of the study (authors, country, year of publication, and study design); (2) sample characteristics (sample size and distribution by sex and age); (3) treatment characteristics (radiation doses, associated chemotherapy, moment of implant placement, and number of implants placed), and (4) main results (follow-up time, implant failure, loss, and survival). In cases lacking information, the corresponding author of the study was reached by email.

Risk of Bias

The JBI critical appraisal tool for systematic reviews²⁴ was used to assess the risk of bias and the individual quality of the selected studies. A specific tool was applied to each study design. Two reviewers (T.E.A.P. and N.T.A.R.) assessed each domain blindly and independently in terms of the potential risk of bias of the articles, as recommended by the PRISMA statement.²³ A third reviewer (L.R.P.) was invited to discuss the risk of bias when consensus was not reached.

Each study was categorized according to the percentage of positive answers to the questions corresponding to the assessment tool. The risk of bias was considered high, moderate, and low when the percentage of positive answers was up to 49%, 50% to 69%, and > 70%, respectively.

Summary Measures and Syntheses of Results

A meta-analysis was performed if the data from the eligible studies were homogenous. However, methodologic and observational differences between the included studies were observed; therefore, conducting a meta-analysis was not appropriate because of significant data heterogeneity. Accordingly, descriptive analysis of the findings from the studies was performed to identify core themes related to the study aim.

RESULTS

Study Selection

The first phase of study selection yielded 3,445 articles distributed over 10 electronic databases, including

the “gray literature.” After removing duplicated results, 1,659 articles were retained for the analysis of the titles and abstracts. After detailed analysis, 100 studies were eligible for full-text analysis; their references were evaluated carefully, but no additional articles were selected. Subsequently, 84 studies were excluded because they did not meet the inclusion criteria or because their full texts were not found (Appendix Table 1; see Appendix in online version of this article at quintpub.com). Thus, 16 studies were selected for qualitative analysis. Figure 1 shows the process of search, identification, inclusion, and exclusion of articles.

Characteristics of Eligible Studies

Four quasi-experimental studies,^{18,25-27} four cohort studies,^{16,17,20,28} and eight case series^{6,7,10,22,29-32} were included. The studies were published between 1996 and 2020 and were undertaken in Austria,¹⁸ Brazil,⁷ France,³² Germany,^{16,25,28} Italy,^{10,22} the Netherlands,^{20,26,31} Poland,²⁷ Spain,¹⁷ and the United States,^{6,29} with one multicenter study conducted in the United States and Japan.³⁰

Most studies (n=10) evaluated implants survival in post-radiated patients without a control group.^{6,7,10,18,22,27,29-32} Four studies compared implant survival between post-radiated patients and nonirradiated patients (control group).^{16,17,25,28} The other two studies compared implant survival between pre-radiated patients and nonirradiated patients (control group).^{20,26}

Five studies reported the use of chemotherapy in part of their sample.^{16,17,25,27,32} The mean radiation doses were 47.1 to 62 Gy, and the number of implants placed was 20 to 830. The total sample included 549 patients: 298, 52, and 199 patients in the post-RDT, pre-RDT, and without-RDT groups, respectively. However, three studies did not reveal the number of post-RDT patients.^{6,27,31} The median patient age was 51 to 66.5 years.

A total of 2,994 implants were placed among patients with HNC in the 16 studies included in this systematic review. Most implants (1,784) were placed post-RDT. In the pre-RDT group, 185 implants were placed. The remaining implants (749) were placed in the without-RDT (control) group. One study²⁸ did not reveal the number of implants placed in each group and only indicated the total number of implants (276). The corresponding author was contacted by email, but no response was received. It was difficult to determine the number of implants placed in patients treated with chemotherapy because the studies did not specify this information. Other information regarding the demographic and clinical characteristics of the population is presented in Table 2. Only five articles^{6,7,10,17,31} were approved by the Ethics Committee of their respective institutions. Informed consent was obtained from patients in six studies,^{6,10,17,25,26,31} and no study described the use of any report guide.

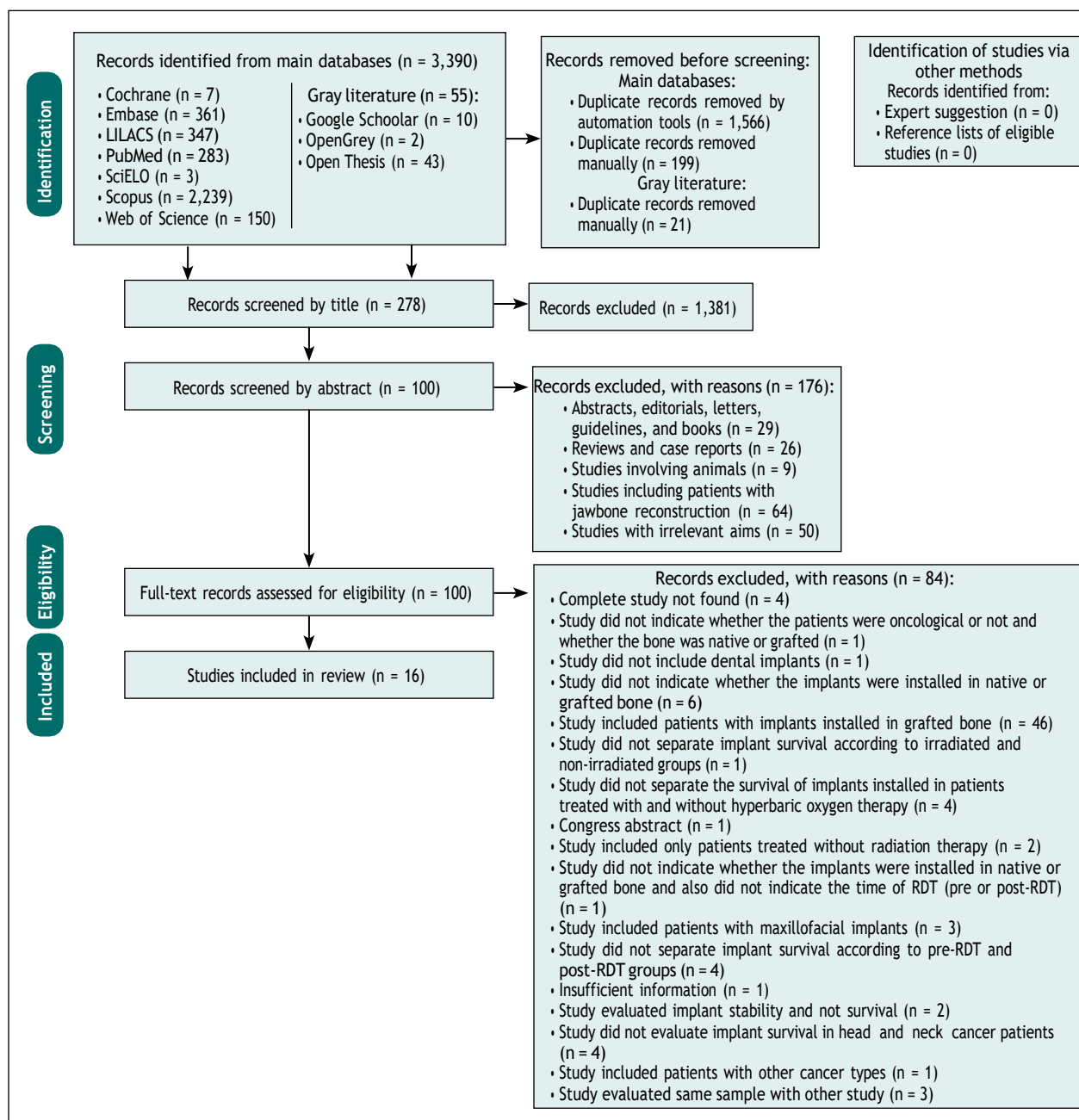


Fig 1 Process of search, identification, inclusion, and exclusion of articles.

Risk of Bias

Table 3 shows detailed information about the risk of bias of the studies included in the qualitative analysis, assessed by the Joanna Briggs Institute assessment tools.³³⁻³⁵ The risk of bias of quasi-experimental studies was classified as low in one article¹⁸ and moderate in three articles.²⁵⁻²⁷ Two studies^{18,27} received “no” for question 4 because their controls did not match the control criteria for this systematic review (nonirradiated patients). All quasi-experimental studies received “no” for question 2 because they had no uniform sample,

with differences in age, HNC subtype, cancer stage, and habits (smoking and alcohol consumption) as well as the presence of associated treatment with chemotherapy.

Only one cohort study¹⁷ had a high risk of bias, and the remaining three studies^{16,20,28} had a moderate risk of bias. None of them clearly identified the confounding factors (question 4), and they did not even indicate the strategies used to deal with those confounding factors (question 5). All cohort studies had complete follow-up, which is why none showed strategies used to address incomplete follow-up (question 10).

Table 2 Summary of the Main Characteristics of the Eligible Studies Included for Qualitative Analysis

Study (year)	Study design	Associated chemotherapy (yes/no)	Groups	Sample	Age range in years (mean $\pm$ SD)	No. of implants placed	Mean radiation doses (range)	Follow-up time (mo)	Survival rate (%)
Watzinger et al (1996) ¹⁸	Quasi-experimental	a	Post-RDT	10	c	60	50 Gy	Mean 36	87.8
Keller et al (1997) ²⁹	Case series	a	Post-RDT	11 (6 male, 5 female)	24-84 (57 $\pm$ 15)	56	56 Gy (27.5-70)	Mean 120	100.0
Niimi et al (1998) ³⁰	Case series	a	Post-RDT	30	d	161	Between < 25 and > 66 Gy	1 to > 49	87.5
Esser et al (1999) ²⁸	Retrospective cohort	a	Post-RDT	28 (23 male, 5 female)	(55.3)	b	60 Gy	Mean 58.2	93.8
			Without RDT	34 (28 male, 6 female)	(54.7)	b			97.0
			Total	62 (51 male, 11 female)	a	276	60 Gy		
Visch et al (2002) ³¹	Case series	a	Post-RDT	c	c	411	Between > 0-50 and > 72 Gy	Maximum 168	83.0
Landes and Kovács (2006) ²⁵	Quasi-experimental	b b Yes (22 patients)	Post-RDT	19	b	72	57 Gy	Mean 24	98.0
			Without RDT	11	b	42			100.0
			Total	30 (22 male, 8 female)	47-83 (63)	114	57 Gy		
Schepers et al (2006) ²⁰	Retrospective cohort	a	Pre-RDT	21	b	61	b (60-68 Gy)	Mean 29.6	97.0
			Without RDT	27	b	78			100.0
			Total	48 (29 male, 19 female)	(66.5 $\pm$ 10.3)	139	b (60-68 Gy)		
Korfage et al (2010) ²⁶	Quasi-experimental	a	Pre-RDT	31 (23 male, 8 female)	41-81 (59.8 $\pm$ 10.7)	124	60.1 Gy (30-70)	Mean 60	89.4
			Without RDT	19 (12 male, 7 female)	43-81 (64.2 $\pm$ 11.9)	71			98.6
			Total	50 (35 male, 15 female)	41-81 (61.5 $\pm$ 11.2)	195	60.1 Gy (30-70)		
Sammartino et al (2011) ²²	Case series	a	Post-RDT	77	a	172	a	> 36	88.3
Buddula et al (2012) ⁶	Case series	a	Post-RDT	c	c	212	60.7 Gy (50.2-67.5)	Mean 84	83.0
Doll et al (2014) ¹⁶	Prospective cohort	b b Yes (55 patients)	Post-RDT	55	b	292	50-72 Gy	Mean 121	89.5
			Without RDT	102	b	538		(37-240)	92.2
			Total	157 (62 male, 95 female)	16-79 (53.7)	830	50-72 Gy		
Curi et al (2018) ⁷	Case series	a	Post-RDT	22	d	95	62 Gy (50-70)	Mean 89.2 (3.6-176.4)	94.1
Desoutter et al (2018) ³²	Case series	Yes (4 patients)	Post-RDT	18 (14 male, 4 female)	42-78 (57.5)	40	51.8 Gy (50-66)	Mean 89 (58-119)	80.0
Flores-Ruiz et al (2018) ¹⁷	Retrospective cohort	b b Yes (5 patients)	Post-RDT	11	b	71	a	Mean 60	87.3
			Without RDT	6	b	20			100.0
			Total	17 (12 male, 5 female)	30- > 60	91			
Di Carlo et al (2019) ¹⁰	Case series	a	Post-RDT	17 (7 male, 10 female)	(51 $\pm$ 19)	84	47.1 Gy (40-50)	Mean 22.9 (SD = 15.5)	90.5
Rolski et al (2020) ²⁷	Quasi-experimental	Yes (a)	Post-RDT	c	c	58	Up to a dose of 50 Gy	Mean 36	94.8

a = Not cited by the authors; b = The study did not separate the data according to the groups; c = Impossible to extract the data of native bone patients only; d = Impossible to extract the data of patients without HBOT only.

Table 3 Risk of Bias Assessed by the Joanna Briggs Institute Critical Appraisal Tools for Cohort Studies,³³ Case Series,³⁴ and Quasi-Experimental Studies³⁵

Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	Q.11	% yes/risk
Cohort studies												
Esser et al (1999) ²⁸	✓	✓	U	U	-	✓	✓	✓	✓	-	✓	63.6
Schepers et al (2006) ²⁰	✓	✓	✓	U	-	✓	U	✓	✓	-	-	54.5
Doll et al (2014) ¹⁶	✓	✓	U	U	-	✓	U	✓	✓	-	✓	54.5
Flores-Ruiz et al (2018) ¹⁷	✓	U	U	U	-	✓	U	✓	✓	-	-	36.4
Quasi-experimental studies												
Watzinger et al (1996) ¹⁸	✓	-	✓	-	✓	✓	✓	U	✓			77.8
Landes and Kovács (2006) ²⁵	✓	-	-	✓	✓	✓	✓	U	-			55.6
Korfage et al (2010) ²⁶	✓	-	-	✓	✓	✓	✓	-	-			55.6
Rolski et al (2020) ²⁷	✓	-	-	-	✓	✓	✓	U	✓			55.6
Case series studies												
Keller et al (1997) ²⁹	U	-	U	-	-	✓	✓	✓	-	-		30.0
Niimi et al (1998) ³⁰	-	-	U	U	U	-	-	-	✓	-		10.0
Visch et al (2002) ³¹	U	✓	✓	✓	✓	✓	U	U	✓	✓		70.0
Sammartino et al (2011) ²²	✓	U	-	U	-	✓	U	✓	✓	-		40.0
Buddula et al (2012) ⁶	✓	✓	✓	U	✓	✓	✓	U	✓	✓		80.0
Curi et al (2018) ⁷	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		100.0
Desoutter et al (2018) ³²	✓	✓	✓	U	U	✓	✓	✓	-	-		60.0
Di Carlo et al (2019) ¹⁰	✓	✓	✓	U	U	✓	✓	✓	✓	-		70.0

✓ = Yes; - = No; U = unclear; N/A = not applicable. Cohort studies: Q.1 = Were the two groups similar and recruited from the same population? Q.2 = Were the exposures measured similarly to assign people to both exposed and unexposed groups? Q.3 = Was the exposure measured in a valid and reliable way? Q.4 = Were confounding factors identified? Q.5 = Were strategies to deal with confounding factors stated? Q.6 = Were the groups/participants free of the outcome at the start of the study (or at the moment of exposure)? Q.7 = Were the outcomes measured in a valid and reliable way? Q.8 = Was the follow-up time reported and sufficient to be long enough for outcomes to occur? Q.9 = Was follow-up complete, and if not, were the reasons for loss to follow-up described and explored? Q.10 = Were strategies to address incomplete follow-up utilized? Q.11 = Was appropriate statistical analysis used? Quasi-experimental studies: Q.1 = Is it clear in the study what is the "cause" and what is the "effect" (ie, there is no confusion about which variable comes first)? Q.2 = Were the participants included in any similar comparisons? Q.3 = Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest? Q.4 = Was there a control group? Q.5 = Were there multiple measurements of the outcome both pre- and post-intervention/exposure? Q.6 = Was follow-up complete, and if not, were differences between groups in terms of their follow-up adequately described and analyzed? Q.7 = Were the outcomes of participants included in any comparisons measured in the same way? Q.8 = Were outcomes measured in a reliable way? Q.9 = Was appropriate statistical analysis used? Case series studies: Q.1 = Were there clear criteria for inclusion in the case series? Q.2 = Was the condition measured in a standard, reliable way for all participants included in the case series? Q.3 = Were valid methods used for identification of the condition for all participants included in the case series? Q.4 = Did the case series have consecutive inclusion of participants? Q.5 = Did the case series have complete inclusion of participants? Q.6 = Was there clear reporting of the demographics of the participants in the study? Q.7 = Was there clear reporting of clinical information of the participants? Q.8 = Were the outcomes or follow-up results of cases clearly reported? Q.9 = Was there clear reporting of the presenting site(s)/clinic(s) demographic information? Q.10 = Was statistical analysis appropriate?

The risk of bias of case series studies was classified as low for four articles,^{6,7,10,31} moderate for one article,³² and high for three articles.^{22,29,30}

Synthesis of Results

The follow-up period in the studies was 1 to 240 months, with a median follow-up period of 60 months in most articles. The survival rates for all groups were 80% to 100%. Implants placed post-RDT, pre-RDT, and without RDT presented survival rates of 80% to 100%, 89.4% to 97%, and 92.2% to 100%, respectively.

Among the studies that directly compared the irradiated to nonirradiated groups, a statistically significant difference was observed in only one study¹⁷ which

showed a higher survival rate for implants placed without RDT compared to implants placed post-RDT. In the remaining studies that made comparisons, no significant differences between groups^{20,28} or no statistical results of the comparison^{16,25,26} were reported (Tables 4 and 5). All studies that directly compared the groups showed better survival for implants placed in patients who did not undergo RDT.

DISCUSSION

The number of patients with HNC treated with RDT and rehabilitated with implant-supported prostheses has

Table 4 Implant Survival (in Months) in the Pre-RDT Group Compared to the Nonirradiated Group, According to the Follow-up Period and Radiation Doses

	Mean radiation doses (Gy)	Mean follow-up period (mo)	Survival rate: without RDT (%)	Survival rate: pre-RDT (%)
Schepers et al (2006) ²⁰	97.0	30	100	97
Korfage et al (2010) ²⁶	60.1	60	98.6	89.4

Table 5 Implant Survival (in Months) in the Post-RDT Group Compared to the Nonirradiated Group, According to the Follow-up Period and Radiation Doses

	Mean radiation doses (Gy)	Mean follow-up period (mo)	Survival rate: without RDT (%)	Survival rate: post-RDT (%)
Esser et al (1999) ²⁸	60	58.2	97.0	93.8
Landes and Kovács (2006) ²⁵	57	24	100	98
Doll et al (2014) ¹⁶	50-72	121	92.2	89.5
Flores-Ruiz et al (2018) ¹⁷	a	60	100	87.3

^aNot cited by the authors.

increased over the last decade,^{7,12} but the most appropriate timing for implant placement (prior to or after RDT) is controversial. This study systematically investigated the survival rate of implants placed pre-RDT and post-RDT compared to that of implants placed without RDT. The exclusion criteria included studies that exclusively involved patients with comorbidities and HBOT. Comorbidities such as diabetes and osteoporosis can significantly interfere with implant survival⁷; therefore, they could be a confounding factor in this study. The use of adjuvant HBOT is controversial, with several studies showing no influence of HBOT on implant survival, and it is an expensive treatment that is not accessible to many populations.^{7,20}

In the present study, chemotherapy was not considered as an exclusion factor since limitations were noted in the included studies. Among the total of 16 studies, only 5 claimed to have included patients treated with chemotherapy (without detailed information about the chemotherapy drugs used or their dosage), and the remaining 11 did not mention whether or not patients treated with chemotherapy were included. Therefore, the exclusion of these studies would significantly decrease the total number of included studies.

Most of the drugs used in chemotherapy do not have a specific mechanism of action against tumor cells, acting on cell proliferation and thus interfering with the metabolism of healthy cells, especially those with high turnover rate (such as bone marrow cells), which can lead to reduced bone turnover, although more modern drugs tend to cause less bone damage.³⁶ According to some studies,^{16,25,36,37} chemotherapy seems to have no significant influence on the survival of dental implants. Additionally, one study found that chemotherapy does not interfere with the number of osteoblasts produced, showing that bone metabolism is not completely

compromised by chemotherapy.³⁸ The significantly lower survival rate of dental implants is mainly due to the effects of RDT rather than chemotherapy.^{10,39}

Analysis of the survival rate according to sex, radiation techniques, and implant location was not performed. Sex does not interfere with the survival of dental implants.²⁸ There are different RDT techniques, such as external beam radiation, radioisotope therapy, and brachytherapy; however, different radiation techniques do not affect the survival of dental implants.⁴⁰ Implant location does not influence implant survival.¹⁶ The results showed that dental implants placed post-RDT had a high survival rate, similar to those placed without RDT. Despite the high survival of implants placed post-RDT, the greatest number of implant failures was reported in this group. Patients have rejected offers of implant placement post-RDT because it may lead to additional surgery when patients are already exhausted.^{5,14}

Implant survival is completely dependent on the osseointegration process²¹; hence, implants placed pre-RDT may show better results since osseointegration is well established before the bone is compromised by RDT, although the late effects of RDT continue for years after completion of initial treatment.^{13,14} These factors may help explain the relatively high survival rate of dental implants placed pre-RDT compared to those placed post-RDT.

One of the most appropriate times for implant placement is during ablative surgery, which combines tumor surgery and implant placement, providing benefits such as implant placement in nonradiated bone (leading to a reduced risk of developing osteoradionecrosis), lack of need for additional surgery, earlier rehabilitation with an implant-supported prosthesis, and initial implant healing occurring before initiating RDT.^{7,41} There

are some disadvantages, such as improper positioning of the implants (especially in patients with large defects) and difficulties in obtaining sufficient keratinized mucosa around the implants.^{7,14} Another disadvantage is the risk of losing the implants due to tumor recurrence,¹⁷ but in the present study, few implants (58) were lost due to tumor recurrence in all included studies.

This review has some limitations. Two articles^{6,21} evaluated the same sample, and therefore only one was included.⁶ The same happened with another set of two articles,^{26,42} and once again only one article was included.²⁶ In both cases, the article with the longest follow-up time was included. Four articles were excluded because their full text was not found (Appendix 1), thus compromising the certainty of evidence for the present study. Only two studies were found that evaluated the survival rate of dental implants placed pre-RDT in the native bone. There was huge variation in the mean follow-up period and radiation doses between all included studies; therefore, meta-analysis could not be performed. Some data could not be extracted because they were not available in the articles. Therefore, the corresponding authors were contacted by email. Some of them responded, but the others did not. The present study did not find randomized clinical studies comparing patients with implants placed pre- or post-RDT to those with implants placed without RDT, which could enrich the study. These aspects decrease the conclusiveness of the presented findings.

CONCLUSIONS

Despite the alterations caused by ionizing radiation in peri-implant tissues, dental implants placed pre- and post-RDT seem to have high survival rates, similar to those placed without RDT, indicating that it is a relatively safe procedure. However, the available evidence is limited, with great heterogeneity in the survival rates; therefore, more well-designed studies should be performed so that accurate conclusions could be drawn.

ACKNOWLEDGMENTS

The authors were supported by the National Counsel of Technological and Scientific Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES; 001), and Research Support Foundation of the State of Minas Gerais (FAPEMIG). The authors deny any conflicts of interest related to this study.

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APPENDIX

Appendix Table 1 Studies Excluded in Reading of Full Texts and Reasons for Exclusion and Full-Text Studies Not Found (n = 84)

	Study excluded	Reason for exclusion
1	Ueda et al (1993)	Complete study not found
2	Lorant et al (1994)	Study did not indicate whether the patients were oncological or not and whether the bone was native or grafted
3	Roumanas et al (1994)	Study did not include dental implants
4	Smatt et al (1995)	Complete study not found
5	Franzén et al (1995)	Study did not indicate whether the implants were placed in native or grafted bone
6	Weischer et al (1996)	Study included patients with implants placed in grafted bone
7	Brogniez et al (1998)	Study included patients with implants placed in grafted bone
8	Arcuri et al (1997)	Study did not indicate whether the implants were placed in native or grafted bone
9	Jisander et al (1997)	Study included patients with implants placed in grafted bone
10	Weischer and Mohr (1997)	Study did not indicate whether the implants were placed in native or grafted bone
11	McGhee et al (1997)	Study included patients with implants placed in grafted bone
12	Esser and Wagner (1997)	Study included patients with implants placed in grafted bone
13	August et al (1998)	Study included patients with implants placed in grafted bone
14	Ihara et al (1998)	Study did not indicate whether the implants were placed in native or grafted bone
15	Wagner et al (1998)	Study did not separate implant survival according to irradiated and nonirradiated groups
16	Andersson et al (1998)	Study included patients with implants placed in grafted bone
17	Betz et al (1999)	Study included patients with implants placed in grafted bone
18	Grötz et al (1999)	Study included patients with implants placed in grafted bone
19	Mericske-Stern et al (1999)	Study included patients with implants placed in grafted bone
20	Schliephake et al (1999)a	Study included patients with implants placed in grafted bone
21	Schliephake et al (1999)b	Study included patients with implants placed in grafted bone
22	Weischer and Mohr (1999)	Study included patients with implants placed in grafted bone
23	Werkmeister (1999)	Study included patients with implants placed in grafted bone
24	Granström et al (1999)	Study did not separate the survival of implants placed in patients treated with and without hyperbaric oxygen therapy
25	Snauwaert et al (2000)	Study included patients with implants placed in grafted bone
26	Kwakman et al (2001)	Congress abstract
27	Kovács (2001)	Study included only patients treated without radiation therapy
28	Van Steenberghe et al (2002)	Study did not indicate whether the implants were placed in native or grafted bone
29	Goto et al (2002)	Study included patients with implants placed in grafted bone
30	Cao and Weischer (2003)	Study included patients with implants placed in grafted bone
31	Schmidt et al (2004)	Study did not indicate whether the implants were placed in native or grafted bone
32	Moy et al (2005)	Study did not indicate whether the implants were placed in native or grafted bone and also did not indicate the time of RDT (pre- or post-RDT)
33	Granström (2005)	Study included patients with maxillofacial implants
34	Shaw et al (2005)	Study included patients with implants placed in grafted bone
35	Garrett et al (2006)	Study included patients with implants placed in grafted bone
36	Bodard et al (2006)	Study did not separate implant survival according to pre-RDT and post-RDT groups
37	Yerit et al (2006)	Study included patients with implants placed in grafted bone
38	Roumanas et al (2006)	Study included patients with implants placed in grafted bone
39	Nelson et al (2007)	Study included patients with implants placed in grafted bone
40	Alsaadi et al (2007)	Insufficient information
41	Schoen et al (2007)	Study included patients with implants placed in grafted bone
42	Adell et al (2008)	Study included patients with implants placed in grafted bone
43	Bauer et al (2008)	Complete study not found
44	Schoen et al (2008)	Study evaluated same sample as Korfage et al (2010)
45	Cuesta-Gil et al (2009)	Study included patients with implants placed in grafted bone
46	Nagy et al (2009)	Study included patients with implants placed in grafted bone

Appendix Table 1 Studies Excluded in Reading of Full Texts and Reasons for Exclusion and Full-Text Studies Not Found (n = 84)

	Study excluded	Reason for exclusion
47	Barrowman et al (2011)	Study included patients with implants placed in grafted bone
48	Buddula et al (2011)	Study evaluated same sample as Buddula et al (2012)
49	Støre et al (2011)	Study did not separate the survival of implants placed in patients treated with and without hyperbaric oxygen therapy
50	Linsen et al (2012)	Study included patients with implants placed in grafted bone
51	Mancha de la Plata et al (2012)	Study included only patients treated without radiation therapy
52	Katsoulis et al (2013)	Study included patients with implants placed in grafted bone
53	Mizbah et al (2013)	Study did not separate implant survival according to pre-RDT and post-RDT groups
54	Dholam et al (2013)	Study included patients with implants placed in grafted bone
55	Gander et al (2014)	Study included patients with implants placed in grafted bone
56	Korfage et al (2014)	Study did not separate the survival of implants placed in patients treated with and without hyperbaric oxygen therapy
57	Reich et al (2015)	Complete study not found
58	Pompa et al (2015)	Study included patients with implants placed in grafted bone
59	Hessling et al (2015)	Study included patients with implants placed in grafted bone
60	Karayazgan-Saracoglu et al (2017)	Study evaluated implant stability and not survival
61	Ch'ng et al (2016)	Study included patients with implants placed in grafted bone
62	Maló et al (2016)	Study did not evaluate implant survival in head and neck cancer patients
63	Ettl et al (2016)	Study included patients with implants placed in grafted bone
64	Wu et al (2016)	Study did not separate implant survival according to pre-RDT and post-RDT groups
65	Cotic et al (2016)	Study did not separate the survival of implants placed in patients treated with and without hyperbaric oxygen therapy
66	Ernst et al (2016)	Study included patients with implants placed in grafted bone
67	Kobayashi et al (2016)	Study included patients with implants placed in grafted bone
68	Rana et al (2016)	Study included patients with implants placed in grafted bone
69	Carr et al (2017)	Study did not evaluate implant survival in head and neck cancer patients
70	Hasegawa et al (2017)	Study included patients with implants placed in grafted bone
71	Nicoli et al (2017)	Study included patients with other cancer types
72	Rolski et al (2017)	Study evaluated implant stability and not survival
73	Wetzels et al (2017)	Study included patients with implants placed in grafted bone
74	Kim et al (2018)	Study did not evaluate implant survival in head and neck cancer patients
75	Takahashi et al (2018)	Study included patients with implants placed in grafted bone
76	Butterworth (2019)	Study included patients with maxillofacial implants
77	Ettl et al (2020)	Study included patients with implants placed in grafted bone
78	Moore et al (2019)	Study included patients with maxillofacial implants
79	Nguyen et al (2019)	Study did not evaluate implant survival in head and neck cancer patients
80	Papi et al (2019)	Study included patients with implants placed in grafted bone
81	Woods et al (2019)	Study included patients with implants placed in grafted bone
82	Laverty et al (2019)	Study included patients with implants placed in grafted bone
83	Alberga et al (2020)	Study did not separate implant survival according to pre-RDT and post-RDT groups
84	Patel et al (2020)	Study included patients with implants placed in grafted bone

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3.2 Capítulo 2.

Artigo a ser submetido para *INTERNATIONAL JOURNAL OF RADIATION ONCOLOGY, BIOLOGY, PHYSIC*

Microtomographic analysis of surgically-created bone defects in rats irradiated after surgery: manual versus predefined region-of-interest definition methods: an experimental study in rats

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ABSTRACT

Radiotherapy (RT) is a strategy used to treat cancer, which can generate significant problems at bone level. Micro-computed tomography is a high-resolution radiographic method widely used to evaluate those problems in animals. This study aimed to assess the difference between the manual and predefined region-of-interest (ROI) definition methods during the analysis of bone defects by micro-CT and evaluate the repair process of bone submitted to RT after surgery. Bone defects with 2.3 mm in diameter were created in both tibiae of rats. After 7 days, they were submitted to RT. Fourteen and 28 days after, the tibiae were scanned. During the analysis by CtAnalyser® software, the ROI was defined in two different ways for each sample: manually and predefined (based on rectangular shape). Data corresponding to bone volume fraction (BV/TV), trabecular number (Tb.N), trabecular thickness (Tb.Th) and trabecular separation (Tb.Sp) were analyzed. The ROI definition method influenced the results of all parameters except Tb.Th. Statistical difference was detected between the periods of 14 and 28 days for BV/TV ($p < 0.001$) and Tb.Th ($p < 0.001$), no significant difference was found for Tb.N ($p = 0.06$) and Tb.Sp ($p = 0.96$). No parameter was influenced by RT. Despite being time-consuming (446.2 ± 49.5 s) compared with the predefined method (131.2 ± 38.8 s). The results provided by the manual method reflected an advanced repair process and no statistically significant difference was detected in the repair process between irradiated and non-irradiated bone.

Key words: X-Ray Microtomography, radiotherapy, bone repair, rats

INTRODUCTION

Radiotherapy (RT) is one of the strategies used to treat head and neck cancer (HNC).¹ It may result in severe adverse side-effects well established in the literature,^{1,2} that compete for changes in the bone remodelling process, which persist for many months.³⁻⁵ Osteoradionecrosis (ORN) is the most severe complication,^{6,7} defined as an ulceration of the mucosa with exposed necrotic bone that does not heal in 3 months, as result of hypoxia, hypovascularization and hypocellularity associated with infection and ischemia,⁸ often precipitated by an additional tissue insult within the radiation field, such as surgery or denture trauma.^{1,5} This complication can lead to mandibular fractures, swallowing and speech disorders.⁹ To obviate the risk of ORN, it is recommended that newly diagnosed HNC patients undergo thorough oral assessment and be rendered dentally fit prior to radiotherapy.² ORN may also occur when the extractions are performed before RT, with an incidence ranging from 0.55% to 20.8%.^{7,10}

Notwithstanding the previous, RT is considered a highly effective cancer treatment that benefits around 50% of all cancer patients.¹¹ The ionizing radiation destroys cancer cells by inducing nuclear DNA damage or forming free radicals with subsequent cell death.^{5,12} Unfortunately, the irradiation can have a systemic reflection, which occurs when an irradiated cell communicates with non-irradiated one via secreted factors and/or the gap junction, and the non-irradiated cells exhibit responses usually characteristic of cells directly exposed to radiation.^{3,13} With the continued increase in life expectancy of HNC patients, radiation-induced long-term adverse effects have become a growing concern for physicians,¹² therefore, the study of the impact of RT on the various biological phenomena is of great importance.

To assess bone tissue properties, bone defects created surgically in animal models are used to better understand the repair process.¹⁴ For that purpose, one of the most used methods is micro-computed tomography (micro-CT), which is a nondestructive sectional imaging and high-resolution method.¹⁵ The images are collected from multiple viewing angles around the animal and reconstructed three-dimensionally (3D) on a micrometric scale, providing information about the 3D geometry and tissue properties^{16,17} in a relatively short turnaround time with a high throughput¹⁸ sharing the same physical principles as that of a clinical computed tomography scanner, but with higher resolution imaging.¹⁷ This methodology is currently considered the gold standard for quantifying 3D bone micro-architecture in animal models.¹⁶ Some variables associated with obtaining, processing, and analyzing scanings with micro-CT can affect the morphological results obtained from bone tissue samples.¹⁹ One of those variables is the segmentation of the analyzed structures, the first step in structural analysis and is critical

for accurately quantifying architectural parameters.²⁰ This is dependent on two steps, firstly of the definition of a region-of-interest (ROI), and then its thresholding to separate the tissues that must be quantified. These are among the most critical steps in micro-CT analysis. The ROI definition can be performed similarly to that of the trabecular and cortical bone.^{19,20}

Despite the existence of studies addressing the micro-CT methodology,^{14,16,20} there is a lack of information about the influence of the ROI definition on morphometric results. In this context, the aim of this study was to (1) assess whether there is any difference between the manual and predefined ROI definition methods during the 3D analysis of bone defects by micro-CT and (2), evaluate the repair process of bone submitted to RT after surgery. The null hypothesis was that the evaluated ROI definition methods would generate no influence on the results of the micro-CT analysis and no difference between the irradiated and non-irradiated bone tissue.

MATERIAL AND METHODS

Animals

This study was approved by the Bioethics Committee for Animal Experimentation (CEUA #045/20) at Federal University of Uberlândia - Brazil, and followed the normative guidelines of the National Council for Animal Control and Experimentation (CONCEA). A total of 24 male *wistar* rats weighing between 300 and 350g with three months old were included. They were acclimatized for two weeks before the experimental procedures and randomly housed in standard cages containing food and water *ad libitum* at an ambient temperature of 22 °C, under controlled humidity and a 12 h light-dark cycle. A previous pilot study determined the total number of included animals.

Surgical procedure

The animals were submitted to intraperitoneal anesthesia combining 100 mg/kg of 10% ketamine hydrochloride (Ketamina Agener®; Agener União Ltda, São Paulo, SP, Brazil) with 10 mg/kg of 2% xylazine hydrochloride (Rompum ® Bayer SA, São Paulo, SP, Brazil), then, both animals' legs were shaved and cleaned with a 2% chlorhexidine solution (Clorexoral® Biodinâmica, Ibioporã, PR) to prevent possible infections. Incisions of 3 cm length were performed over the legs and, the soft tissue, including the periosteum, was removed to expose proximal regions of the tibias. Finally, 2.3 mm diameter bone defects were created in the exposed region using a spherical drill #8 moved by a low-speed straight headpiece cooled with

physiological saline solution. The depth of perforation was limited to the rupture of the cortical bone. Subsequently, the animals received the due post-surgical care.

Radiotherapy

Seven days after surgical procedure, half of the sample (12 animals) was aleatory submitted to external RT. The other half was submitted to RT simulation procedure (*Sham* method). The animals were anesthetized in the same way as in the surgical procedure. Both legs were positioned laterally and fixed using adhesive tape. A wax bolus with a thickness of 15 mm was positioned over the legs to allow optimal delivery of the radiation dose. The beam was collimated and irradiation was delivered using a linear accelerator with a direct electron beam of 6 MeV electrons (Varian 600- C®; Varian Medical Systems Inc., Palo Alto, CA, USA). The photons were delivered by a single dose of 30 Gy (dose rate of 400 cGy/min), with a source–skin distance of 60 cm. The irradiation was restricted to the region the tibias using a Laser-guided system to define and limit the field (15 × 15), as is usually practised in patients. If signs of animal suffering were verified, it was excluded and submitted to euthanasia.

Animal Sacrifice and Sample Processing

Fourteen or 28 days after RT, animals were sacrificed, they were anaesthetized with 2.5% thiopental and sacrificed with an intravenous injection of 19% potassium chloride (Ariston Chemical and Pharmaceutical Industry Ltda. São Paulo, SP, Brazil). The tibias were removed by disarticulation and fixed in 4% paraformaldehyde solution in phosphate-buffered saline for 48h.

Micro CT analyses

The tibias were scanned by a single operator, at an energy of 70 kV and an intensity of 278 µA with an isotropic voxel of 9 µm using an aluminum filter (1 mm of thickness) and 180° rotation with an angular increment of 0.4° using micro-CT (Skyscan-1272 X-ray microtomography; Bruker, Kontich, Belgium). *NRecon*® software (version 1.7.4.2; SkyScan, Bruker, Belgium) was used for sample reconstruction, following the same parameters of smoothing (0), ring artifacts reduction (12) and beam-hardening (40%) for all samples. ROI definition was also carried out by a single operator, using two different methods (manual and predefinid), totaling a number of three study factors (RT, evaluation period and ROI definition method) and 6 Groups.

Dataviewer® software (version 16.0.0; SkyScan, Bruker, Belgium) was used to select sagittal slices (Fig 1). Top and bottom selection by *CT-Analyzer*® software (version 1.20.8.0; SkyScan, Bruker, Belgium) was performed, including the same number of slices (200) located in the central region of the lesion, for both ROI definition methods. In the manual method, the ROI delimitation was performed following the contours of the newly formed bone (Fig 2A), slice by slice. For the predefined method, a rectangular shape was used (2.3X0.5mm, considering the size of the lesion and the average of rat tibia cortical thickness, respectively) (Fig 2B), fitting it to the center of the bone lesion, and checking slice by slice to correct any possible displacement of the rectangle, since, as the evaluation approached the periphery of the lesion, the size of the lesion reduced progressively. For both methods, ROI delimitation was performed, including only the lesion located in the cortical region. The interpolation tool between the slices was used, allowing for the delimitation in a smaller number of slices after careful inspection. A standard threshold (upper 255 and lower 62) was applied, ensuring that differences between the study groups were inherent to the experiment itself and not to image processing, and finally, the 3D morphometric analysis of the cortical bone lesion was performed. Data related to bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp) and trabecular number (Tb.N) were obtained. The time consumed for ROI definition of each sample of both groups was carefully counted.

Statistical Analysis

The statistical analysis were performed using the package JAMOV (version 2.4.1). Data of all evaluated parameters were tested for normal distribution (Shapiro-Wilk) and equality of variances (Levene's test), followed by a three-way ANOVA test to assess the difference between groups, considering the three factors tested. When $\alpha = 0.05$ the difference was considered statistically significant. The time consumed for ROI definition (in seconds) of each sample was registered then, the median and standard deviation was obtained.

RESULTS

The means and standard deviation of all evaluated parameters are presented in table 1. For BV/TV, differences between the ROI definition methods ($p < 0.001$) and the period ($p < 0.001$) were detected. The manual method and 28 days period tended to present higher results than the predefined method and 14 days period. Tb.N was significantly influenced by the ROI definition method ($p < 0.001$) and evaluation period ($p = 0.02$), but not by RT ($p = 0.64$).

Evaluation period was the only factor that showed significant difference for Tb.Th ($p<0.001$), where the later period presented thicker trabeculae, ROI definition method showed no statistically difference ($p=0.96$). Tb.Sp was significantly influenced only by the ROI definition method ($p<0.001$), but not by the period ($p=0.96$) and RT ($p=0.87$). The comparison between irradiated and nono-irradiated groups using the manual method, showed that RDT did not significantly interfere in the repair process, for all parameters: BV/TV ($p=0.46$), Tb.N ($p=0.92$), Tb.Th ($p=0.56$) and Tb.Sp ($p=0.39$). The manual method was more time consuming (446.2 ± 49.5 s) than the predefined (131.2 ± 38.8 s).

DISCUSSION

The results showed that the ROI definition method influenced on the microtomographic analysis, thus, the null hypothesis was rejected. Although microtomography is a widely used methodology, some variables related to sample analysis need to be studied. BV/TV, Tb.Th, Tb.Sp, Tb.N are the minimal set of parameters that should be reported when describing trabecular bone morphology,¹⁶ for this reason, the present study was based on the results of these parameters, aiming to clarify what is the best way to define ROI. It's essential to measure trabecular parameters such as Tb.Th, Tb.Sp, Tb.N, because the woven bone is the first bone formed during the repair process due to rapid deposition of osteoid matrix, which will posteriorly be replaced by lamellar bone.^{16,21}

One of the reasons that may be behind the differences between the manual and predefined groups is the fact that, in the manual group, a contour was made involving only the bone area that was observed, while in the predefined group, a rectangle was used to fill the region correspondent to the lesion, therefore, not only the newly formed bone was included. This may have meant that, during parameter calculation, the software used different proportions, especially for BV/TV which is considered a critical morphometric parameter to describe trabecular microstructure¹⁵ and gives the ratio of the delineated bone volume to the total volume of the region-of-interest, which helps to determine new bone formation,¹⁶ and it has been considered the main outcome of several studies.^{21,22} Although the 28-day period showed signs of a more advanced repair process, it was not completely corticalized, and regions of mixing with trabecular bone remained. For these reasons, in the present study, trabecular parameters were used to evaluate the repair process in both periods ensuring a comparison between them. The separation of bone for cortical-only analyzes requires the ability to clearly distinguish the two structures. Algorithms have been proposed to separate both bone types with many relying

on a combination of threshold-based techniques combined with morphological opening/closing or erosion/dilation operations.²³

The manual method showed results that reflected a more advanced bone repair, for this reason, that method was used to compare the repair process taking into account the RT factor. Tb.Th tends to be thinner in irradiated bone than non-irradiated, but Tb.N and BV/TV tends to be the same, as shown in previous study.²¹

The response of cells to ionizing radiation depends on several factors, such as dose rate, radiosensitivity of cell type, cell age and internal and external microenvironments. The largest impact of radiation is on the least differentiated cells and the shortest cell cycle time such as bone marrow cells, and the least effect of radiation is on the most differentiated cells such as nervous system.²⁴ Damage caused by ionizing radiation to bone is dose- and time-dependent, the higher the radiation dose, the greater the level of damage.^{13,24,25} Dose-fractionation is an effective strategy and better than the single-dose treatment.²⁶ Most HNC patients treated with curative intent receive fractional doses of radiation, usually 2 Gy daily, five days a week for a period of five to seven weeks, totaling a dose between 50 and 70 Gy.⁵ For animal models like the one used in this study, its recommended to use a 30Gy single dose, classified as a mild procedure, which is equivalent to 2 or 3 times the routine doses used for treatment in humans, especially for treatment of squamous cell carcinomas of the upper aerodigestive tract in patients.^{13,21,25} Unfortunately, due to the difficulties in subjecting the animal to several fractional doses, animal studies avoid dose fractionation, opting for single doses.^{9,21} These single dose is enough to produce effects on bone microstructure, collagen maturation, and osteocytes, arresting the bone repair process.²¹ Fractioning the doses administered to the animals included in this study would not be sufficient to produce damage that we seek to detect through this study.^{5,25} Fractionation would be very costly and several animals would have been lost due to exposure to repeated procedures inherent to RT such as anaesthesia and animal transport, and we wouldn't have enough time to wait for the late effects of the RT, the animal could die before that moment. Multiple ketamine administration can diminish the bone remodelling potential, falsifying the deleterious effects of RT and consequently, the results of the study.²⁷ Anaesthesia also reduces the animal's blood flow and breathing rate and therefore may put the animal's life at risk, especially if associated with hypothermia and inadequate monitoring.²⁸

In the first two weeks after RT, there is an increase in the local number of osteoclasts and later there is a depletion.²⁹ This may explain the fact that the microtomographic parameters evaluated showed advanced repair process in the 28-day than in the 14-day period, although,

after long periods, a reduction in bone formation is expected, as the bone marrow population is surprising, also affecting mesenchymal stem cells.²¹ Furthermore, Arnold *et al*³⁰ proved that irradiation performed 4 days or more after surgery does not significantly interfere with the bone healing process even with very high doses of radiation. We chose the most proximal region, of the tibia (metaphysis, very close to the growth disc) for performing the lesion because this region also has woven bone, similarly to the jaws. A more central location could imply carrying out the lesion on a bone merely cortical, removing the mimetic effect of the study.²¹ Even though no statistically significant difference was detected between the irradiated and their respective non-irradiated groups, some irradiated tibia showed a much poorer repair than the non-irradiated ones, however, this number is reduced and they were considered outliers. This is probably due to their debilitation that interfered with the systemic state of the animal caused by the process of preparing the animals for RT (transport and aesthesia), but not the RT itself.

The acute effects are clinically observed during or within a few weeks after treatment. Late effects are typically seen after a latent period of six months or more.⁵ The evaluation periods of 14 and 28 days in rats would be equivalent to 2-3 months and 3-6 months in humans, respectively, since rats have a four to six times higher metabolic rate than humans.³¹ These short periods (14 and 28 days) did not allow complete repair of the lesion, facilitating its identification during microtomographic analysis.

The bony defects mimic dental extractions, as some HNC patients need to go through this procedure before RT, which is potentially dangerous.¹³ It is established that the radiation dose at which patients would prophylactically remove teeth to prevent ORN is 70 Gy in the maxilla and 60 Gy in the mandible, with healing period of 7–14 days between dental extractions and the start of RT avoiding delays to commencement of RT.¹ Some patients that undergo tumor ablative surgery before RT may be submitted to bone resection, which is considered a major challenge in oncological head and neck surgery because of the need of its reconstruction³² that may be required in oral cavity tumours and mandibular primary tumours where medullary bone invasion, inferior alveolar nerve involvement, aggressive tumor pattern of invasion or extensive invasion of peri-mandibular soft tissues are present.³³ Oromandibular reconstruction is now reliable due to the use of vascularized autogenous bonegraft³² which shows low rates of bone resorption and low complication and failure rates.³⁴ Despite RT can damage small vessels and potentially affect microvascular anastomoses of the bone flap,³⁵ studies have shown that perioperative radiotherapy does not interfere the bone flap viability.³⁶

CONCLUSION

Within the study's limitations, it was possible to conclude that:

The results of manual ROI definition method traduced a better repair process than the predefinid one. This method should be used whenever possible, despite being time-consuming.

No statistically significant difference in the repair process between irradiated and non-irradiated bone was observed. The evaluated parameters indicated an advanced bone repair in the 28-day period than in the 14-day period.

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ACKNOWLEDGMENTS

The authors were supported by the National Counsel of Technological and Scientific Development (CNPq), Coordination for the Improvement of Higher Education Personnel (CAPES; 001), and Research Support Foundation of the State of Minas Gerais (FAPEMIG).

FIGURES

Fig. 1 Selection of sagittal section on *Dataviewer*®

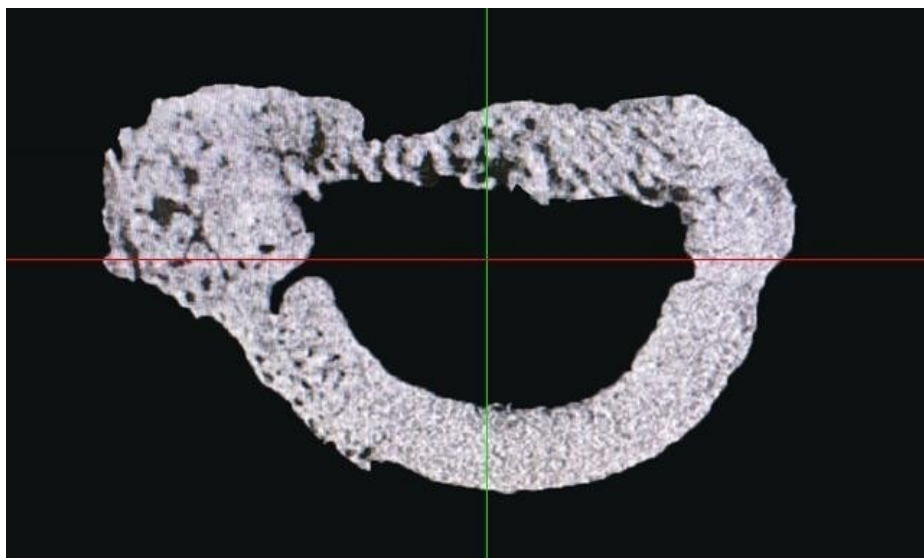


Fig. 2 ROI delimitation using *CT-Analyzer*® software. A) manual delimitation; B) automatic delimitation (predefined).

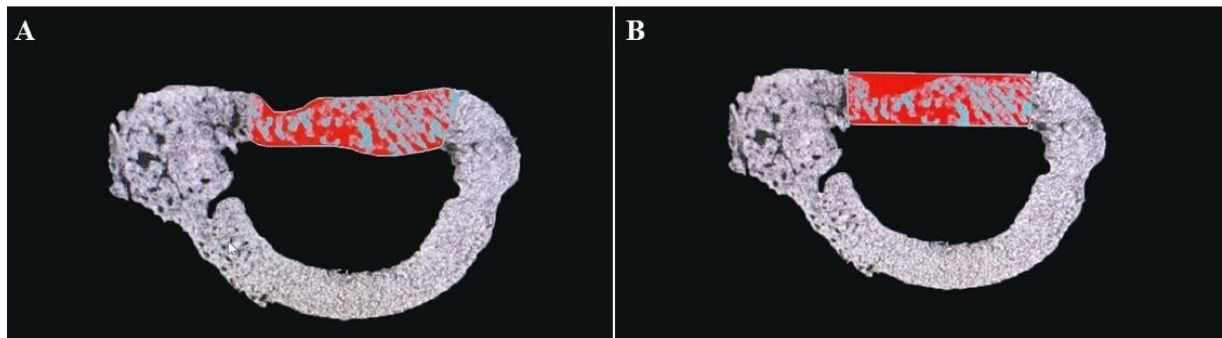


Table 1. Mean and standard deviation of BV/TV, Tb.N, Tb.Th and Tb.Sp parameters.

		14d		28d	
		A	M	A	M
BV/TV	NIr	59.12±12.53 ^{Ba}	75.04±7.63 ^{Aa}	68.62±6.93 ^{Ba}	87.01±6.51 ^{Aa}
	Ir	58.51±12.51 ^{Ba}	76.53±3.88 ^{Aa}	70.89±21.92 ^{Ba}	90.79±4.47 ^{Aa}
Tb.N	NIr	6.43±1.50 ^{Ba}	7.94±1.27 ^{Aa}	5.63±1.91 ^{Ba}	7.24±2.33 ^{Aa}
	Ir	5.96±1.27 ^{Ba}	8.14±1.20 ^{Aa}	5.38±1.89 ^{Ba}	7.13±1.16 ^{Aa}
Tb.Th	NIr	0.09±0.02 ^{Ba}	0.09±0.02 ^{Ba}	0.13±0.05 ^{Aa}	0.14±0.06 ^{Ba}
	Ir	0.10±0.02 ^{Ba}	0.10±0.02 ^{Ba}	0.13±0.02 ^{Ba}	0.13±0.02 ^{Ba}
Tb.Sp	NIr	0.11±0.03 ^{Ba}	0.10±0.15 ^{Aa}	0.13±0.04 ^{Aa}	0.05±0.01 ^{Ba}
	Ir	0.14±0.09 ^{Aa}	0.06±0.01 ^{Ba}	0.18±0.11 ^{Aa}	0.04±0.01 ^{Ba}

Different capital letters: significant differences within the same line; Different lowercase letters: significant differences within the same column.

3.3 Capítulo 3.

Artigo a ser submetido para *INTERNATIONAL JOURNAL OF ORAL AND MAXILLOFACIAL SURGERY*

Biological and biomechanical evaluation of the effects caused by postoperative radiotherapy on the osseointegration of titanium implants in rats

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ABSTRACT

Purpose: Radiotherapy (RT) is one of the strategies used to treat head and neck cancer patients, which may interfere with the osseointegration of dental implants. The aim of the present study was to assess the influence of ionizing radiation on the osseointegration of titanium implants installed previous to RT.

Methods: Custom-made grade IV titanium implants (2.7 x 1.4mm), coated with nanoparticulated hydroxyapatite (nHA) or treated with double-acid etching (DAE), were installed in the right and left tibias of 64 *wistar* rats, respectively. Seven days after surgical procedure, half of the sample (32 animals) was aleatory selected to RT with a dose of 30 Gy, the other half was submitted to RT simulation. Fourteen or 28 days after RT, animals were sacrificed. Microtomographic, histomorphometric and removal torque tests were performed. All data was tested for normal distribution (Shapiro-Wilk) and the Three-way ANOVA test was used for statistical analysis, followed by post hoc Tukey test for multiple comparisons among the groups. Differences were considered statistically significant if $\alpha < 0.05$.

Results: All testes performed showed no statistical difference between non-irradiated (Nlr) and irradiated (Ir) groups, 14- and 28-days, and between nHA and DAE surfaces, although the nHA surfaces and Nlr group showed better results in most comparisons. In general, the values of Nlr group tended to increase between the period of 14 and 28 days, while Ir seemed to decreased.

Conclusions: The osseointegration of implants installed previous to RT was similar to that of implants installed without associated RT.

KEY WORDS: Dental implants, radiotherapy, osseointegration

INTRODUCTION

Radiotherapy (RT) is a treatment strategy used in 60-70% of patients with cancer, and 40% of cancers can be treated exclusively by radiotherapy.¹ Surgery is another treatment modality, which can cause anatomical changes that will interfere with the retention and functionality of removable prostheses.² Implant supported prothesis are indicated for this patients,²⁻⁴ since osseointegrated implants contribute to the retention of bone height, while conventional prostheses are associated with gradual bone loss.³ A large number of patients become edentulous during the oral screening previous to RT,⁵ and the rehabilitation of these patients using dental implants is a great challenge, which is partially mitigated by the use of bioactive surfaces.⁶ Osseointegrated dental implants are made of commercially pure titanium with a roughened modified surface, which enhance the osseointegration potential, compared the smooth surfaces.^{4,7} Establishing a firm bone/implant interface is an important prerequisite for the short- and long-term clinical function of implants.⁸ This is influenced by surface geometry and topography,^{9,10} appropriate surgical techniques,^{7,11} biocompatibility, quality and bone quantity, loading conditions¹¹ and wettability.⁶

The placement of osseointegrated implants in irradiated patients was formerly contraindicated (before 1986).^{12,13} It was observed that the implant installation caused damage at the level of bone, periosteum, mucosa and vascular endothelium, which can lead to hypoxia, hypocellularity, hypovascularity and progressive fibrosis.^{14,15} Osteoradionecrosis (ORN) is one of the risks associated with the placement of dental implants in irradiated field, affecting more often the mandible than the maxilla, with an incidence in the mandible between 2% and 22%, being often diagnosed within the first 2 years after RT,¹⁶ and is related to doses greater than 60 Gy.¹⁷

Implant placement during the ablative tumour surgery or prior to RT, has already proven to be a strategy that provides good results.^{18,19} The advantage is that the patient will not have to wait long for the implant placement and rehabilitation, avoiding the need of a second surgical intervention (when the implant is installed during ablative surgery).^{4,20}

Although the original micro and macrostructural aspects of the standard Brånemark implant (Nobel Biocare, Goteborg, Sweden) were initially considered essential to ensure adequate bone formation around the implant,¹¹ one of the main focus of implantology today is the design of active surfaces, seeking to accelerate implant osseointegration,

through changes both in the roughness of the implant surface and in its chemical composition,^{7,8,21} which can be very useful in specific clinical situations, such as in the case of patients with deficiencies in the repair process (e.g. patients undergoing RT).²²

To help establishing a successful and predictable treatment protocol with implant-supported prostheses to rehabilitate HNC patients that will be treated with RT, consistent information about the osseointegration is necessary. In that context, the aim of the present study was to assess the influence of ionizing radiation on the osseointegration of titanium implants installed previous to RT, in two evaluation periods (14 and 28 days), using two different implant surfaces: coated with nanoparticulated hydroxyapatite (nHA) and treated with double acid etching (DAE). The null Hypotheses is that there is no difference in the osseointegration between the non-irradiated (NIr) and irradiated (Ir) groups, both surfaces and both evaluation periods.

MATERIAL AND METHODS

Animals

This study was approved by the Bioethics Committee for Animal Experimentation (CEUA #045/20) at Federal University of Uberlândia - Brazil, and was carried out in accordance with the ethical principles adopted by the National Council for Animal Control and Experimentation (CONCEA). Sixty-four male *Wistar* rats weighing between 300 and 350g were included. Thirty-two animals were used for microtomographic and histomorphometric analyses and same number was used for biomechanical test. They were randomly housed in standard cages containing food and water *ad libitum* at an ambient temperature of 22°C, under controlled humidity and a 12-hour light-dark cycle. The sample size calculation was based on previous studies,^{23,24} using the G-Power software (Version 3.1.9) with type I (α) and type II (β) errors of 5% and 20% respectively.

Surgical procedure

The animals were submitted to intraperitoneal anesthesia combining 100 mg/kg of 10% ketamine hydrochloride (Ketamina Agener®; Agener União Ltda, São Paulo, SP, Brazil) with 10 mg/kg of 2% xylazine hydrochloride (Rompum®; Bayer SA, São Paulo, SP, Brazil), then, trichotomy and antisepsis using 2% chlorhexidine solution (Clorexoral®; Biodinâmica, Iporã, PR) in both legs were performed, followed by incisions of 3 cm length over the legs and, the soft tissue (including the periosteum), was

removed to expose proximal regions of the tibias. Custom-made grade IV titanium implants with 2.7 mm in length and 1.4 mm in diameter (S.I.N. – Sistema Nacional de Implantes; São Paulo, SP, Brazil), coated with nanoparticulated hydroxyapatite or treated with double-acid etching, were installed in the right and left tibias, respectively. The site for installing the implant was prepared in proximal metaphysis of each tibia through a light initial drilling with a spear drill (2.0x18.0 mm) followed by the enlargement with a 1.2 x 15.0 mm drill, under copious sterile saline irrigation, using 800 rpm and 35 N torque. The implant was installed using a hexagonal screwdriver (1.2 x 24.0mm) until all the threads were inside the bone. The internal tissues were sutured in layers using a 5.0 bioabsorbable (Categute®; Technofio, Goiânia, GO, Brasil) and the 4.0 nylon sutures (Nylon®; Technofio, Goiânia, GO, Brasil) was used in the external layer. The animals received the due post-surgical care.

Radiotherapy

Seven days after surgical procedure, half of the sample (32 animals) was aleatory selected to external RT. The other half was submitted to RT simulation (*Sham* method). The animals were anesthetized in the same way as in the surgical procedure. Both legs were positioned laterally and fixed using adhesive tape. A wax bolus with a thickness of 15 mm was positioned over the legs to allow an optimal delivery of the radiation dose. The beam was collimated and irradiation was delivered using a linear accelerator with a direct electron beam of 6 MeV electrons (Varian 600- C®; Varian Medical Systems Inc., Palo Alto, CA, USA). The photons were delivered by a single dose of 30 Gy (dose rate of 400 cGy/min), with a source–skin distance of 60 cm. The irradiation was restricted to the region the tibias using a laser-guided system to define and limit the field (15 × 15), as is usually practised in patients. If signs of animal suffering were verified, it was excluded and replaced by another animal.

Animal Sacrifice and Sample Processing

The animals were sacrificed fourteen or 28 days after RT. They were anaesthetized with 2.5% thiopental and sacrificed with an intravenous injection of 19% potassium chloride (Ariston Chemical and Pharmaceutical Industry Ltda. São Paulo, SP, Brazil). One hundred and twenty-eight blocks of tibias containing the implants were collected and fixed in 10% formaldehyde for 48h. Half of these blocks (n=64) were randomly selected and used for microtomographic and histomorphometric analysis. The other half was

intended for the reverse torque removal test. Each half consisted of 32 tibias with nHA implants and the same number of DAE implants. All sample were identified with a random numerical sequence and separated by Microsoft Office Excel software (Microsoft, Redmond, WA, USA) into eight experimental groups (n = 8) according to factors under study (Implant surface, Radiotherapy and Evaluation period): 1.NIr14nHA; 2.NIr14DAE; 3.NIr28nHA; 4.NIr28DAE; 5. Ir14nHA; 6. Ir14DAE; 7. Ir28nHA; 8.Ir28DAE. Where: NIr – Non-irradiated; Ir -Irradiated; 14 or 28 - Interval between RT and sacrifice in days; nHA – Surface coated with nanoparticulated hydroxyapatite; DAE – Double acid etching surface.

Micro-CT analyses

Micro-computed tomography (micro-CT) (Skyscan-1272 X-ray microtomography; Bruker, Kontich, Belgium) was used for 3-dimensional radiographic analysis. The sample were scanned by a single operator, at an energy of 80 kV and an intensity of 125 μ A with an isotropic voxel of 9 μ m, using an aluminum filter (1 mm of thickness) and 360° rotation with an angular increment of 0.6° with a scanning time of 36 minutes for each sample.

NRecon® software (version 1.7.4.2; SkyScan, Bruker, Belgium) was used for sample reconstruction, following the same parameters of smoothing (0), ring artifacts reduction (3) and beam-hardening (30%) for all samples. *Dataviewer*® software (version 16.0.0; SkyScan, Bruker, Belgium) was used to place the implant in the correct position and select transaxial slices (Fig. 1). Top and bottom selection by *CT-Analyzer*® software (version 1.20.8.0; SkyScan, Bruker, Belgium) was performed, including 170 slices (total length of the implant portion that was inside the bone). A circular shape with 2.4 mm in diameter (0.5 mm away from the surface of the implant, totalling 1 mm more in the implant diameter) was used to delimit the region-of-interest (Fig. 2). The minimum gray-scale threshold value was set at 130 to 255 for implant and 62 to 180 for the bone surrounding the implant. Data related to bone volume fraction (BV/TV, %), trabecular thickness (Tb.Th; mm), trabecular separation (Tb.Sp; mm) and trabecular number (Tb.N, 1/mm), issue volume (TV, mm³) and bone volume (BV, mm³) was obtained.

Histological procedure

The samples were prepared by dehydration in an increasing solution of ethylic alcohol (70%, 80%, 90% and 100%), 7 days in each solution, at 5 °C. The samples were then imbibed in a methacrylate-based resin (LR White hard grade acrylic resin®; Agar

Scientific, Stansted, Essex, UK). Polymerization was achieved by mixing the accelerator and catalyst with the resin under the vacuum, following the manufacturer's instructions. Then, the excess resin was removed using an electric cutter (Skil 3385[®], China) and samples were subjected to a wear process using sandpaper (Tigre; Joinville-SC, Brazil) with progressively smaller granulation (#120, 220, 320, 500, 600, 1200 and 2000 μm), until reaching the centre of the implant, using a polishing machine (AROPOL 2 V[®]; Arotec, Cotia-SP, Brazil). Then, the previously sanded and polished side was glued over an acrylic slide, using an ethyl-cyanoacrylate-based adhesive (Superbonder[®]; Loctite, São Paulo, SP, Brazil). On the other side of the slide, coated with a plastic film, a plastic soda cap was glued and used as a handle during the wear process of the other side of the sample, until reach a thickness of approximately 30 μm . At the end, the soda cap and the plastic film were removed, the slide was identified and the staining with toluidine blue was performed.

Histomorphometry

Digital images of the histological sections obtained using a microscope (Leica DM500, Germany) attached to a digital camera (Leica ICC50, Germany) were used for calculating percentage of bone-to-implant contact (BIC) and the percentage of mineralized bone area between threads (BAPT), using ImageJ software, version 1.34s (NIH, Bethesda, MD, USA), to quantify the osseointegration.

Removal torque test

The hexagonal screwdriver used for implant installation was connected to the terquimeter (Tohnichi STC200CN; M.Shimizu, Japan) with a scale range from 0.1 to 10.0 Ncm. A counterclockwise force was applied until the bone-implant interface was disrupted and the corresponding value was registered.

Statistical Analysis

Analysis were performed using the package JAMOV (version 2.4.1). All data was tested for normal distribution (Shapiro-Wilk). The Three-way ANOVA test was used for statistical analysis, followed by the Tukey post-hoc test for the multiple comparisons among the groups and significance level of $\alpha = 0.05$.

RESULTS

Microtomographic analysis

The micro-CT means and standard deviation values are shown in Table 1. There was no significant differences for all microtomographic parameters, although, in general, all Nlr groups presented higher results compared with Ir groups, for TV ($p=0.84$), BV ($p=0.06$), BV/TV ($p=0.10$), Tb.Th ($p=0.46$), Tb.N ($p=0.38$) and Tb.Sp ($p=0.97$).

Regarding the surface, nHA showed higher results than DAE for TV ($p=0.84$), BV ($p=0.86$), BV/TV ($p=0.84$), Tb.Th ($p=0.32$). DAE surface presented higher result for Tb.N ($p=0.89$) and Tb.Sp ($p=0.43$).

The period of 28 days presented higher values than 14 days, for TV ($p=0.15$), BV ($p=0.41$), BV/TV ($p=0.49$), Tb.Th ($p=0.46$), but the 14 days period presented higher Tb.N ($p=0.73$) and Tb.Sp ($p=0.43$) values. It was also observed that in the Nlr groups, the values of TV, BV (Except for DAE surface), BV/TV (except for NlrDAE and IrDAE groups), Tb.Th (except IrDAE group), Tb.N (except Ir DAE surface) and Tb.Sp (only for NlrDAE and IrDAE) tended to increase over time. The figure 3 represents three-dimensional models obtained from micro-Ct, using CTvox® software.

Histomorphometric analysis

BIC and BAPT values are shown in table 2 and 3, respectively. The three-way ANOVA test showed that RT ($p=0.38$), evaluation period ($p=0.18$) and type of surface ($p=0.12$) did not influence the BIC. However, Nlr groups presented higher values than the Ir ones, except for DAE14d group. As time passed, BIC increased in all Nlr and Ir groups. nHA presented higher BIC values than DAE in all the groups.

For BAPT, the only statistically difference detected was regarding the evaluation period ($p=0.03$), the values increased over time for all, except for IrnHA group. No statistically difference was detected for RT ($p=0.46$) and type of surface ($p=0.57$). Values were higher in the Nlr group, comparing to the Ir one, except for nHA14d and DAE28 groups. All the nHA surface presented higher values than DEA, except the IrnHA28d group.

The histological evaluation, showed bone in direct contact with the implant surface, in all groups, especially in areas close to the trabecular bone (contact osteogenesis). The nHA-bone contact seemed to be more extensive than DAE and the period of 28 days had more contact than 14 days period for Nlr and Ir groups (Fig. 4).

Reverse torque removal test

This test showed that in the 14d period, the NIr groups presented higher values than Ir groups and the opposite was observed in the 28 days period (nHA surface). The results of all groups tended to increase over time. nHA surface presented higher values than DAE in all the groups, except in Ir14d. The only statistically significant difference was observed in the 28d period, between IrnHA and IrDAE groups, where the first presented higher result ($p = 0.03$) (Table 4).

During the interval between the surgery and sacrifice, the NIr and Ir animals lost an average of 67.56 ± 15.34 g and 75.93 ± 18.45 g, respectively. Eight animals died during the study, all belonging to the Ir group, which was promptly replaced.

DISCUSSION

In general, no difference was observed when comparing NIr with Ir groups, regardless of the evaluation period or surface used, although the 28d period and nHA showed results relatively higher than the DAE surface and 14d period. Then, the null Hypotheses has been accepted.

The interval of 7 days between the implant placement and RT allowed the bone to begin its formation around the bioactive implant surfaces, since, it has been shown that a period of 5 days is enough to detect new bone formation in rat model, and at 28 - 30 days, the newly formed bone in contact with the implant surface is almost completed.²⁵ In a longer interval between implantation and RT (more than 14 days), normal osteocytes can be found in the newly formed bone around the implant, but the amount of osteoid matrix formed may be smaller compared to the NIr group,²⁶ becoming evident that the longer the interval between implantation and RT, the better the osseointegration process and the smaller the probability of finding differences between the Ir and NIr groups, since more differentiated osteoblasts are known to be less sensitive to irradiation than the immature ones.^{26,27} Therapeutic irradiation shortly after implantation increases the risk of fibrous encapsulation.²⁶ Probably this is why RT did not interfere significantly in the results of the present study.

A single radiation dose was administrated, based on previous study, which suggests that 30 Gy is enough to interfere with the bone repair process, causing morphological changes in the trabecular arrangement of newly formed bone, the characteristics of osteocytes, increasing their probability of death.²⁸ In humans, the total radiation dose in

the range of 50 to 60 Gy is considered borderline for rehabilitation with dental implants, without the need for additional treatments such as hyperbaric oxygen therapy.^{2,14,29} Fortunately, radiation dose frequently administered to patients ranges between 47.1 to 62 Gy.¹⁸ The higher radiation dose and the duration of RT, the worse the results of osseointegration are expected, especially in the case of patients who received implants after RT.¹³ High-dose irradiation induced the initial extinction of bone cells, decreases bone turnover activities in dogs, and an accelerated resorption process was observed 4 to 10 weeks after irradiation. Fractional dose administration after surgery allowed most Haversian canals to preserve unplugged blood vessels in dogs, even with relative high doses (43 Gy), therefore, the ischemia, which is responsible to accelerate the formation of osteoclasts, may not be evident.³⁰ That may also justify the persistence of bone formation in the Ir group of the present study, despite the irradiation dose was not fractionated, but was lower (30 Gy).

A preclinical study showed that fourteen days after irradiation, most of the nHA surface was in contact with newly formed bone but, bone remodelling was observed after 28 days.²⁶ Most of the parameters evaluated in the present study were better at the 14-day period than at the 28-day period, in the Ir group, for both surfaces as a result of the described remodelling process that is marked at the 28-day period.

The histomorphometric parameters evaluated in the present study (BIC and BABT) reflect the formation of new bone in contact with the implant surface. However, their proportion depend on several factors, including surgical technique, implantation site, timing and implant design.²² As in the present study, Kudo *et al*²⁶ also observed that the BIC occurring before irradiation was minimally affected when compared to the NIr group. This may be associated with the preservation of mesenchymal cells due to their high radioresistance properties and their capacity of repairing the DNA double-strand breaks caused RT.⁴ The radioresistance of MSCs is even greater when they are exposed to low and fractionated irradiation dose.³¹ Fractionation leads to higher tissue tolerance, persisting the osteogenic potential of the medullary compartment.³⁰ Mesenchymal stem cells derived from human bone marrow exposed to irradiation doses of 30 or 60 Gy showed significant decrease in proliferation, however, the subpopulation of living cells was able to differentiate after irradiation.³²

The histomorphometric analysis showed that the new bone formation in Ir group tended to increase over time but, the microtomographic analysis showed the opposite. This difference can be explained by the fact that the histology is a bidimensional method

showing only a slice, while micro-CT involves a three-dimensional analysis of all the sample.³³ The reverse torque removal test has been used to measure the implant anchorage or osseointegration, higher torque rotation forces required to remove implants can mean an increase in bone-implant contact.¹¹ It provides an indirect measure of the shear force required to disrupt the bone-implant interface.³⁴

Regarding the implant surface, it has been shown that different implant surfaces can affect protein adsorption, platelet activation and aggregation, fibrin retention and cell-to-surface interaction at the bone/implant interface.^{22,35} Rougher surfaces stimulate the differentiation, growth and attachment of bone cells and increase mineralization.³⁶ There is a certain range of roughness that can be perceived by the undifferentiated cell, in which it cannot flatten and differentiate into a fibroblastic morphology, instead, it assumes an osteoblastic morphotype.³⁵ Several studies show that acid etched implants offer greater resistance to reverse torque removal and better osseointegration compared to machined surface implants.^{7,10,11,37} This type of surface has proven to favourably affect angiogenesis as well as cell migratory behaviour, cell alignment, orientation, attachment, and ultimately cell activity and function.¹¹

Hydroxyapatite is a very stable biological form of CaP.⁶ It has a great protein adsorption capacity, improves osteoblastic proliferation, increases bone formation and reduces bone loss, which makes the connection between the bone and the implant faster and stronger compared to uncoated implants.⁸ On the other hand, calcium ions create a biochemical bond between the implant and the bone, resulting in the acceleration of the osseointegration process.⁸ The presence of nHA on implant surfaces increases osteogenic markers such as alkaline phosphatase and osteocalcin while at the same time suppressing inflammation.³⁸ The nHA surface has the potential to promote a better environment to trigger cell spread, with a significant increase in osteoblastic adhesion when compared with machined surfaces. Both DAE and nHA do not interfere with the viability of osteoblasts when compared to the machined surface.³⁹

While the microtopography of the implant surface acts at the cellular level, it is believed that nanotopography influences the interactions between the cell and the implant at the cellular and protein level (at the chemical level), thanks to its similarity between the nanotopography and the extracellular matrix.^{6,9,10} Therefore, nanotopography is linked to increased gene expression and is indicative of faster osteoblastic differentiation. In addition, the nanoscale induces a selectivity of cell adhesion, showing a relative reduction of fibroblast adhesion increasing the osteoblast adhesion when compared with

microstructured surfaces.¹⁰ It has also been proven in a pre-clinical study,³⁸ through the nanoindentation test, that the quality of the bone tissue is significantly improved around implants coated with nHA, in comparison with implants treated with acids.

Having in mind the above, it was expected that the results of the nHA surface would be better than the DAE, however, both surfaces had similar performance. The difference in the osseointegration process of between implants DAE and nHA seems to be more evident in the first moments after implantation than in the long term, favouring early osseointegration events. In the long term, the performance of both surfaces tends to be similar, as confirmed in the present study.⁹

The fact that the implants were not loaded is one of the factors that prevents the direct comparison of the results of the present study with the real clinical situation where the implants are loaded, since, as, controlled loads on implants increase bone formation and inhibit resorption.⁴⁰ Another important issue is fractionation or hyper fractionation, which is performed clinically,⁴¹ but for logistical reasons and to avoid the excessive exposure of the animal to the anaesthesia which can cause death,⁴² it was not possible to use this strategy in the animal model used in the present study. Despite the absence of differences between the groups, it seems that the RT interfered with the weight of the animals.

CONCLUSION

Within the limitation of the study design, it was possible to conclude that the osseointegration of implants installed previous to RT is similar to that of implants installed without associated RT, regardless of the period evaluated or the type of surface of the implant.

The progress in the field of implant dentistry, which aims to create coatings with growth factors that actively influence the bone repair process is quite promising, and future research will be able to evaluate its effect on the osseointegration of implants in irradiated patients, with a view to shortening even more the interval between implant placement and the start of the RT, achieving good results in its osseointegration.

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TABLES

Table 1: Mean and standard deviation of microtomographic parameters.

		14d		28d	
		nHA	DAE	nHA	DAE
TV (mm ³)	NIr	4.39±0.08 ^{Aa}	4.23±0.08 ^{Aa}	4.43±0.13 ^{Aa}	4.29±0.06 ^{Aa}
	Ir	4.34±0.16 ^{Aa}	4.21±0.29 ^{Aa}	4.22±0.22 ^{Aa}	4.14±0.09 ^{Aa}
BV (mm ³)	NIr	0.77±0.24 ^{Aa}	0.74±0.23 ^{Aa}	0.78±0.23 ^{Aa}	0.82±0.18 ^{Aa}
	Ir	0.68±0.15 ^{Aa}	0.64±0.12 ^{Aa}	0.75±0.14 ^{Aa}	0.78±0.14 ^{Aa}
BV/TV (%)	NIr	16.21±7.92 ^{Aa}	18.21±7.59 ^{Aa}	16.41±7.04 ^{Aa}	15.31±4.12 ^{Aa}
	Ir	14.84±5.33 ^{Aa}	17.62±5.44 ^{Aa}	16.87±6.32 ^{Aa}	13.02±6.08 ^{Aa}
Tb.Th (mm)	NIr	0.04±0.01 ^{Aa}	0.03±0.01 ^{Aa}	0.04±0.01 ^{Aa}	0.03±0.01 ^{Aa}
	Ir	0.03±0.01 ^{Aa}	0.04±0.01 ^{Aa}	0.03±0.01 ^{Aa}	0.03±0.01 ^{Aa}
Tb.N (1/mm)	NIr	5.22±1.21 ^{Aa}	3.42±0.44 ^{Aa}	5.52±1.94 ^{Aa}	3.81±1.23 ^{Aa}
	Ir	3.01±1.38 ^{Aa}	5.64±2.01 ^{Aa}	4.12±1.12 ^{Aa}	3.24±1.48 ^{Aa}
Tb.Sp (mm)	NIr	0.11±0.02 ^{Aa}	0.10±0.04 ^{Aa}	0.11±0.02 ^{Aa}	0.14±0.01 ^{Aa}
	Ir	0.13±0.02 ^{Aa}	0.11±0.04 ^{Aa}	0.11±0.03 ^{Aa}	0.12±0.03 ^{Aa}

Different capital letters: significant differences within the same line; Different lowercase letters: significant differences within the same column.

Table 2: Mean and standard deviation of the values corresponding to the BIC (%).

	14d		28d	
	nHA	DAE	nHA	DAE
NIr	35.22±7.74 ^{Aa}	19.37±7.16 ^{Aa}	43.68±9.16 ^{Aa}	32.38±17.9 ^{Aa}
Ir	31.44±11.20 ^{Aa}	22.79±9.10 ^{Aa}	23.43±8.27 ^{Ba}	28.18±7.17 ^{Aa}

Different capital letters: significant differences within the same line; Different Lowercase letters: significant differences within the same column.

Table 3: Mean and standard deviation of the values corresponding to BABT (%).

	14d		28d	
	nHA	DAE	nHA	DAE
NIr	42.26±10.35 ^{Aa}	40.14±12.03 ^{Aa}	50.94±13.32 ^{Aa}	52.72±16.53 ^{Aa}
Ir	53.67±9.14 ^{Aa}	37.50±9.35 ^{Ba}	50.5±14.06 ^{Aa}	60.66±12. ^{Aa}

Different capital letters: significant differences within the same line; Different Lowercase letters: significant differences within the same column.

Table 4: Mean and standard deviation of the values corresponding to the reverse torque removal test.

	14d		28d	
	nHA	DAE	nHA	DAE
NIr	1.85±0.89 ^{Aa}	1.77±0.56 ^{Aa}	2.35±0.60 ^{Aa}	2.21±0.39 ^{Aa}
Ir	1.25±0.55 ^{Aa}	1.57±0.63 ^{Aa}	2.90±0.61 ^{Aa}	2.03±0.88 ^{Ba}

Different capital letters: significant differences within the same line; Different Lowercase letters: significant differences within the same column.

FIGURES

Figure 1. Placement of the implant in correct position to select the transaxial slices, using *Dataviewer*® software.

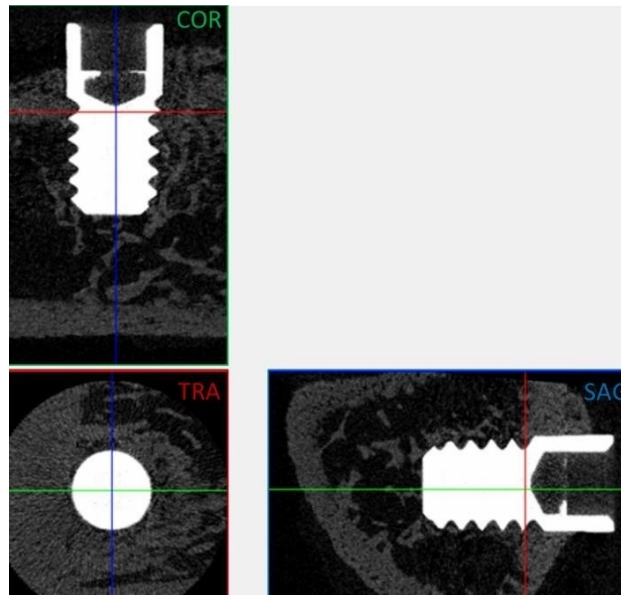


Figure 2. Delimitation of a circular region-of-interest with 0.5 mm away from the implant surface using *CT-Analyzer*® software.

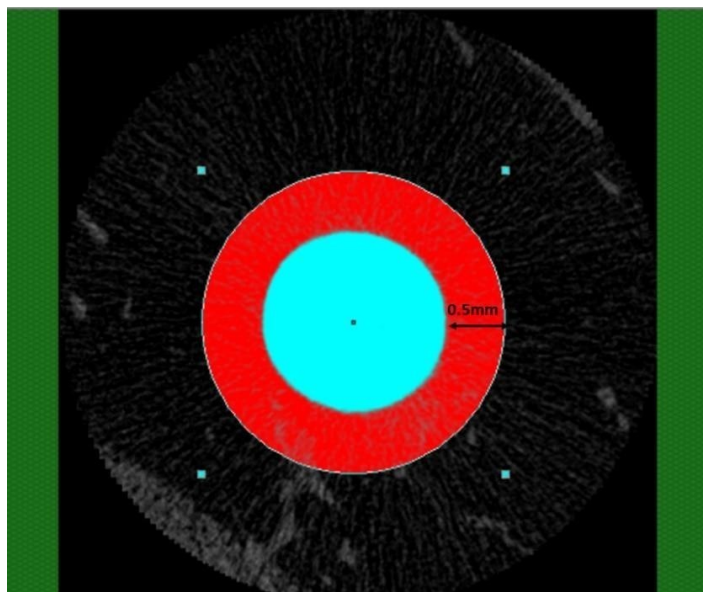


Figure 3. Representative three-dimensional models of bone with implant, obtained from micro-CT using *CTvox®* software.

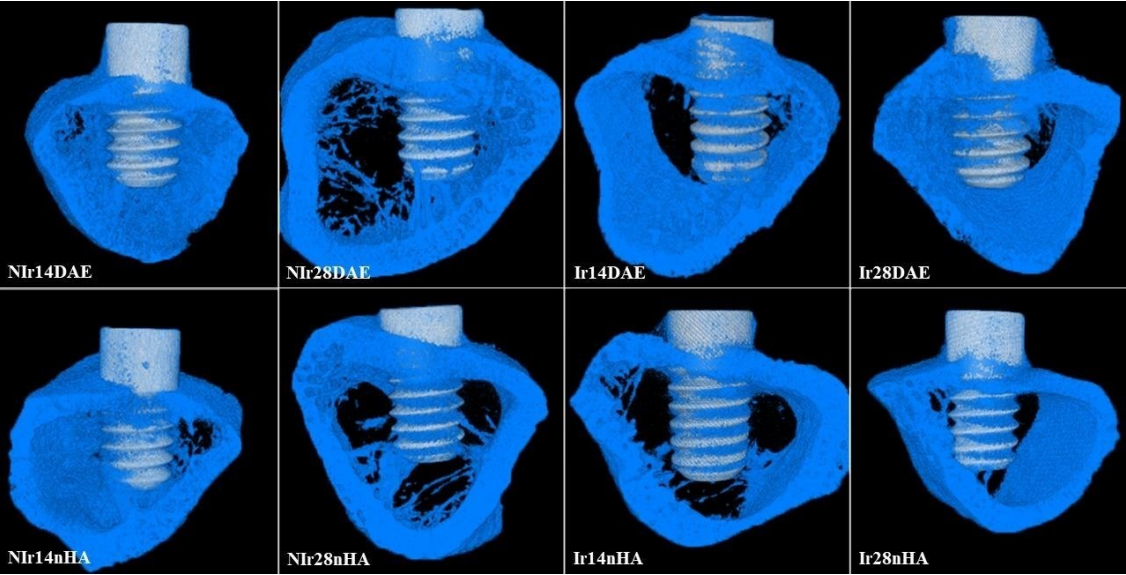
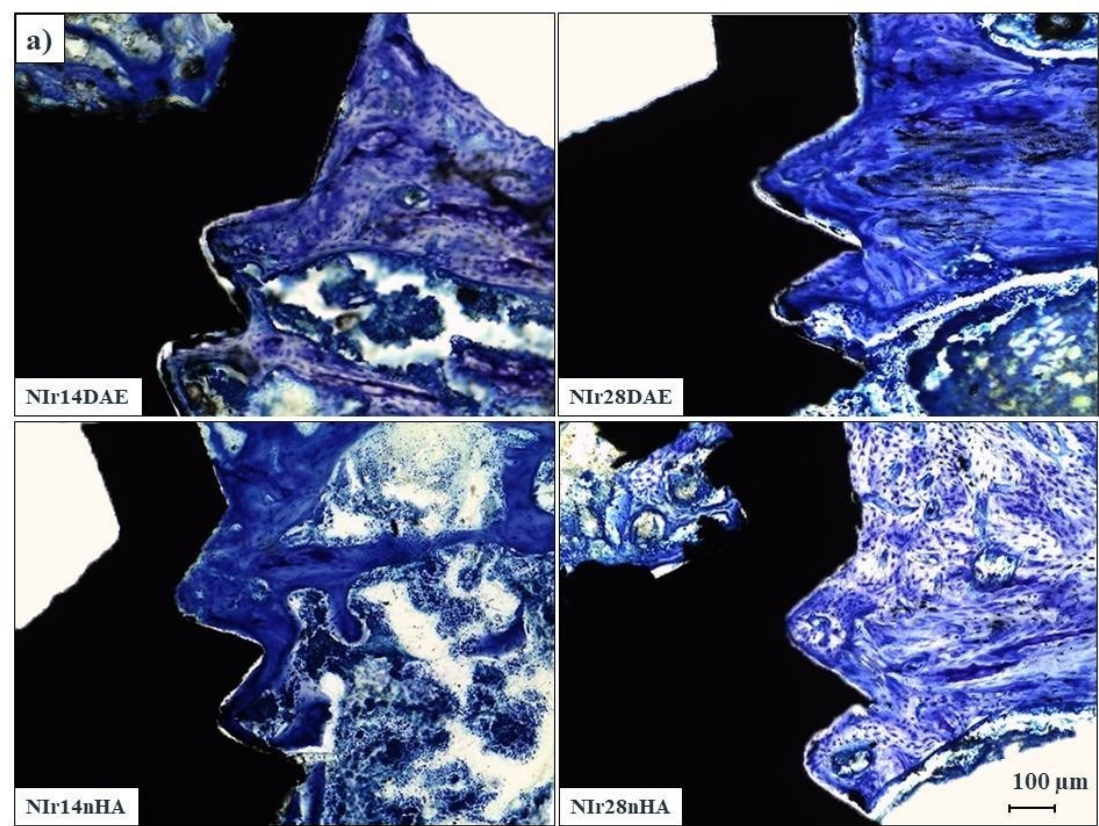
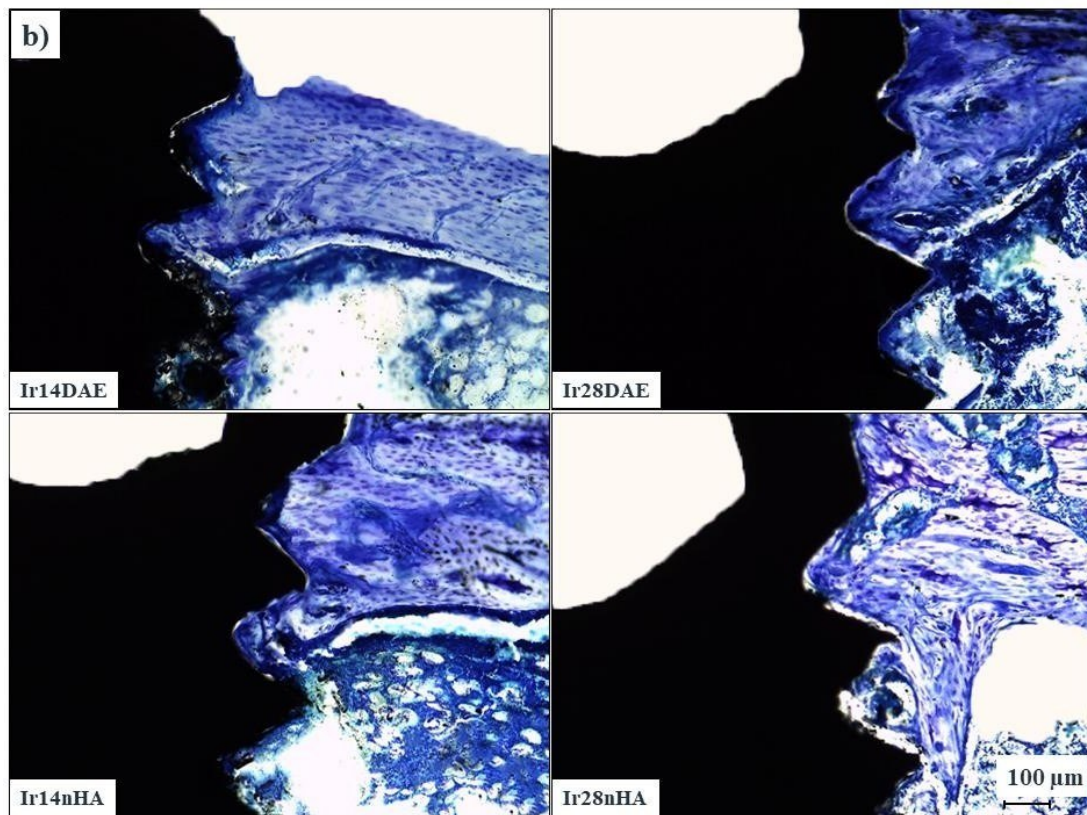


Figure 4. Histological images of non-irradiated (a) and irradiated (b) groups – 10x.





4. CONCLUSÕES

Com base nas metodologias e resultados obtidos nos diferentes estudos, pode-se concluir:

- Apesar das alterações causadas pela radiação ionizante nos tecidos peri-implantares, os implantes dentários instalados pré- e pós-radioterapia tiveram uma elevada taxa de sobrevida, semelhante à dos implantes instalados sem radioterapia associada.
- O método de definição da região de interesse interfere nos resultados obtidos através da análise por micro-CT.
- Não foi observada diferença estatisticamente significativa no processo de reparo entre osso irradiado e não irradiado. Os parâmetros avaliados indicaram reparo ósseo mais avançado no período de 28 dias do que no período de 14 dias.
- A osseointegração de implantes instalados previamente à radioterapia foi semelhante à de implantes instalados sem radioterapia associada, independente do período de avaliação ou do tipo de superfície do implante.

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¹ De acordo com a Norma da FOUFU, baseado nas Normas de Vancouver. Abreviaturas dos periódicos com conformidade com Medline (Pubmed).

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CERTIFICADO

Certificamos que o projeto intitulado “Efeito da radiação ionizante no processo de reparo ósseo periimplantar – análises biológicas, biomecânicas e morfológicas”, protocolo nº **045/20**, sob a responsabilidade de **Priscilla Barbosa Ferreira Soares** – que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata, para fins de pesquisa científica – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela COMISSÃO DE ÉTICA NA UTILIZAÇÃO DE ANIMAIS (CEUA) da UNIVERSIDADE FEDERAL DE UBERLÂNDIA, em reunião **26 de Março de 2021**.

(We certify that the project entitled “Efeito da radiação ionizante no processo de reparo ósseo periimplantar – análises biológicas, biomecânicas e morfológicas” protocol **045/20**, under the responsibility of **Priscilla Barbosa Ferreira Soares** - involving the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata, for purposes of scientific research - is in accordance with the provisions of Law nº 11.794, of October 8th, 2008, of Decree nº 6.899 of July 15th, 2009, and the rules issued by the National Council for Control of Animal Experimentation (CONCEA) and it was **approved** for ETHICS COMMISSION ON ANIMAL USE (CEUA) from FEDERAL UNIVERSITY OF UBERLÂNDIA, in meeting of March 26th, 2021).

Vigência do Projeto	Início: 05/05/2021 Término: 01/08/2023
Espécie / Linhagem / Grupos Taxonômicos	Rato heterogênico Wistar
Número de animais	108 animais
Peso / Idade	300g / 90 dias
Sexo	Macho
Origem / Local	REBIR – UFU
Local onde serão mantidos os animais:	REBIR - UFU

Uberlândia, 05 de Abril de 2021.


Prof. Dr. Luiz Fernando Moreira Izidoro
UNIVERSIDADE FEDERAL DE UBERLÂNDIA
Comissão de Ética na Utilização de Animais
Coordenador da CEUA
Portaria R Nº 1114/2020