LUIZ HENRIQUE FERREIRA JÚNIOR

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Effect of different therapies on Bone Tissue Repair and Microstructure.

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UBERLÂNDIA, 2020

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A seguir o senhor(a) presidente concedeu a palavra, pela ordem sucessivamente, aos(às) examinadores(as), que passaram a arguir o(a) candidato(a). Ultimada a arguição, que se desenvolveu dentro dos termos regimentais, a Banca, em sessão secreta, atribuiu o resultado final, considerando o(a) candidato(a):

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SUMÁRIO

RESUMO

A estrutura óssea possui um processo fisiológico de regeneração complexo, visto que este tecido possui inúmeras particularidades estruturais além de sofrer remodelação contínua ao longo da vida. No entanto, existem condições clínicas adquiridas em que o processo de reparo ósseo é comprometido. A morfologia e a constituição óssea são alteradas quando associadas a determinados medicamentos ou à radiação ionizante, terapias médicas essenciais para tratamento de algumas patologias. A radioterapia é usualmente empregada para tratamentos de câncer, no entanto, altas doses de radiação provocam danos metabólicos em tecidos sadios. No tecido ósseo, a radiação ionizante promove alterações em sua composição química e física, resultando em um colapso imediato da qualidade óssea, produzindo efeitos colaterais graves como a osteorradionecrose mandibular (ORN), o aumento do risco de fraturas e a hipovascularização do tecido, resultando em uma capacidade de cicatrização reduzida. A osteorradionecrose acomete pacientes que recebem radiação terapêutica para o tratamento do câncer da cabeça e pescoço. A área afetada pela radioterapia possui o processo de renovação do tecido ósseo prejudicado devido a alterações teciduais hipovasculares, hipocelulares e hipóxicas. O uso de bifosfonatos é regularmente indicado para condições relacionadas ao câncer e para o manejo de doenças ósseas metabólicas, atuando sobretudo na inibição da atividade osteoclástica e consequentemente inibindo a reabsorção óssea. A osteonecrose dos maxilares relacionada ao bisfosfonato é uma comum complicação que afeta adversamente a qualidade de vida, produzindo morbidade significativa em pacientes afetados prejudicando o metabolismo de renovação óssea. A oxigenação hiperbárica (OH) tem sido utilizada como uma estratégia eficiente para minimizar os prejuízos relacionados a osteorradionecrose e a osteonecrose associada ao uso de bifosfonatos. Considerando esse contexto, a presente tese de doutorado propôs investigar o efeito do OH na regeneração óssea através de revisão sistemática e por meio de experimentação em ratos submetidos a radiação ionizante e a protocolos medicamentosos. Em conclusão as análises indicaram que a OH favorece o processo de reparo ósseo comprometido, através de efeitos benéficos como: porcentagem de neoformação óssea, resposta angiogênica aumentada, assim como atividades relacionadas à osteogênese e remodelação óssea.

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

Palavras-chave: Regeneração óssea, Osteonecrose associada a bisfosfonatos, osteorradionecrose, radiação ionizante, Oxigenação hiperbárica.

ABSTRACT

The bone structure has a complex physiological process of regeneration, since this tissue has many particular characteristics in addition to undergoing continuous remodeling throughout life. However, there are acquired clinical conditions where bone repair process is compromised. Bone morphology and constitution are altered when associated with some specific use of medication or ionizing radiation, essential medical therapies for the treatment of some pathologies. Radiotherapy is currently used to control cancer, however, high doses of radiation cause metabolic damage to healthy tissues. In bone tissue, ionizing radiation promotes changes in its chemical and physical composition, resulting in immediate collapse of bone quality, producing serious effects such as mandibular osteoradionecrosis (ORN), increased risk of fractures and tissue hypovascularization, resulting in a reduced healing capacity. Osteoradionecrosis affects patients who receive ionizing radiation as therapeutic treatment for head and neck cancer. The affected area by radiotherapy has the process of restoring bone tissue impaired due to hypovascular, hypocellular and hypoxic tissue changes. The use of bisphosphonates is regularly indicated for conditions related to cancer and metabolic bone diseases, acting mainly in inhibiting osteoclastic activity and consequently inhibiting bone resorption. Jaw osteonecrosis related to the use of bisphosphonate is a common complication that adversely affects quality of life, producing significant morbidity in patients affected injuring the metabolism of bone repair. Hyperbaric oxygenation (OH) has been used as an efficient strategy to minimize the damage related to osteoradionecrosis and osteonecrosis associated with the use of bisphosphonates. In this context, the present phd dissertation proposed an study on the effect of OH on bone regeneration through systematic review and through experiments in rats submited to ionization and medication protocols. In conclusion, the analyzes indicated that OH favored the bone repair process once compromised, through beneficial effects such as: percentage of new bone formation, increased angiogenic response, as well as activities related to osteogenesis and bone remodeling.

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

Kew-words: Bone regeneration, Bisphosphonate-Associated Osteonecrosis of the Jaw, Osteoradionecrosis, Ionizing radiation, Hyperbaric oxygenation.

INTRODUÇÃO E REFERENCIAL TEÓRICO

1 - INTRODUÇÃO E REFERENCIAL TEÓRICO

Os ossos apresentam funções essenciais para o funcionamento do corpo humano. Sua arquitetura, em conjunto com os músculos esqueléticos, propicia dinamismo mecânico, sustentação e proteção de órgãos internos. Os ossos também contribuem para o armazenamento e equilíbrio de minerais e triglicerídeos, bem como para a produção de células mesenquimais, eritrócitos, leucócitos e plaquetas por meio da medula óssea, que é um tecido conjuntivo especializado presente no interior dos ossos longos (1).

O tecido ósseo, componente importante dos ossos, é uma forma especializada de tecido conjuntivo constituído por uma fase mineral ou inorgânica relacionada à uma matriz orgânica. A fase inorgânica é composta, essencialmente, por cristais de fosfato de cálcio sob a forma de hidroxiapatita, enquanto a fase orgânica é composta por colágeno tipo I, proteoglicanas e proteínas não colágenas. A combinação entre os componentes minerais e os componentes orgânicos confere ao tecido ósseo rigidez com boa maleabilidade, tornando-o resistente às demandas funcionais(2).

Quatro diferentes tipos de células compõem o tecido ósseo: osteoblastos, osteoclastos, osteócitos e células osteoprogenitoras. As células responsáveis pela síntese e deposição da matriz orgânica são os osteoblastos, que também participam do processo

de mineralização dessa matriz. Adjacente aos osteoblastos existem células osteogênicas ou osteoprogenitoras que se diferenciam em osteoblastos quando necessário. Durante a formação da matriz óssea, alguns osteoblastos ficam aprisionados na matriz, sendo então denominados osteócitos. Estas células comunicam-se entre s por meio de prolongamentos citoplasmáticos localizados em uma rede canalicular, a qual permite a passagem de nutrientes e outras substâncias essenciais para a manutenção da matriz óssea (3). Os osteoclastos, por sua vez, são células multinucleadas localizadas na superfície da matriz óssea e responsáveis pela reabsorção da mesma, sendo ativos no processo de remodelação óssea(4,5).

Histologicamente, o tecido ósseo pode ser classificado em primário (imaturo) ou secundário (maduro ou lamelar). O tecido ósseo imaturo apresenta fibrilas colágenas sem organização definida, maior número de osteócitos incluídos na matriz óssea e menor conteúdo mineral. No adulto ele está presente nas suturas dos ossos do crânio, nos alvéolos dentários e em alguns pontos de inserção de tendões. Ele é o primeiro tecido ósseo formado, seja na constituição do esqueleto ou em processos de reparação óssea. Por sua vez, o osso maduro possui uma arquitetura organizada em camadas denominadas lamelas, que podem ser paralelas entre si ou concêntricas em torno dos canais de Havers formando os ósteons. No interior desses canais transitam nervos e vasos sanguíneos, essenciais para o metabolismo do tecido Entre as lamelas existem pequenas lacunas onde estão alojados os osteócitos, interconectados pelos canalículos. Em um indivíduo adulto e saudável, o osso maduro adquire estrutura lamelar, com menor número de osteócitos incluídos na matriz e maior conteúdo mineral, o que lhe garante maior resistência à demanda mecânica(2,4,6)

Macroscopicamente, o tecido ósseo apresenta importantes diferenças na organização estrutural, podendo ser compacto ou esponjoso. O osso cortical ou compacto não apresenta cavidades em seu interior com medula óssea e localiza-se nas superfícies ósseas. Por sua vez, o osso esponjoso ou trabecular, possui um arranjo macroscópico em trabéculas delimitando pequenas cavidades, preenchidas por medula óssea, na qual há produção ativa de células sanguíneas a partir de células mesenquimais. Esse arranho macroscópico é definido pelas demandas biomecânicas que incidem no

osso desde sua formação, e ao longo da vida. Embora macroscopicamente diferentes, o osso cortical e o osso esponjoso possuem a mesma constituição histológica(2,7).

Em condições fisiológicas, os ossos são formados por dois processos diferentes: ossificação intramembranosa e/ou ossificação endocondral. A ossificação intramembranosa ocorre durante o desenvolvimento dos ossos chatos ou laminados, especialmente aqueles que se encontram no crânio. Nesse processo, células mesenquimais de membranas conjuntivas diferenciam-se em osteoblastos, os quais produzem a matriz óssea. Por sua vez, a ossificação endocondral acontece a partir de um modelo de cartilagem hialina, o qual é progressivamente substituído por tecido ósseo. Esse é um processo mais lento que a ossificação intramembranosa e ocorre em muitas regiões do esqueleto, principalmente nos ossos longos(8,9). Independentemente do processo de osteogênese, o crescimento ósseo, bem como a manutenção de sua anatomia, é determinado pela combinação de formato de novo tecido ósseo e reabsorção de tecido pré-existente.

Apesar do seu aspecto aparentemente inerte e estático, os ossos são altamente dinâmicos e apresentam um processo combinado de formação e reabsorção, denominado de remodelação óssea. Este processo quando alterado, gera mudanças nas propriedades estruturais e na composição óssea, resultando em alterações da geometria macroscópica, bem como da microarquitetura(10–12). Por meio da remodelação, o osso se adapta aos estímulos mecânicos, modificando e determinando a arquitetura do esqueleto, estabelecendo uma relação entre o desenvolvimento e o estresse sofrido. Assim os ossos têm a capacidade de adaptarem-se às modificações de tamanho, estrutura e forma de acordo com a intensidade e variação do estresse mecânico recebido(13,14).

O processo de reparo é um fenômeno que ocorre para reconstituir áreas desorganizadas ou destruídas por traumas aos tecidos, envolvendo células e vários mediadores químicos. A resposta de um tecido vivo à agressão, onde houve destruição dos componentes teciduais, é denominado de reparação, ocorrendo por regeneração ou por cicatrização. No processo de regeneração, ocorre a reconstituição da parte danificada ou perdida, resultando em estruturas com a mesma arquitetura e função teciduais originais. Já a cicatrização, é a reparação da região lesada por tecido,

geralmente conjuntivo denso que não restaura completamente a arquitetura ou função da parte danificada, apesar de restabelecer a continuidade tecidual (15).

Basicamente, o processo de reparo tecidual envolve as seguintes etapas: inflamação e coagulação sanguínea, formação de tecido de granulação, e maturação e remodelação tecidual. Os eventos iniciais do processo de reparo são caracterizados pela formação do coágulo sanguíneo, com a presença de plaquetas carregadas de fatores de crescimento, além de células inflamatórias. Rapidamente, inicia-se a formação de uma rede de fibrina, que auxilia a migração de células circundantes e estabelece-se um infiltrado inflamatório. Gradualmente, ocorre invasão de fibroblastos que têm origem a partir da diferenciação das células mesenquimais locais, bem como proliferação de capilares sanguíneos, originados a partir de células endoteliais, caracterizando a formação do tecido de granulação. A neovascularização também é característica do processo, sendo que novos vasos sanguíneos podem ser identificados no tecido de granulação. Com o passar do tempo, o tecido de granulação origina um tecido conjuntivo fibroso, ainda não totalmente maduro, e que sofrerá processo de maturação e remodelação, com reorganização das fibras colágenas(16).

O reparo da lesão óssea se dá por meio de um processo fisiológico similar ao que ocorre em tecidos não mineralizados, porém com algumas especificidades. Quando o osso é lesionado, ocorre o rompimento do periósteo, de vasos sanguíneos, destruição de matriz, da medula óssea, morte de células ósseas e lesão em tecidos vizinhos. No local da lesão ocorre hemorragia e a formação de um coágulo. O reparo segue com o início da resposta inflamatória ocorrendo recrutamento de polimorfo nucleares, macrófagos e monócitos(17). Tais células secretam mediadores químicos para resolver a inflamação e promover a angiogênese. Continuamente, há uma resposta proliferativa intensa do periósteo e do endósteo, com neoformação de tecido conjuntivo e de novos capilares, caracterizando a formação de um tecido de granulação. Os osteoblastos, diferenciados a partir de células mesenquimais, sintetizam colágeno formando inicialmente um tecido ósseo imaturo, que apresenta fibrilas colágenas agrupadas sem organização lamelar e muitos osteócitos incluídos na matriz. O processo de reparo continua, ocorrendo a remodelação óssea, na qual o tecido primário (imaturo) é lentamente substituído por tecido secundário (maduro) por atividade de reabsorção pelos osteoclastos. O tecido ósseo maduro apresenta organização das fibrilas colágenas formando lamelas, menor

número de osteócitos incluídos na matriz mineralizada e maior conteúdo mineral, apresenta melhor desempenho biomecânico (7,18,19).

O reparo e a remodelação óssea são complexos e sofrem influência das características ósseas intrínsecas, além de fatores extrínsecos, como traumas(11),o uso de medicações/drogas e terapias frequentemente indicadas para o tratamento de algumas patologias, como a radioterapia(20,21) e o uso de bisfosfonatos(22).

A radioterapia é frequentemente usada no tratamento de câncer de cabeça e pescoço(23),buscando o máximo de erradicação das células tumorais com o mínimo efeito deletério aos tecidos saudáveis adjacentes ao tumor. Entretanto, a radiação não é capaz de atingir apenas as células tumorais(23), sendo as alterações nos tecidos circunvizinhos inevitáveis(24). Os efeitos da radiação atingem principalmente os tecidos com altas taxas de proliferação, que respondem com reações agudas à radioterapia (efeitos precoces). Todavia, é importante lembrar que os efeitos da radiação ionizante podem perdurar durante meses ou anos após o tratamento (efeitos tardios)(25), podendo modificar a dinâmica de crescimento e remodelação óssea, e, consequentemente, resultar em alterações estruturais e mecânicas.

O osso, quando submetido à altas doses de radiação, pode apresentar desequilíbrio da atividade osteoblástica e osteoclástica, favorecendo a reabsorção óssea(26–28). A radiação ionizante também reduz a população de osteócitos e osteoblastos, com um declínio no processo de mineralização óssea (29,30). Além disso, há um comprometimento da nutrição tecidual, devido à hipóxia, por diminuição da vascularização e fibrose vascular (31,32). Além disso, a radiação ionizante reduz a matriz óssea, altera a morfologia dos canais ósseos (33) e aumenta a fragilidade óssea(32,34). Sendo assim, a reparação e a resistência mecânica são comprometidas no osso irradiado(35,36), o que pode, ao longo do tempo, gerar fraturas ósseas espontâneas ou osteorradionecrose(32).

A Osteoradionecrose (ORN) é uma das mais sérias complicações bucais do tratamento do câncer da cabeça e pescoço. É conceituada pela American Association of Oral and Maxillofacial Surgeons (AAOMS) como uma área de exposição óssea que não se repara espontaneamente em oito semanas e acomete pacientes que estejam recebendo ou que receberam tratamento radioterápico. Trata-se do resultado de uma soma de

fatores: hipóxia, hipovascularização, hipocelularridade decorrentes das altas doses de radiação. Histologicamente, a osteoradionecrose é caracterizada por destruição de osteócitos e ausência de osteoblastos da área marginal; clinicamente, há variações entre pequenas exposições de tecido ósseo assintomáticas a processos agressivos e agudos que progridem rapidamente para fraturas patológicas da área afetada(27,28,37,38). Quadros semelhantes de Osteonecrose tem sido observados associados em algumas terapias medicamentosas com bisfosfonatos.

Os bisfosfonatos (BFs) são fármacos prescritos devido à sua comprovada eficácia na inibição da atividade osteoclástica, sendo frequentemente indicados no tratamento das doenças ósseas metabólicas e do paciente oncológico(39). Os BFs possuem alta afinidade pelos tecidos mineralizados e atuam em sítios de grande formação e reabsorção óssea. Sua ação resulta na inibição tanto da diferenciação celular das células precursoras dos osteoclastos quanto na atuação dos osteoclastos plenamente diferenciados. Ademais, induzem a apoptose desses osteoclastos, modificando sua estrutura celular nos momentos que precedem esse fenômeno(40,41). Além dessas atividades, propriedades antiangiogênicas como redução no número de vasos sanguíneos e resposta endotelial aos hormônios angiogênicos têm sido descritas(42,43). Na literatura há relatos de que o desenvolvimento de Osteonecrose por uso de bisfosfonatos é bastante frequente quando comparado às demais medicações que podem ser associadas com osteonecrose dos maxilares(44).

A Osteonecrose dos Maxilares ou Osteonecrose Associada ao uso de Medicamentos (OAM) é uma séria reação adversa à algumas medicações e que acomete, por mecanismo ainda desconhecido, os ossos maxilares provocando grande destruição tecidual(45). A OAM foi conceituada em 2014 pela American Association of Oral and Maxillofacial Surgeons (AAOMS) como uma área de exposição óssea na maxila ou na mandíbula que não se repara em oito semanas e acomete pacientes que estejam recebendo ou que receberam bisfosfonatos, inibidores de Rank e inibidores de angiogênese sistemicamente e não sofreram irradiação no complexo maxilomandibular. As causas da OAM ainda são obscuras, mas parecem advir de uma complexa interação entre o metabolismo ósseo, trauma local, infecção, hipovascularização e o uso desses medicamentos. Em especial, os pacientes que fazem uso de BFs administrados por via

parenteral parecem ser mais susceptíveis à OAM do que os tratados por via oral. Além disso, fatores sistêmicos como diabetes mellitus, imunossupressão, uso de outras medicações concomitantes, como agentes quimioterápicos e corticoesteróides também parecem ter relação com a manifestação da OAM(45).

Ainda não existem formas totalmente eficientes de prevenção e controle da OAM e ORN, porém, vários recursos têm sido testados, como o uso de antissépticos bucais, tal como a clorexidina a 0,12%; antibioticoterapia sistêmica; procedimentos cirúrgicos, como curetagem e ressecção ósseas (46); ou ainda terapias alternativas como oxigenação hiperbárica(47,48), laserterapia de baixa potência(30) e ozônio(49). Entre estes, atualmente, a oxigenação hiperbárica tem merecido destaque.

A oxigenação hiperbárica (OH) é uma modalidade terapêutica que consiste na administração de oxigênio puro a uma pressão ambiente maior do que ao nível do mar, utilizando-se de equipamentos denominados câmaras hiperbáricas. A OH já tem sido empregada como terapia coadjuvante em casos de embolias gasosas, gangrena, infecções necrotizantes de tecidos moles, isquemias agudas, queimaduras e osteomielites(50). Essa terapia promove uma elevação da quantidade de oxigênio dissolvido no sangue em decorrência da elevação do aporte de oxigênio inspirado e da pressão dentro da câmara, auxiliando a oxigenação tecidual.

Os princípios atribuídos à eficiência da OH estão relacionados à sua capacidade de estimular a vascularização, a angiogênese e a capacidade osteogênica devido à maior disponibilidade de oxigênio para as células. Além disso, ela estimula a proliferação celular, interferindo na síntese de colágeno(51) com efeitos positivos no reparo tecidual (48,52), especialmente no que se refere ao reparo de áreas irradiadas(53–56).

O estímulo à osteogênese pela OH já foi relatado em pesquisas com animais e situações clínicas. Kawada et al.(55), por meio de experimentos realizados com animais, notaram que o aumento da tensão de oxigênio influencia também o metabolismo ósseo. Em seu estudo, a OH aumentou significativamente as taxas de deposição mineral e de formação óssea, acelerando o reparo de fraturas ósseas. Os autores acrescentam que essa melhora do reparo resultou em maior rigidez e força máxima até a falha das amostras analisadas.

Pedersen et al.(56), ao estudarem o reparo ósseo em defeitos críticos de calvária de ratos, também observaram resultados promissores. Os autores observaram melhora da vascularização e da neoformação óssea após oxigenação hiperbárica.

Em 1996, Sawai et al.(57) realizaram um estudo para avaliar o efeito da terapia em enxertos ósseos autógenos livres transplantados de crista ilíaca para as mandíbulas de coelhos e os resultados indicaram que a OH acelera a união de enxertos ósseos autógenos livres. Outros estudos também demonstraram que a OH eleva a atividade da fosfatase alcalina, um marcador da formação óssea, em ratos após osteotomia(58), aumenta a atividade dos osteoblastos e a angiogênese em mandíbulas submetidas à distração(31) e favorece a expressão do fator de crescimento endotelial vascular durante o reparo ósseo(59).

Assim sendo, terapias coadjuvantes que favoreçam o restabelecimento da normalidade da morfologia e função tecidual, reduzam possíveis prejuízos ao processo natural do reparo ósseo, são interessantes em diversas situações clínicas. Dessa forma, neste estudo foi avaliado, por meios de estudos laboratoriais em animais e revisões sistemáticas da literatura, métodos para otimizar o reparo do tecido ósseo normal, ou comprometido pela radioterapia ou uso de bisfosfonatos.

Capítulos

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2 CAPÍTULOS

2.1 CAPÍTULO 1

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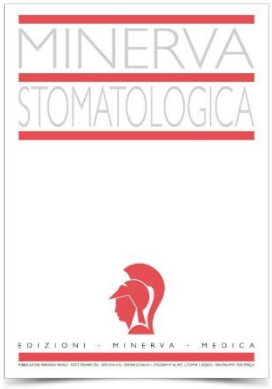

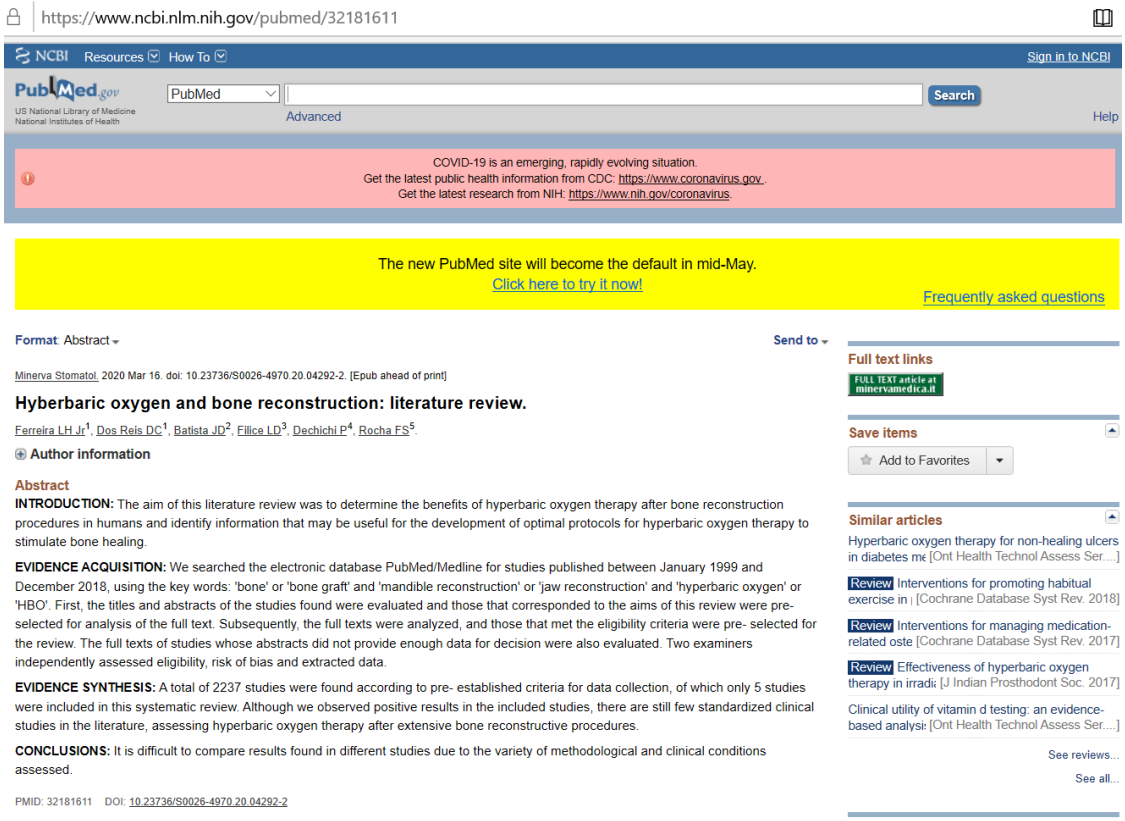

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Hyberbaric oxygen and bone reconstruction: literature review.

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Author information

Abstract

INTRODUCTION: The aim of this literature review was to determine the benefits of hyperbaric oxygen therapy after bone reconstruction procedures in humans and identify information that may be useful for the development of optimal protocols for hyperbaric oxygen therapy to stimulate bone healing.

EVIDENCE ACQUISITION: We searched the electronic database PubMed/Medline for studies published between January 1999 and December 2018, using the key words: 'bone' or 'bone graft' and 'mandible reconstruction' or 'jaw reconstruction' and 'hyperbaric oxygen' or 'HBO'. First, the titles and abstracts of the studies found were evaluated and those that corresponded to the aims of this review were pre-selected for analysis of the full text. Subsequently, the full texts were analyzed, and those that met the eligibility criteria were pre- selected for the review. The full texts of studies whose abstracts did not provide enough data for decision were also evaluated. Two examiners independently assessed eligibility, risk of bias and extracted data.

EVIDENCE SYNTHESIS: A total of 2237 studies were found according to pre- established criteria for data collection, of which only 5 studies were included in this systematic review. Although we observed positive results in the included studies, there are still few standardized clinical studies in the literature, assessing hyperbaric oxygen therapy after extensive bone reconstructive procedures.

CONCLUSIONS: It is difficult to compare results found in different studies due to the variety of methodological and clinical conditions assessed.

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Title: HYBERBARIC OXYGEN AND BONE RECONSTRUCTION – LITERATURE REVIEW

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ABSTRACT:

Objective: The aim of this literature review was to determine the benefits of hyperbaric oxygen therapy after bone reconstruction procedures in humans and identify information that may be useful for the development of optimal protocols for hyperbaric oxygen therapy to stimulate bone healing. Evidence acquisition: We searched the electronic database PubMed/Medline for studies published between January 1999 and December 2018, using the key words: 'bone' or ‘bone graft’ and 'mandible reconstruction' or 'jaw reconstruction' and ‘hyperbaric oxygen’ or ‘HBO’. First, the titles and abstracts of the studies found were evaluated and those that corresponded to the aims of this review were pre-selected for analysis of the full text. Subsequently, the full texts were analyzed, and those that met the eligibility criteria were pre-selected for the review. The full texts of studies whose abstracts did not provide enough data for decision were also evaluated. Two examiners independently assessed eligibility, risk of bias and extracted data. Evidence synthesis: A total of 2237 studies were found according to pre-established criteria for data collection, of which only 5 studies were included in this systematic review. Although we observed positive results in the included studies, there are still few standardized clinical studies in the literature, assessing hyperbaric oxygen therapy after extensive bone reconstructive procedures.

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Conclusions: It is difficult to compare results found in different studies due to the variety of methodological and clinical conditions assessed.

KEYWORDS: bone reconstruction, autogenous bone graft, hyperbaric oxygen therapy.

INTRODUCTION:

Bone grafting is a clinical tool that allows reconstruction of the facial region and craniofacial skeleton. Different grafts, both natural and synthetic, are used to promote bone regeneration, however, the characteristics of autogenous bone grafts best meet the requisites of an ideal graft[1,2,3,4,5]. Several factors may influence the integration and maintenance of autogenous bone graft volume, such as the use of rigid fixation[6,7], vascularization of the receptor site[8], direct contact between bone graft and receptor bed, and physiological stress suffered by the graft[5,6].

Advances in tissue repair research and the development of new reconstructive techniques transformed autogenous bone grafts into more predictable procedures[1,2,3,4,9]. These grafts are still the gold standard for reconstructive techniques due to their excellent potential for revascularization, immunological compatibility and effective osteogenic capacity[1,2,5].

Good vascularization of the soft tissues of the face has allowed the use of free autogenous bone grafts with good success rates; however, larger free grafts may increase the risk of bone resorption with unfavorable results. Recently, hyperbaric oxygen therapy (HBO) was used to optimize bone repair. The increase in oxygen availability to the tissues induced by HBO allows better wound repair[10,11,12,13,14,15], since this therapy promotes reduction in hypoxia, promotes angiogenesis, and the differentiation of fibroblasts[10,11,16,17]. More recently, studies have determined that HBO

also generates reactive oxygen species (ROS) which, in turn, interfere with the synthesis of inflammation mediators, antioxidants and growth factors resulting in improved repair[12,13,14].

In oral and maxillofacial surgery, HBO can be used as adjunctive therapy in reconstructive procedures[3,18]. After maxillofacial trauma, ruptured blood vessels lead to the formation of a hypoxic zone. While hypoxia is required to stimulate angiogenesis and revascularization, prolonged hypoxia will reduce tissue repair. Therefore, increasing the available oxygen stimulates important biological events such as angiogenesis and osteogenesis[10,11,12,17,18,19,20,21], enhancing tissue repair and increasing the overall success of extensive free bone reconstruction procedures.

Although the potential of HBO is promising, there is still no consensus in the literature regarding the clinical protocol for its use, and the period of use after bone reconstruction procedures. The aim of this study, conducted through a systematic review, was to determine the benefits of hyperbaric oxygen after mandibular reconstruction procedures in humans, by identifying information that may be useful for the development of optimal HBO protocols to stimulate bone repair. Our hypothesis was that HBO treatment increases bone formation and improve success of bone reconstruction procedures.

EVIDENCE ACQUISITION - MATERIALS AND METHODS:

Search Strategy and Eligibility Criteria

The review was designed to answer the guiding question, based on the PICO strategy: Population (individuals submitted to bone reconstruction); Intervention

(Hyperbaric oxygen therapy and bone reconstruction); Control (individuals not undergoing Hyperbaric oxygen therapy); Outcome (positive results and complications).

The literature search was conducted in Pubmed/MEDLINE electronic databases, using the search terms: 'bone' or ‘bone graft’ and 'mandible reconstruction' or 'jaw reconstruction' and ‘hyperbaric oxygen’ or ‘HBO’, in advanced mode (search parameters were the title and abstract). The search was limited to studies published from January 1999 to December 2018 as a full-length article in the English language. Studies were included if they met all the following criteria:

(1) Clinical studies or case reports;

(2) Studies evaluating bone reconstruction (bone defect, bone fracture or grafting) assessing hyperbaric oxygen therapy with reports of the therapy protocols.

The exclusion criteria set for selection of the studies were:

(1) Animal or *in vitro* studies;

(2) Review studies, brief communications, editorials or letters to the editor, monographs, conference summaries, and book / book chapters;

(3) Out-of-objective studies.

In addition, a manual search was performed on the references of the eligible studies after electronic search.

Study selection, Data Extraction and Quality Assessment

At first, as a calibration, three reviewers discussed the eligibility criteria and applied them to a sample of the studies. After obtaining an adequate level of agreement,

the titles and abstracts were reviewed by two eligibility reviewers independently. In case of disagreement, the decision was solved by consensus and if necessary, a third reviewer performed the evaluation. First, the titles and abstracts of the studies found were evaluated and those that corresponded to the aims of this review were pre-selected for analysis of the full text. Subsequently, the full texts were analyzed, and those that met the eligibility criteria were pre-selected for the review. The full texts of studies whose abstracts did not provide enough data for decision were also evaluated.

All the relevant data were extracted and tabulated in an electronic spreadsheet independently by reviewer one and reviewer two. To ensure consistency between the reviewers, a training exercise was conducted where information was extracted by both reviewers from the same eligible study. Any disagreement was resolved through discussion, and when these two reviewers could not reach agreement, a third reviewer was consulted to make a final decision. The data included bibliographic references, study type/design, description of bone defect/reconstructive procedure, HBO protocol, interval between HBO sessions, number of HBO sessions, results, and possible complications.

The risk of bias, individual quality of evidence and the strength of recommendation of the selected studies were assessed based on study design, sample size, methodological limitations and inconsistencies.

EVIDENCE SYNTHESIS:

Results

Briefly, a total of 2237 studies were identified in the initial database search. Of the potentially relevant studies, 2227 were excluded during title and abstract reading,

and only 10 were chosen for full text complete reading. Later 2 studies selected manually were included, totaling 12 studies assessed for eligibility criteria. After assessing the full text, another 7 studies were excluded and only 5 met the inclusion criteria for the present systematic review. The literature screening process is outlined in Figure 1.

The studies were heterogeneous as regards subject characteristics, study desing and clinical sequence. The general characteristics of the included studies are listed in Table 1. Only 3 studies evaluated the use of HBO in bone reconstruction procedures with free autogenous grafts. However, surgical conditions were very different between the studies. Jisander et al.[22] described 8 cases of free autogenous bone reconstruction for the treatment of osteoradionecrosis. In their patients the HBO was used only during osteoradionecrosis (ORN) treatment, and afterwards, the bone graft was used to restore the bone defect without additional HBO sessions. Sawhney & Ducic[23] described the use of free bone grafts to reconstruct areas after ORN resection. However, their 37 patients received different HBO protocols: 20 HBO sessions just prior to bone reconstruction with fibula (22 patients), 10 HBO sessions right after bone reconstruction with fibula (10 patients) and the others were distinct protocols because of insurance problems or patient preferences. The osteoradionecrosis occurred after deleterious effects of radiotherapy in all described cases, but HBO was indicated only during reconstruction planning. On the other hand, Oliveira et al.[18] described a single case of free autogenous bone reconstruction and concomitant use of hyperbaric oxygen, after resection of ameloblastoma, a benign tumor.

In 2 studies[24,25], the authors retrospectively evaluated the use of HBO during repair after implant placement in a grafted area. In both studies the graft was

autogenous, microvascularized and performed in previously irradiated areas. There were a higher number of cases described, because both were retrospective studies.

All the authors mentioned in this review used the HBO protocol of 2.4ATM for 90 minutes in each session. Only Sawhney & Ducic[23] did not mention this information. However, the number of sessions varied considerably among authors. The reasons for the choice of the protocols used were not reported. Only Oliveira et al.[18] informed the interval between sessions.

Between the studies that evaluated the use of HBO in bone reconstruction procedures with free autogenous graft, Oliveira et al.[18] found positive results in volume and stability of the bone graft. They did not report any complications after a period of 6 months of follow-up. Jisander et al.[22], in a longer follow-up period, noted satisfactory closure after resection of osteoradionecrosis. The authors concluded that it was important to use HBO during ORN control prior to free graft reconstruction. They reported complications such as fistulas (3 patients), mandibular fracture (2 patients) and recurrence of injury (1 patient). Sawhney & Ducic[23] suggested that 20 HBO sessions just prior bone reconstruction presented better results. They reported complications such as loss of skin (2 patients), plate exposure (3 patients) and non-union of graft (4 patients) in the group of patients that received only 10 HBO sessions right after bone reconstruction (Table 2).

Among the studies that evaluated the use of HBO after implant placement in reconstructed areas, Shaw et al.[24] found success rate of 92% after 1 year, 79% after 2 years and 74% after 3 years. The main causes of loss of implants reported by the authors were peri-implantitis (25 implants), failure of osseointegration (9 implants), graft

fracture (7 implants) and tumor recurrence (6 implants). The failures due to problems with the prostheses (without other associated factors) were rare and occurred in only 2 patients (Table 3). Salinas et al.[25], after a period of 4-108 months of follow-up, found success rate of 82.4% when the implants were placed in the grafted area, and 88% when placed in adjacent native bone. The early loss (considering a period up to 6 months after insertion) was a more common occurrence (21 implants); however, late loss was also seen (10 implants).

Discussion

The reconstruction of large bone defects is of great importance for rehabilitating individuals mutilated by resective surgery, major trauma or even by extensive bone resorption, and improving their quality of life. However, extensive bone defects are a major challenge because of the increased possibility of complications and reduced predictability of the graft. In this context, hyperbaric oxygen therapy appears to be a supplementary therapy in cases of bone reconstruction, improving the prognosis and increasing the predictability of large free autogenous bone grafts. In this systematic review we evaluated the benefits of hyperbaric oxygen after bone reconstruction procedures for the purpose of identifying information that might be useful for the development of optimal HBO protocols in bone tissue. In general, the results of HBO were positive in the few studies included in this review. However, the literature still lacks standardized clinical studies evaluating HBO after bone reconstructive procedures making it difficult to obtain satisfactory levels of evidence.

Cells respond to changes in oxygen tension after HBO via the oxygen-dependent degradation of hypoxia-inducible transcription factors (HIFs) resulting in improved

angiogenesis, energy metabolism and cell proliferation. Moreover, the increase in oxygen leads to the production of reactive oxygen species (ROS) which act on signaling pathways that modulate the synthesis of inflammation mediators, antioxidants and growth factors contributing to the healing process[12,13,15]. Exposure to adequate levels of oxygen in tissues is therefore osteogenic and highly related to the level of vascularization[10,11,12,13,17,18,19,20,21]. Histological studies in animals have shown favorable results regarding the acceleration of bone graft incorporation[26] or bone repair[10] when compared with control groups that did not use the oxygen therapy. However, the wide variety of HBO protocols in the literature, both in humans and in animals, hinders proper interpretation of the effects of this therapy. This diversity occurs because HBO protocols are usually chosen considering the individual experience of the authors, since there are no universally accepted parameters. In this review we found only 5 studies that met the inclusion criteria; they evaluated different types of patients, as well as different types of grafts, making it difficult to compare the results observed by the authors.

Jisander et al.[22], Sawhney & Ducic[23] and Oliveira et al.[18] described the effects of therapy after jaw reconstructive procedures with free autogenous bone grafts. Free grafts, when removed from the donor area, represent a hypoxic environment, therefore, these grafts could be improved with the complementary use of HBO. Initially we intended to compare these 3 studies due to the similarity of the grafting procedures, however, the characteristics of patients evaluated by each of the authors differed, influencing the results.

Jisander et al.[22] and Sawhney & Ducic[23] reported bone reconstruction after resection of osteoradionecrosis (ORN). The ORN is a sequela of radiotherapy, characterized by loss of oral mucosal lining and consequent exposure of necrotic bone

tissue for a prolonged period even in the absence of local neoplastic changes. It is a difficult condition to treat, with a poor prognosis[27]. The possibility of post-operative complications in these irradiated patients is more common as observed by the authors[22,23] who reported the occurrence of fistula, mandibular fracture, loss of skin, plate exposure, graft nonunion and recurrence of injury. On the other hand, Oliveira et al.[18] reported a single case using HBO associated with free bone reconstruction after resection of a benign tumor, with favorable results and without complications.

Both Jisander et al.[22] and Oliveira et al.[18] adopted the same HBO protocol (2.4ATM for 90 minutes), however, differentiating the number of sessions applied during the postoperative period. This difference may also be related to radiotherapy because irradiated bone has pronounced hypovascularization, with reduction in the number of osteoblasts and progressive fibrosis[28,29], which may explain the poor response to surgical manipulation. Therefore, it is reasonable to believe that the bone subjected to radiation would benefit from a higher number of HBO sessions.

An interesting question was to compare the authors Shaw et al.[24] and Salinas et al.[25], who used similar HBO protocols (2.4 ATM for 90 minutes), the same number and frequency of sessions, and evaluated the use of the therapy during implant placement in irradiated areas, after bone reconstruction with microvascularized grafts. Although there are similarities between bone repair events post-implant placement and the repair of extensive free grafts, some considerations must be made due to the inherent differences between the two procedures, especially regarding surgical morbidity. The implant placement generates a small trauma resulting from the drilling and subsequent implant placement procedures. These procedures would present less morbidity than an extensive reconstructive procedure.

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Shaw et al.[24] reported 91% success at 1 year, and among the main causes of loss of implants they found: peri-implantitis (25 implants), failure of osseointegration (9 implants), graft fracture (7 implants) and tumor recurrence (6 implants). However, in their study, the authors did not report whether the implants were placed in grafted areas or native bone and the hygiene protocol maintenance. It has been widely shown that patients undergoing implant treatment included in maintenance programmes benefit more than those who are not included, or who do not follow consistently this programme[30]. Salinas et al.[25] found a success rate of 82.4% when the implants were placed in the grafted area, and 88% when placed in adjacent native bone, with early loss being the most frequent complication for loss of implants. Both authors used a long follow-up time and achieved a success rate higher than 85%, which led us to believe that the HBO favored the success of implants placed in grafted areas under the conditions reported by the authors.

One of the most important questions regarding HBO indication referred to the number and frequency of sessions required to obtain good results, since the technique is time consuming (each session lasts about 90 minutes) and has a high cost. However, apparently, the clinical indication also appeared to interfere in the HBO application protocol, therefore, further studies with groups of patients in similar conditions are needed to establish a proper guideline for the use hyperbaric oxygen therapy after bone reconstructive procedures.

CONCLUSIONS:

The quality of the evidence was limited by the very small number of trials, the small sample sizes, and the risks of bias in the included trials. The results of HBO were

positive in the studies included in this review; however, in the literature there is still a lack of randomized clinical studies evaluating HBO after extensive bone reconstructive procedures.

REFERENCES:

1. Fearon JA, Griner D, Ditthakasem K, et al. Autogenous Bone Reconstruction of Large Secondary Skull Defects. Plast Reconstr Surg. 2017 Feb;139(2):427-438.
2. Sakkas A, Wilde F, Heufelder M, et al. Autogenous bone grafts in oral implantology-is it still a "gold standard"? A consecutive review of 279 patients with 456 clinical procedures. Int J Implant Dent. 2017 Dec;3(1):23.
3. Dorosz N, Dominiak M. Mandibular ridge reconstruction: A review of contemporary methods. Adv Clin Exp Med. 2018 Aug;27(8):1159-1168.
4. Pace F, Randelli F, Ayeni OR, et al. Debridement, internal fixation and staged autogenous bone graft for the management of infected femoral non-union. Injury. 2018 Dec;49 Suppl 4:S48-S57.
5. Bow A, Anderson DE, Dhar M. Commercially available bone graft substitutes: the impact of origin and processing on graft functionality. Drug Metab Rev. 2019 Nov;51(4):533-544
6. Latrenta Gs, Mccarthy Jg, Breitbart As, et al. The role of rigid skeletal fixation in bone-graft augmentation of the craniofacial skeleton. Plast Reconstr Surg. 1989; 84(4):578-88.
7. Phillips Jh, Rahn Ba. Fixation effects on membranous and endochondral onlay bone graft revascularization and bone deposition. Plast Reconstr Surg. 1990; 85(6):891-7.

8. Michel G, Blery P, Pilet P, et al. Analysis of Radiation-Induced Osteopenia and Bone Hypovascularization in Rat. Calcif Tissue Int. 2015 Jul;97(1):62-8. Epub 2015 May 8.
9. Pistilli R, Felice P, Piatelli M, et al. Blocks of autogenous bone versus xenografts for the rehabilitation of atrophic jaws with dental implants: preliminary data from a pilot randomised controlled trial. Eur J Oral Implantol. 2014 Summer;7(2):153-71.
10. Rocha FS, Gomes Moura CC, Rocha Rodrigues DB, et al. Influence of hyperbaric oxygen on the initial stages of bone healing. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015 Nov;120(5):581-7.
11. Grassmann JP, Schneppendahl J, Hakimi AR, et al. Hyperbaric oxygen therapy improves angiogenesis and bone formation in critical sized diaphyseal defects. J. Orthop. Res. 2015;33(4):513-20.
12. Marenzana M, Arnett TR. The Key Role of the Blood Supply to Bone. Bone Res. 2013 Sep 25;1(3):203-15.
13. Tejada S, Batle JM, Ferrer MD, et al. Therapeutic Effects of Hyperbaric Oxygen in the Process of Wound Healing. Curr Pharm Des. 2019;25(15):1682-1693.
14. Silva ML, Tasso L, Azambuja AA, et al. Effect of hyperbaric oxygen therapy on tooth extraction sites in rats subjected to bisphosphonate therapy-histomorphometric and immunohistochemical analysis. Clin Oral Investig. 2017 Jan;21(1):199-210.

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

15. Godman CA, Chheda KP, Hightower LE, et al. Hyperbaric oxygen induces a cytoprotective and angiogenic response in human microvascular endothelial cells. Cell Stress Chaperones. 2010 Jul;15(4):431-42.
16. Tandara AA, Mustoe TA. Oxygen in wound healing--more than a nutrient. World J Surg. 2004 Mar;28(3):294-300.
17. Schneppendahl J, Jungbluth P, Sager M, et al. Synergistic effects of HBO and PRP improve bone regeneration with autologous bone grafting. Injury. 2016 Dec;47(12):2718-2725.
18. Oliveira MT, Rocha FS, de Paulo LF, et al. The approach of ameloblastoma of the mandible: a case treated by hyperbaric oxygen therapy and bone graft reconstruction. Oral Maxillofac Surg. 2013 Dec;17(4):311-4. Epub 2013 Jan 17.
19. Fok TC, Jan A, Peel SA, et al. Hyperbaric oxygen results in increased vascular endothelial growth factor (VEGF) protein expression in rabbit calvarial critical-sized defects. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2008 Apr;105(4):417-22.
20. Jacobson AS, Buchbinder D, Hu K, et al. Paradigm shifts in the management of osteoradionecrosis of the mandible. Oral Oncol. 2010 Nov;46(11):795-801. Epub 2010 Sep 16. Review.
21. Chang H, Oh SE, Oh S, et al. Four-week histologic evaluation of grafted calvarial defects with adjunctive hyperbaric oxygen therapy in rats. J Periodontal Implant Sci. 2016 Aug;46(4):244-53.

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

22. Jisander S, Grenthe B, Salemark L. Treatment of Mandibular Osteoradionecrosis by Cancellous Bone Grafting. J Oral Maxillofac Surg. 1999 Aug;57(8):936-42; discussion 942-3.
23. Sawhney R, Ducic Y. Management of pathologic fractures of the mandible secondary to osteoradionecrosis. Otolaryngol Head Neck Surg. 2013 Jan;148(1):54-8.
24. Shaw RJ, Sutton AF, Cawood JI, et al. Oral Rehabilitation after treatment for head and neck malignancy. Head Neck. 2005 Jun;27(6):459-70.
25. Salinas TJ, Desa VP, Katsnelson A, et al. Clinical Evaluation of implants in Radiates Fibula Flaps. J Oral Maxillofac Surg. 2010 Mar;68(3):524-9.
26. Neves PC, Abib Sde C, Neves RF, et al. Effect of hyperbaric oxygen therapy combined with autologous platelet concentrate applied in rabbit fibula fraction healing. Clinics (Sao Paulo). 2013 Sep;68(9):1239-46.
27. Nabil S, Samman N. Risk factors for osteoradionecrosis after head and neck radiation: a systematic review. Oral Surg Oral Med Oral Pathol Oral Radiol. 2012 Jan;113(1):54-69.
28. Da Cunha SS, Sarmento V, Ramalho LM, et al. Effect of laser therapy on bone tissue submitted to radiotherapy: experimental study in rats. Photomed Laser Surg. 2007 Jun;25(3):197-204.
29. Batista JD, Zanetta-Barbosa D, Cardoso SV, et al. Effect of low-level laser therapy on repair of the bone compromised by radiotherapy. Lasers Med Sci. 2014 Nov;29(6):1913-8.

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

30. Matarese G, Ramaglia L, Fiorillo L, et al. Implantology and Periodontal Disease: The Panacea to Problem Solving? Open Dent J. 2017 Aug 30;11:460-465. doi: 10.2174/1874210601711010460.

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Tables:

Table 1: Parameters Obtained of the studies included in this review.

Author/Year	Study Type	Patientts	Type of Defects	HBO Protocol	HBO number of sessions	Interval between sessions
Jisander et al. (1999)[22]	Series of cases	8	Free graft in the resection of osteoradionecrosis area	2.4 ATM during 90 min	35 to 75 sessions prior to bone reconstruction	Not informed
Shaw et al. (2005)[24]	Retrospective	81	Microvascular graft in tumor resection area and later implant insertion	2.4 ATM during 90 min	20 sessions prior and 10 sessions post implant installation	Not informed
Salinas et al. (2010)[25]	Retrospective	44	Microvascular graft in tumor resection area and later implant insertion	2.4 ATM during 90 min	20 sessions prior and 10 sessions post implant installation	Not informed
Sawhney & Ducic (2013)[23]	Series of cases	37	Free graft in the resection of osteoradionecrosis area	Uninformed	20 sessions prior and 10 sessions post bone reconstruction	Not informed
Oliveira et al. (2013)[18]	Case report	1	Free graft in the resection area of ameloblastoma	2.4 ATM during 90 min	10 sessions prior and 40 sessions post bone reconstruction	Daily

Table 2: Results of studies evaluating the use of hyperbaric oxygen therapy in bone reconstruction procedures with free graft.

Author/Year	Results	Complications	Follow-up time
Jisander et al. (1999)[22]	Satisfactory closure in the injury area	Fistula, mandibular fracture and lesion recurrence.	20 to 93 months
Sawhney & Ducic (2013)[23]	Reduced incidence of nonunion with HBO.	Loss of skin, plate exposure and graft nonunion.	10 months to 12 years
Oliveira et al. (2013)[18]	Good size and graft stability	None	6 months

Table 3: Results of studies evaluating the use of hyperbaric oxygen therapy in implant insertion after microvascular free graft.

Author/Year	Implant Success Rate	Causes of implant loss	Follow-up time
Shaw et al. (2005)[24]	92% (1 year); 79% (2 years) and 74% (3 years)	Peri-implantitis and failed osseointegration	3 months - 14 years
Salinas et al. (2010)[25]	85% (82,4% graft area / 88% native bone)	Early loss of implants, especially in grafted area.	4 - 108 months

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Figure Legends:

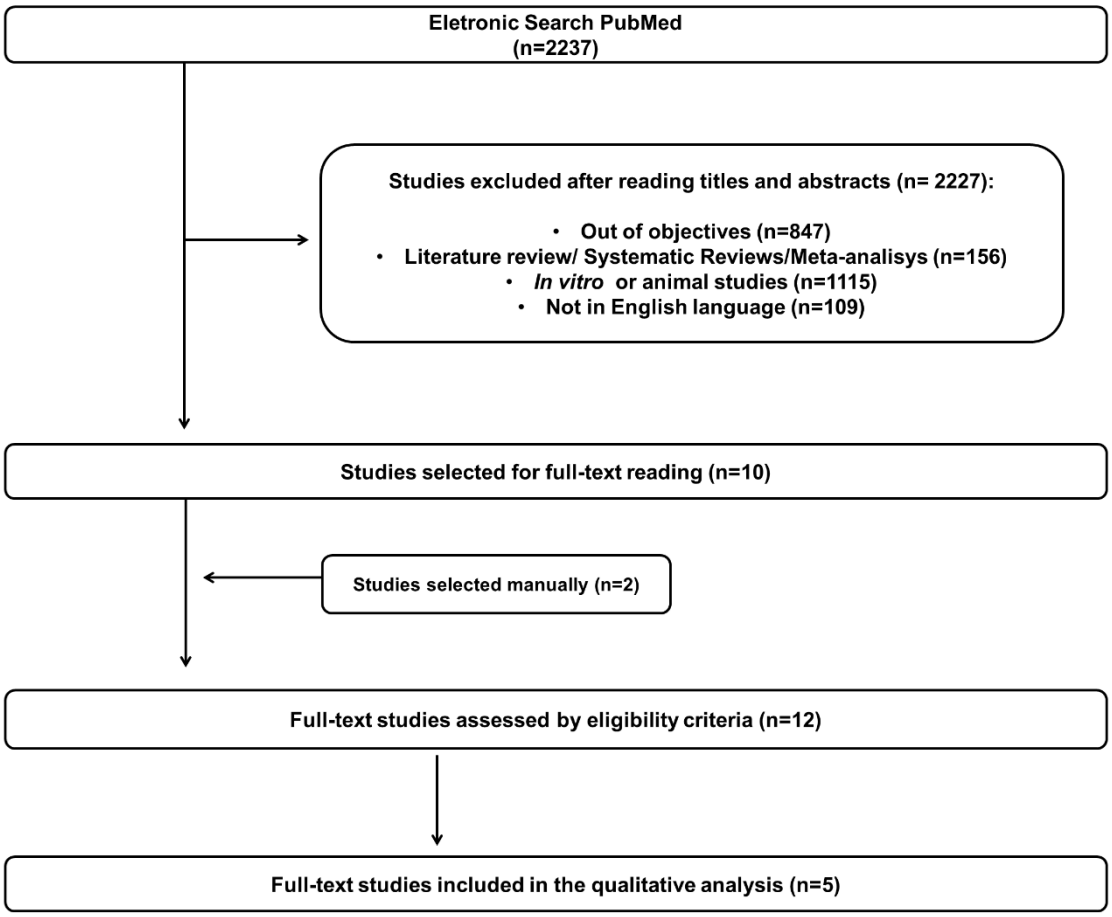

Figure 1: literature screening process.

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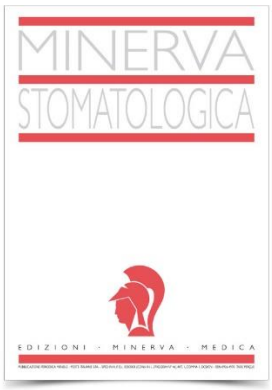
2.2 CAPÍTULO 2

Comprovante de publicação – Revista: Minerva Stomatologica



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Dear Prof. FLAVIANA ROCHA,

I am pleased to inform you that your manuscript entitled

BISPHOSPHONATE-ASSOCIATED OSTEONECROSIS OF THE JAW: SYSTEMATIC REVIEW.

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BISPHOSPHONATE-ASSOCIATED OSTEONECROSIS OF THE JAW: SYSTEMATIC REVIEW.

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ABSTRACT

Objective: The purpose of this systematic review was to determine the possible risk factors related to pathophysiology of Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ) and identify adequate treatment strategies available and success rates. **Evidence acquisition:** We performed a search for publications about the treatment of BRONJ, published between 2003 and 2018 in the PubMed/Medline data base using the key words: 'Bisphosphonate-Associated Osteonecrosis of the Jaw' OR 'Bisphosphonate Osteonecrosis' OR 'BRONJ', based on the list of MeSH and DeCS. **Evidence synthesis:** According to pre-established criteria for data collection concerning the treatment of BRONJ, we found 19 articles covering a total of 400 patients. Treatments that showed good outcomes were Ozone, PRF, PRP/Debridement/Laser bio-stimulation, Laser surgery and Laser surgery/Laser bio-stimulation. HBO did not achieve good results and was used in only 10 patients. BRONJ is a rare condition in patients with osteoporosis/other pathologies using BP orally. These patients had long exposure time

and cumulative doses of BPs until onset of the lesion. The oncological patients were exposed to more potent intravenously administered BPs such as pamidronate and zoledronate. These patients had a shorter exposure time until onset of the lesion **Conclusions:** The treatment of BRONJ is still under debate and there are promising treatments that need randomized trials with larger numbers of patients to confirm their results. Patients receiving BPs or those who will start treatment should be encouraged to perform preventive dental treatment and maintain good oral hygiene.

Keywords: Bisphosphonate-Related Osteonecrosis of the Jaw, Bisphosphonate, Bone.

INTRODUCTION

Bisphosphonates (BPs), potent inhibitors of bone resorption[1], cause suppression of osteoclast activity that impairs physiological bone remodeling[2-3]. There are two classes of BPs: nitrogenated (zoledronic acid, disodium pamidronate, sodium residronate, sodium alendronate and sodium ibandronate) and non-nitrogenated (disodium clodronate, disodium etidronate, disodium tiludronate) types[4]. The indications include treatment of osteoporosis, osteitis deformans (Paget's disease), control of osteolysis in bone metastases (with or without hypercalcemia), multiple myeloma and other conditions that present bone fragility. They are medications structurally analogous to pyrophosphate[5].

In 2003, Marx firstly reported a series of 36 cases of osteonecrosis of the jaw after bisphosphonate treatment for cancer. This condition is defined as the presence of exposed and necrotic bone in the oral cavity that persists for more than six weeks in patients with a history of bisphosphonate use and with no history of head and neck

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radiation therapy, generally refractory to conservative treatment with local debridement and antibiotic medications[6].

The scientific medical literature contains descriptions of risk factors for the development of Bisphosphonate-Related Osteonecrosis of the Jaw (BRONJ). Among these risk factors, medication-dependent factors, such as the timing of bisphosphonate treatment are important[3,7-8]. The concomitant use of other drugs, such as corticosteroids and antiangiogenic drugs, increases the risk of osteonecrosis. Local factors, such as tooth extractions, pre-existing periodontal problems, and prosthesis trauma, can also predispose patients to bisphosphonate-related osteonecrosis of the jaw[7-8].

Clinically, exposed areas of necrotic bone may be observed; in other cases, only fistulas in the bone are detected[7]. BRONJ can generate complications such as swelling, dental mobility, soft tissue infections, cutaneous fistula, oral mucosal fistula, extraoral oral exposure and oroantral communication[2,4].

The purpose of this systematic review was to determine the possible risk factors related to pathophysiology of BRONJ and identify adequate treatment strategies available and success rates.

EVIDENCE ACQUISITION - MATERIALS AND METHODS

The literature search was conducted in association with research librarian in 2019. Articles published in journals indexed in the PubMed/Medline databases were selected using the following filters: Clinical Trial Phase I, Clinical Trial Phase II, Clinical Trial Phase III, Clinical Trial Phase IV, Controlled Clinical Trial, Randomized Controlled Trial, Multicenter Study, Case Reports. The search was limited to articles published during the period from January 2003 to December 2018. The keywords used

for research were 'Bisphosphonate-Associated Osteonecrosis of the Jaw' OR 'Bisphosphonate Osteonecrosis' OR 'BRONJ', based on the list of MeSH and DeCS. All references were gathered and screened for eligibility.

The inclusion criteria defined for selection of articles were:

A. clinical studies with humans.
B. articles published in English language confirming the diagnosis of BRONJ in accordance with the American Association of Oral and Maxillofacial Surgeons (AAOMS) or American Society of Bone and Mineral Research (ASBMR) definitions.
C. types of study: controlled and uncontrolled clinical trials, and case series.
D. themes: risk factors/prevention of BRONJ, treatment and outcomes.
E. studies with a sample of five or more patients with BRONJ containing detailed information about each patient, the type of treatment performed and clinical response to the treatment.

The exclusion criteria defined for selection of articles were:

A. experimental studies using animals.
B. *in vitro* studies.
C. single case reports.
D. case series with fewer than 5 patients.
E. literature reviews.

The selection of studies on BRONJ treatment was done in two phases using a data collection protocol: 1st) evaluation of titles and abstracts to identify studies that could be relevant for review, 2nd) evaluation of the full text and data collection. The

reference lists of all the articles were manually searched for appropriate studies related to the review topic. Each publication was critically appraised to assess the study validity, and the following data were extracted and assembled in a spreadsheet: reference and year, number of patients, age, gender, location of lesions, primary cause of BRONJ, stage of BRONJ lesion, types of BP used and duration, treatment methods, follow-up period and response to treatment.

EVIDENCE SYNTHESIS - RESULTS

Initially, 519 articles were selected. Of these, 65 articles were chosen for complete reading. After evaluation of the full texts, only 19 described patient characteristics and were accepted according to criteria predetermined in the methodology, resulting in a sample of 400 patients with BRONJ (Figure 1).

The mean age of the 400 patients with BRONJ was 65.85 years, ranging from 28 to 90 years (Table1). In the 340 cases in which the sex distribution was reported, 64.71% (n=220) were females and 35.29% (n=120) were males, showing a female predilection.

The mandible was the most affected bone accounting for 67.03% (n=246) of cases, followed by the maxilla 28.88% (n=106) and both jaws 4.09% (n=15). The site was not reported for only 33 patients. BRONJ most frequently occurred after dentoalveolar surgery (82.46%; n=141). In only 3.51% (n=6) of the cases, the initial event was triggered by traumatic pressure caused by dental prosthesis, whereas in 13.45% (n=23) of the cases, the injury occurred spontaneously. There was only 1 report of BRONJ due to peri-implantitis (0.58%). In 229 patients, the initial event was not reported.

The majority of patients received BPs due to cancer or metastatic disease (n=151 multiple myeloma; n=89 breast cancer; n=28 prostate cancer; n=43 other cancers). The remaining patients presented osteoporosis (n=49) or other pathologies (n=9) such as osteitis deformans (Paget's disease), rheumatoid arthritis and other conditions that present bone fragility. In 42 patients this information was not available. The type of BP prescribed was specified for 275 patients with BRONJ as demonstrated in Table 2.

There was variability in the duration of BP therapy until BRONJ development in Cancer or Osteoporosis/Other (Table 3). The time of exposure to BPs until BRONJ development in months was higher for Osteoporosis/Other patients. On the other hand, Oncological patients, who frequently use intravenous Nitrogenated BPs, presented shorter exposure time until the development of BRONJ.

Regarding the management of BRONJ, the studies reported Drug treatment, especially with antibiotics, isolated or in addition to debridement and surgery in the majority of patients. Various adjunctive treatments such as Ozone, Platelet-rich Plasma (PRP), Platelet-rich Fibrin (PRF), Surgical Laser, Laser Bio-stimulation, Hyperbaric Oxygen (HBO), and other medications were also mentioned with interesting results (Table 4).

The response to treatment was classified as Total Response (TR) when the patient had complete healing of the BRONJ lesion with absence of pain; Partial Response (PR) when the patient presented stable BRONJ lesion or reduced bone exposure, without pain; and the Non-response (NR) when patient presented BRONJ lesion with persistent bone exposure and pain. Among the evaluated studies, the various treatments performed in 298 Oncological patients and 58 Osteoporosis/Other patients

resulted in 137 (45.97%)/36 (62.07%) patients with TR, 60 (20.14%)/8 (13.79%) with PR, 101 (33.89%)/14 (24.14%) with NR. This indicated that irrespective of treatment, underlying disease or time of exposure to BP, BRONJ was refractory to treatment. There was resolution of the symptomatology in many patients, although they still presented some degree of bone exposure that was stable.

Patients with Osteoporosis/Other presented a higher TR rate to BRONJ treatment when compared with patients with cancer or metastatic disease. Factors such as chemotherapy, BP potency used, and time of exposure to the specific BP used, Stage of BRONJ, among other variables may have influenced this difference. In spite of its great importance, many authors did not present this detailed information for each patient, making it impossible to cross these data.

DISCUSSION

After the use of BPs, changes in bone metabolism occur, bone resorption is inhibited resulting in accumulation of non-vital osteocytes[1], accumulation of micro fractures in the bone matrix[27] and direct toxicity to osteoclasts, which may lead to apoptosis[2]. After using more potent medications, angiogenesis is also compromised due to the delay in the formation of new blood vessels, which makes it difficult to repair the oral mucosa exposed by BPs[28]. This damage to bone tissue compromises repair and may trigger more severe changes such as BRONJ, which occurs mainly in patients undergoing procedures involving bone tissue.

First-generation bisphosphonates included etidronate, tiludronate, and clodronate; these lacked a nitrogen side chain or hydroxyl group and did not have such strong specificity for bone[21]. These bisphosphonates are metabolized into cytotoxic

analogues of adenosine triphosphate (ATP), which accumulate intracellularly and compete with ATP for binding sites, eventually starving osteoclasts from lack of energy. Because osteoclasts are responsible for bone resorption, fewer functional osteoclasts result in a lower bone resorption rate[20,29]. The second-generation bisphosphonates include alendronate, pamidronate, ibandronate, risedronate, and zoledronate. These agents affect osteoclast activity by inhibiting farnesyl diphosphate synthase, an enzyme in the mevalonate pathway, which is essential for cholesterol and another sterol production[30].

In our study, patients with cancer predominantly used intravenous BPs for an average of 41.32 months until diagnosis of BRONJ, while patients with osteoporosis/other had a longer exposure time (46.41 months) as detailed in Table 3. According to Marx[2], it is possible to consider suspension of the use of BPs for patients who use the medication to treat benign pathologies, or if the primary indication of BP treatment has been resolved. However, when the drug is used to control bone metastasis due to the long half-life of the drug and its great effectiveness in stabilizing bone lesions, there is no reason to discontinue the therapy with BPs. Patients with bone metastasis use more potent BPs such as pamidronate and zoledronate, known to irreversibly inhibit osteoclast activity. In the treatment of osteoporosis/other, less potent BPs such as alendronate are used, which affect osteoclast function with less severity, but the risk increases with cumulative doses and prolonged use[2]. This difference in the time of exposure to BPs between cancer and osteoporosis/other patients has also been observed in other publications[2,8,31]. In a study conducted in Australia[31], the estimated frequency of BRONJ for osteoporosis patients using monthly or weekly alendronate was from 1 case in 2206 to 8470 (0.01% to 0.04%). When tooth extractions were

performed, the frequency was between 0.09% to 0.34%. In patients with bone metastasis using zoledronate or pamidronate, the frequency was 1 case in 87 to 114 (0.88% to 1.15%) and when tooth extractions were performed, the frequency was between 6.67% and 9.1%.

Treatment of BRONJ varied with the stage of the disease[8,32]. The literature includes many types of therapeutic approaches with varying degrees of success, including hyperbaric oxygen, ozone therapy, laser, bio-stimulator laser, PRF, use of PRP associated with surgery, use of pentoxifylline and tocopherol, and surgery for debridement and use of the buccal adipose cushion[17-18,21-22,24,33]. However, irrespective of the stage, the protocol must include the treatment of dental and periodontal diseases, maintenance and improvement of oral health with antibacterial mouthwash, and systemic administration of antibacterial agents[32].

In a multicenter retrospective study with a sample of 347 patients with BRONJ[33], 66% underwent surgical debridement that resulted in clinical improvement of the lesion in 49%, stabilization in 35% and worsening in 16%. The remaining patients underwent surgery with bone resection showing better results, as 68% of the patients had improved clinical status, 27% had a stable ORB and 5% had clinical worsening[33].In our study, many patients who underwent debridement or surgery had a total response (TR), which was a reasonable clinical response when compared with patients who received only drug treatment; however, this was far from being an effective treatment in all cases.

The use of antibiotics may be important during the treatment of BRONJ; however, this should not be indicated alone but associated with other forms of

treatment. In this study, only 16.98% of the patients had total response when treated with antibiotics and / or topical medication alone. In fact, the use of antibiotics did not lead to significant changes in the total amount of bacteria present in the *in vivo* biofilm of patients with BRONJ when compared with the patient who did not use antibiotics. Prescription of antibiotics seemed to lead to a differentiation in the biofilm population, but not significant enough to reduce or eliminate infection[34]. This could occur due to the presence of avascular and necrotic bone[2], or the formation of biofilm itself, which would prevent effective action of the drug[34]. There are no evidence-based recommendations regarding the type of antibiotics or length of administration for BRONJ treatment[32]. Although antibiotic therapy alone is not effective in the resolution of BRONJ, the control or decrease in the occurrence of opportunistic infections, frequent in these patients, helps the treatment.

BRONJ was seen by Marx & Tursun[35] as a non-inflammatory drug toxicity to bone tissue leading to osteoclast death, which resulted in suppression of bone remodeling, and microorganisms played only a secondary opportunistic role. The presence of actinomyces in BRONJ lesions and osteoradionecrosis is a recently described complication and may be interpreted as being an opportunistic infection. To us, BRONJ would seem to be an alteration, whose cause is not related to damage to the bone system alone, and auxiliary therapies are necessary.

The use of ozone has shown good results in the treatment of BRONJ with total cure of the 27 treated cases. The therapy has antimicrobial capacity and properties that help with mucosal repair. The patients had complete repair of the osteonecrosis lesion or expulsion of spontaneous bone sequestration and closure of the underlying mucosa. In 11 cases, surgery was required to remove sequestration without the need to remove

underlying bone[21,25]. However, it is important to remember that despite the effectiveness of the treatment in these cases, there was no distinction between the stages of BRONJ specifically in these cases reported by Ripamonti et al., (2011)[21] and (2012)[25], which could modify the response to treatment. More advanced stages might suggest the need for invasive procedures or may present fewer promising results.

The surgical technique performed with Er: YAG laser has the advantages of being bactericidal, with minimum production of smear layer in the surgical bed, and possible bio-stimulatory effects. The surgical laser causes thermal ablation, vaporizing necrotic bone tissue until it reaches healthy bone tissue. Surgical laser treatment is promising, and approximately 80% of the patients treated in this study obtained total response[14,18]. The laser used for tissue bio-stimulation has properties that act on the proliferation of cells, blood vessels, lymphatic tissues, and stimulate the healing of wounds. We observed its use as adjuvant therapy along with debridement/surgery, surgical laser, PRP or antibiotic therapy[18-19,24].

PRP associated with surgery/debridement of necrotic bone and laser biostimulation was used in a single study[24]. PRP contains several proteins that are growth factors present in platelets and was applied after debridement of the necrotic bone to improve soft tissue healing and stimulate the formation of bone tissue. Patients undergoing this procedure had 86% total response.

The treatment with pentoxifylline and tocopherol seemed to be effective in the treatment of patients with BRONJ, accelerating healing of the lesion due to reversal of the fibro-necrotic process[36-38]. In this review, we included only one article that used pentoxifylline and tocopherol to treat a sample of 6 patients with BRONJ[17]. Of these, 1

patient had complete response to treatment while the remainder showed a partial response. After the treatment began, all the patients presented no pain, erythema or pus drainage[17].

Hyperbaric oxygen has not yet demonstrated consistent clinical efficacy for BRONJ treatment[5,12,28]. Freiberger et al., (2012)[39] conducted a randomized clinical trial testing HBO in patients with BRONJ. They demonstrated that when the group receiving HBO was compared with the group receiving conventional treatment no statistically significant differences in treatment success were shown. Despite these results, the test group showed faster decrease in the number and size of the lesions and reduction in pain, showing that the therapy was useful as an adjunctive treatment for BRONJ, especially for more severe cases. The HBO does not target the necrotic bone, but rather the soft tissue and bone that is still viable. The region of bone necrosis can only be removed surgically. HBO is then used to restrict surgical removal of non-vital bone to improve healing of the mucosa and to prepare the bone tissue for reconstruction.

Preventive dental care in the beginning of BP therapy and during therapy are effective measures in reducing the incidence of BRONJ[2-4,40-42]. In the mentioned studies, after the implementation of preventive dental care, a reduction from 5.5% to 2.8% in the incidence of BRONJ was observed in patients who used IV BPs for treatment of bone metastases[41]; and from 26.3% to 6.7% in patients with multiple myeloma who used zoledronic acid[42]. Patients who will receive treatment with intravenous BPs should be referred to the dentist to detect possible outbreaks of infection and to perform treatment prior to beginning with BPs therapy[39]. Patients using BPs should make return visits to the dentist every 4-6 months for careful clinical

examination of the oral mucosa, perform preventive treatment and control risk factors such as smoking, alcohol intake and decompensated diabetes[2,41].

In a study conducted with guinea pigs, osteonecrosis lesions occurred only after the oral surgical procedure in animals exposed to BP, and the parenteral use of zoledronate was a sufficient condition for the development of BRONJ after dental extractions[43]. In our study dentoalveolar surgery represented the event that led to the onset of BRONJ in 82.46% of the cases. The same was observed in other publications[2-4,31] suggesting that elective surgeries involving bone tissue should be avoided, alternative therapies such as root interment, splinting of teeth with mobility grade 1 and 2 and endodontic treatment should be tried if possible, leaving tooth extraction only as a last option.

In the articles, it was possible to find different classifications for BRONJ. When reviewing the literature, data collected from articles that used distinct classifications made it difficult to standardize the data, since it was impossible to propose reclassification of the patient. Even in recent publications[21,25], different classifications for BRONJ are still used, or this information is even absent in most of the studies. We believe that staging of the lesion is important to enable the therapeutic decision to be made. The American Association of Oral and Maxillofacial Surgeons[44] have proposed classification and treatment strategies for each stage of BRONJ. Using a unified classification would help to compare the results obtained from BRONJ studies.

CONCLUSIONS

BRONJ was a less common condition in patients with osteoporosis/other pathologies, who used oral BPs. These patients had a long time of exposure to BP and

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cumulative doses until the onset of the lesion. The treatment of osteonecrosis in patients with osteoporosis/other pathologies obtained better results than those obtained in cancer patients, who in turn were exposed to more potent BP, such as pamidronate and zoledronate. Patients who will start treatment with BPs should cut out risk factors such as smoking and alcoholism, control their diabetes, and avoid having dental treatment performed. After starting the medication, periodic visits to the dentist for preventive treatment, routine clinical and radiographic examination are important. There are several types of treatment and those with the best results were ozone therapy, PRP, PRF, debridement and laser biostimulation, laser surgical treatment and laser surgical treatment associated with the laser biostimulation.

REFERENCES

1. Marx RE. Pamidronate (Aredia) and zoledronate (Zometa) induced avascular necrosis of the jaws: a growing epidemic. J Oral Maxillofac Surg 2003;61(9):1115-7.
2. Marx RE, Sawatari Y, Fortin M, et al. Bisphosphonate-induced exposed bone (osteonecrosis/osteopetrosis) of the jaws: risk factors, recognition, prevention, and treatment. J Oral Maxillofac Surg 2005;63(11):1567-75.
3. Mânea HC, Urechescu HC, Balica NC, et al. Bisphosphonates-induced osteonecrosis of the jaw - epidemiological, clinical and histopathological aspects. Rom J Morphol Embryol. 2018;59(3):825-831.
4. Woo SB, Hellstein JW, Kalmar JR. Narrative [corrected] review: bisphosphonates and osteonecrosis of the jaws. Ann Intern Med. 2006 May 16;144(10):753-61.

5. Bamias A, Kastritis E, Bamia C, et al. Osteonecrosis of the jaw in cancer after treatment with bisphosphonates: incidence and risk factors. J Clin Oncol. 2005 Dec 1;23(34):8580-7.
6. Migliorati CA, Epstein JB, Abt E, et al. Osteonecrosis of the jaw and bisphosphonates in cancer: a narrative review. Nat Rev Endocrinol. 2011 Jan;7(1):34-42. doi: 10.1038/nrendo.2010.195. Epub 2010 Nov 16. Review.
7. Bagan L, Jiménez Y, Leopoldo M, et al. Exposed necrotic bone in 183 patients with bisphosphonate-related osteonecrosis of the jaw: Associated clinical characteristics. Med Oral Patol Oral Cir Bucal. 2017 Sep 1;22(5):e582-e585. doi: 10.4317/medoral.22133.
8. Petrovic M, Jelovac DB, Antic S, et al. Medication-Related Osteonecrosis of the Jaws: Two Center Retrospective Cohort Studies. Biomed Res Int. 2019 Mar 18;2019:8345309. doi: 10.1155/2019/8345309.
9. Dimitrakopoulos I, Magopoulos C, Karakasis D. Bisphosphonate-induced avascular osteonecrosis of the jaws: a clinical report of 11 cases. Int J Oral Maxillofac Surg. 2006 Jul;35(7):588-93. Epub 2006 May 9.
10. Wutzl A, Eisenmenger G, Hoffmann M, et al. Osteonecrosis of the jaws and bisphosphonate treatment in cancer patients. Wien Klin Wochenschr. 2006;118(15-16):473-8.
11. Yarom N, Yaholom R, Shoshani Y, et al. Osteonecrosis of the jaw induced by orally administered bisphosphonates: incidence, clinical features, predisposing factors and treatment outcome. Osteoporos Int. 2007 Oct;18(10):1363-70. Epub 2007 Jun 28.

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

12. Magopoulos C, Karakinaris G, Telioudis Z, et al. Osteonecrosis of the jaws due to bisphosphonate use. A review of 60 cases and treatment proposals. Am J Otolaryngol. 2007 May-Jun;28(3):158-63.
13. Boonyapakorn T, Schirmer I, Reichart PA, Sturm I, Massenkeil G. Bisphosphonate-induced osteonecrosis of the jaws: Prospective study of 80 patients with multiple myeloma and other malignancies. Oral Oncol. 2008 Sep;44(9):857-69. doi: 10.1016/j.oraloncology.2007.11.012. Epub 2008 Feb 20.
14. Angiero F, Sannino C, Borloni R, et al. Osteonecrosis of the jaws caused by bisphosphonates: evaluation of a new therapeutic approach using the Er:YAG laser. Lasers Med Sci. 2009 Nov;24(6):849-56. doi: 10.1007/s10103-009-0654-7. Epub 2009 Mar 11.
15. Alons K., Kuijpers SC, de Jong E, et al. Treating low- and medium-potency bisphosphonate-related osteonecrosis of the jaws with a protocol for the treatment of chronic suppurative osteomyelitis: report of 7 cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009 Feb;107(2):e1-7. doi: 10.1016/j.tripleo.2008.09.021. Epub 2008 Dec 13.
16. Stubinger S, Dissmann J, Pinho NC, Saldamli B, Seitz O, Sader R. A preliminary report about treatment of bisphosphoante related osteonecrosis of the jaw with Er:YAG laser abration. Lasers Surg Med. 2009;41(1):26-30.
17. Epstein MS, Wicknick FW, Epstein JB, et al. Management of bisphosphonate-associated osteonecrosis: pentoxifylline and tocopherol in addition to antimicrobial therapy. An initial case series. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010 Nov;110(5):593-6. doi: 10.1016/j.tripleo.2010.05.067.

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

18. Vescovi P, Manfredi M, Merigo E, et al. Surgical approach with Er:YAG laser on osteonecrosis of the jaws (ONJ) in patients under bisphosphonate therapy (BPT). Lasers Med Sci. 2010 Jan;25(1):101-13. doi: 10.1007/s10103-009-0687-y. Epub 2009 Jun 19.
19. Ataley B, Yalcin S, Emes Y, et al. Bisphosphonate-related osteonecrosis: laser-assisted surgical treatment or conventional surgery? Lasers Med Sci. 2011 Nov;26(6):815-23. doi: 10.1007/s10103-011-0974-2. Epub 2011 Aug 2.
20. Moretti F, Pelliccioni GA, Montebugnoli L, et al. A prospective clinical trial for assessing the efficacy of a minimally invasive protocol in patients with bisphosphonate-associated osteonecrosis of the jaws. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011 Dec;112(6):777-82. doi: 10.1016/j.tripleo.2011.07.004. Epub 2011 Oct 14.
21. Ripamonti CI, Cislaghi E, Mariani L, Maniezzo M. Efficacy and safety of medical ozone (O3) delivered in oil suspension applications for the treatment of osteonecrosis of the jaw in patients with bone metastases treated with bisphosphonates: Preliminary results of a phase I–II study. Oral Oncol. 2011 Mar;47(3):185-90. doi: 10.1016/j.oraloncology.2011.01.002.
22. Pautke C, Bauer F, Otto S, et al. Fluorescence-Guided Bone Resection in Bisphosphonate-Related Osteonecrosis of the Jaws: First Clinical Results of a Prospective Pilot Study. J Oral Maxillofac Surg. 2011 Jan;69(1):84-91. doi: 10.1016/j.joms.2010.07.014. Epub 2010 Oct 25.
23. Malden N, Lopes V. An epidemiological study of alendronate-related osteonecrosis of the jaws. A case series from the south-east of Scotland with

attention given to case definition and prevalence. J Bone Miner Metab. 2012 Mar;30(2):171-82. doi: 10.1007/s00774-011-0299-z. Epub 2011 Aug 20

24. Martins MA, Martins MD, Lascala CA, et al. Association of laser phototherapy with PRP improves healing of bisphosphonate-related osteonecrosis of the jaws in cancer patients: a preliminary study. Oral Oncol. 2012 Jan;48(1):79-84. doi: 10.1016/j.oraloncology.2011.08.010. Epub 2011 Sep 21.
25. Ripamonti CI, Maniezzo M, Boldini S, et al. Efficacy and tolerability of medical ozone gas insufflations in patients with osteonecrosis of the jaw treated with bisphosphonates—Preliminary data Medical ozone gas insufflation in treating ONJ lesions. Journal of Bone Oncology 2012;1-7. http://dx.doi.org/10.1016/j.jbo.2012.08.001.
26. Dincă O, Zurac S, Stăniceanu F, et al. Clinical and histopathological studies using fibrin-rich plasma in the treatment of bisphosphonate-related osteonecrosis of the jaw. Rom J Morphol Embryol. 2014;55(3):961-4.
27. Hoefert S, Schmitz I, Tannapfel A, et al. Importance of microcracks in etiology of bisphosphonate-related osteonecrosis of the jaw: a possible pathogenetic model of symptomatic and non-symptomatic osteonecrosis of the jaw based on scanning electron microscopy findings. Clin Oral Investig. 2010 Jun;14(3):271-84. doi: 10.1007/s00784-009-0300-6. Epub 2009 Jun 18.
28. Wehrhan F, Stockmann P, Nkenke E, et al. Differential impairment of vascularization and angiogenesis in bisphosphonate-associated osteonecrosis of the jaw-related mucoperiosteal tissue. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2011 Aug;112(2):216-21. doi: 10.1016/j.tripleo.2011.02.028. Epub 2011 Jun 12.

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

29. Stubinger S, Dissmann J, Pinho NC, et al. A preliminary report about treatment of bisphosphoante related osteonecrosis of the jaw with Er:YAG laser abration. Lasers Surg Med. 2009 Jan;41(1):26-30. doi: 10.1002/lsm.20730.
30. Shannon J, Shannon J, Modelevsky S, et al. Bisphosphonates and Osteonecrosis of the Jaw. J Am Geriatr Soc. 2011 Dec;59(12):2350-5. doi: 10.1111/j.1532-5415.2011.03713.x. Epub 2011 Nov 8..
31. Mavrokokki T, Cheng A, Stein B, et al. Nature and frequency of Bisphosphonat-associated osteonecrosis of the jaws in Australia. J Oral Maxillofac Surg 2007;65:415-23.
32. Yoneda T, Hagino H, Sugimoto T, et al. Antiresorptive agent-related osteonecrosis of the jaw: Position Paper 2017 of the Japanese Allied Committee on Osteonecrosis of the Jaw. J Bone Miner Metab. 2017 Jan;35(1):6-19. doi: 10.1007/s00774-016-0810-7. Epub 2016 Dec 29. Review
33. Graziani F, Vescovi P, Campisi G, et al. Resective Surgical Approach Shows a High Performance in the Management of Advanced Cases of Bisphosphonate-Related Osteonecrosis of the Jaws: A Retrospective Survey of 347 Cases. J Oral Maxillofac Surg. 2012 Nov;70(11):2501-7. doi: 10.1016/j.joms.2012.05.019. Epub 2012 Aug 9.
34. Ji X, Pushalkar SLY, Glickman R, et al. Antibiotic effects on bacterial profile in osteonecrosis of the jaw. Oral Dis. 2012 Jan;18(1):85-95. doi: 10.1111/j.1601-0825.2011.01848.x. Epub 2011 Aug 29.
35. Marx RE, Tursun R. Suppurative osteomyelitis, bisphosphonate induced osteonecrosis, osteoradionecrosis: a blinded histopathologic comparison and its

implications for the mechanism of each disease. Int J Oral Maxillofac Surg. 2012 Mar;41(3):283-9. doi: 10.1016/j.ijom.2011.12.016. Epub 2012 Jan 11.

36. Delanian S, Depondt J, Lefaix J. Major Healing of refractory mandible osteoradionecrosis after treatment combining pentoxifylline and tocopherol: a phase II trial. Head neck. 2005;27:114-23.
37. Delanian S, Chatel C, Porcher R, et al. Complete restoration of refractory mandibular osteoradionecrosis by prolonged treatment with a pentoxifilline-tocopherol-clodronate combination (pentetoclo): a phase II trial. Int J Radiat Oncol Biol Phys. 2011 Jul 1;80(3):832-9. doi: 10.1016/j.ijrobp.2010.03.029. Epub 2010 Jul 16.
38. Mcleod NMH, Pratt CA, Mellor TK, et al. Pentoxifylline and tocopherol in the management of patients with osteoradionecrosis, the Portsmouth experience. Br J Oral Maxillofac Surg. 2012 Jan;50(1):41-4. doi: 10.1016/j.bjoms.2010.11.017. Epub 2011 Jan 19.
39. Freiberger JJ, Padilla-Burgos R, McGraw T, et al. What is the role of hyperbaric oxygen in the management of bisphosphonate-related osteonecrosis of the jaw: a randomized controlled trial of hyperbaric oxygen as an adjunct to surgery and antibiotics. J Oral Maxillofac Surg. 2012 Jul;70(7):1573-83. doi: 10.1016/j.joms.2012.04.001.
40. Bagán JV, Diz-Dios P, Gallego L, et al. Recomendaciones para la prevención de la osteonecrosis de los maxilares (ONM) en pacientes con cáncer tratados con bisfosfonatos intravenosos. Med Oral Patol Oral Cir Bucal. 2008;13:161-7.
41. Vandone AM, Donadio M, Mozzati M, et al. Impact of dental care in the prevention of bisphosphonate-associated osteonecrosis of the jaw: a single-

center clinical experience. Ann Oncol. 2012 Jan;23(1):193-200. doi: 10.1093/annonc/mdr039. Epub 2011 Mar 22.

42. Dimopoulos MA, Kastritis E, Bamia C, et al. Reduction of osteonecrosis of the jaw (ONJ) after implementation of preventive measures in patients with multiple myeloma treated with zoledronic acid. Ann Oncol. 2009 Jan;20(1):117-20. doi: 10.1093/annonc/mdn554. Epub 2008 Aug 9.
43. Maahs MP, Azambuja AA, Campos MM, et al. Association between bisphosphonates and jaw osteonecrosis: a study in Wistar rats. Head Neck. 2011 Feb;33(2):199-207. doi: 10.1002/hed.21422.
44. Ruggiero SL, Mehrotra B, Rosenberg TJ, et al. Osteonecrosis of the Jaws Associated with the Use of Bisphosphonates: A Review of 63 Cases. J Oral Maxillofac Surg. 2004 May;62(5):527-34

TABLES

Table 1: Summary of the publications included in this review: study design, total number of patients with BRONJ and mean patient age.

Reference	Study design	Number of patients	Mean Age (years)
Bamias (2005)[5]	Prospective	17	Not informed
Dimitrakopoulos (2006)[9]	Prospective	11	66.63
Wutzl (2006)[10]	Retrospective	16	65.31
Yarom (2007)[11]	Retrospective	11	69.72
Magopoulos (2007)[12]	Retrospective	60	Not informed
Boonyapakorn (2008)[13]	Prospective	22	61.14
Angiero (2009)[14]	Case series	49	69.75
Alons (2009)[15]	Case series	7	66.86
Stübinger (2009)[16]	Case series	8	68.5
Epstein (2010)[17]	Case series	6	75.66
Vescovi (2010)[18]	Prospective	55	69.31

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Atalay (2011)[19]	Retrospective	20	55.40
Moretti (2011)[20]	Prospective	33	69.09
Ripamonti (2011)[21]	Prospective phase I-II	10	62.0
Pautke (2011)[22]	Prospective	15	63.2
Malden (2012)[23]	Case series	11	69.54
Martins (2012)[24]	Retrospective	22	58.09
Ripamonti (2012)[25]	Retrospective	17	62.29
Dinca (2014)[26]	Retrospective	10	59.5
Total		400	65.85

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Table 2 – Type of bisphosphonate used in each group of patients with BRONJ.

Oncological patients			Osteoporosis/Other patients		
	N	**%**		**N**	**%**
Zoledronate	168	67.74%	Zoledronate	5	18,52%
Zoledronate+ Pamidronate	38	15.32%	Zoledronate+ Alendronate	1	3,70%
Pamidronate	19	7.66%	Pamidronate	1	3,70%
Alendronate	6	2.42%	Alendronate	18	66,67%
Zoledronate+ Pamidronate+ Ibandronate	5	2.02%	Pamidronate+ Alendronate	2	7,41%
Zoledronate+ Ibandronate	3	1.21%			
Ibandronate	8	3.22%			
Clodronate	1	0.41%			
Total	248*	100%	**Total**	27*	100%

*125 patients did not provide information on BP used.

Table 3 - Mean period of bisphosphonate exposure until diagnosis of BRONJ in each group of patients.

Oncological patients		Osteoporosis/Other patients	
	Mean (months)		**Mean (Months)**
Zoledronate	23.53	Zoledronate	36.35
Zoledronate+ Pamidronate	44.67	Zoledronate+ Alendronate	18.0
Pamidronate	27.76	Pamidronate	uninformed
Alendronate	19.0	Alendronate	41.13
Zoledronate+ Pamidronate+ Ibandronate	29.0	Pamidronate+ Alendronate	90.0
Zoledronate+ Ibandronate	66.6		
Ibandronate	84.0		
Clodronate	36.0		
Total	41.32 months (n=133) *	**Total**	46.41 months (n=25) *

PT: Mean period time of use of BFs until diagnosis of BRONJ (* 242 patients did not provide time information).

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Table 4 - Type of treatment and response to therapy in each group of patients with BRONJ.

Oncological patients					Osteoporosis/Other patients				
	N	**TR**	**PR**	**NR**		**N**	**TR**	**PR**	**NR**
Ozone	25	25	0	0	Ozone	2	2	0	0
Biostimulation Laser	10	4	0	6	Biostimulation Laser	5	3	0	2
PRF	10	10	0	0	PRF	0	-	-	-
Surgical Laser	27	22	5	0	Surgical Laser	14	12	2	0
Surgical Laser + Biostimulation Laser	10	7	0	3	Surgical Laser + Biostimulation Laser	0	-	-	-
Debridement or Surgery	51	13	2	36	Debridement or Surgery	12	9	2	1
PRP + Biostimulation Laser + Debridement or surgery	14	12	2	0	PRP + Biostimulation Laser + Debridement or surgery	0	-	-	-
HBO + Debridement or Surgery	10	4	0	6	HBO + Debridement or Surgery	0	-	-	-
Debridement or Surgery + Drug treatment	45	23	11	11	Debridement or Surgery + Drug treatment	15	9	1	5
Drug treatment	96	17	40	39	Drug treatment	10	1	3	6
Total	298	137	60	101	**Total**	58	36	8	14

*6 patients used pentoxifylline and tocopherol as a treatment with TR for 1 patient and PR for 5 patients, however it was not possible to distinguish whether they were osteoporosis/other or oncological patients.

**34 patients used only Drug treatment with TR for 19 patients, PR for 8 patients and NR for 6 patients, however it was not possible to distinguish whether they were osteoporosis/other or oncological patients.

***14 patients did not provide treatment information.

PS: Drug treatment was the type in which the patient received a systemic antibiotic (such as amoxicillin, amoxicillin associated with clavulonate, penicillin G, ciprofloxacin, clindamycin, doxycycline, azithromycin, metronidazole, piperacillin, ceftazidime), systemic antifungal agents (such as fluconazole, ketoconazole), topical application of oral miconazole gel, mouthwash with nystatin and the association of mouthwashes with antiseptics (such as chlorhexidine) or hydrogen peroxide.

Figure Legends:

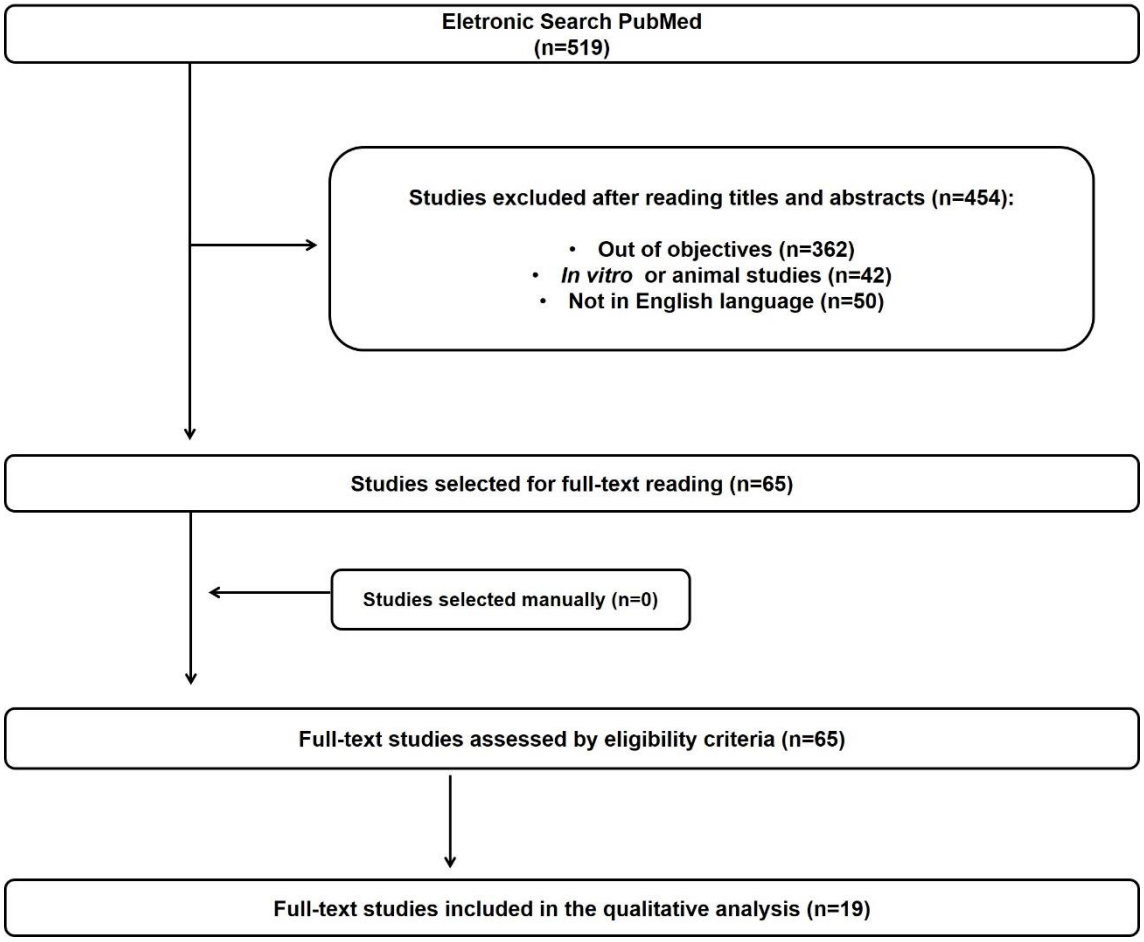

Figure 1: Literature screening process.

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2.3 CAPÍTULO 3

Comprovante de publicação – Revista: Radiation Oncology

29/04/2020 View Letter

Date: 20 Apr 2020
To: "Flaviana Rocha" flavianasoares.rocha@gmail.com
From: "Radiation Oncology Editorial Office" marjoshua.jemina@springernature.com
Subject: Decision has been reached on your submission to Radiation Oncology - RAON-D-19-00642R2

RAON-D-19-00642R2
The effect of hyperbaric oxygen therapy on bone macroscopy, composition and biomechanical properties after ionizing radiation injury
Luiz Henrique Ferreira Júnior; Pedro Henrique Justino Oliveira Limirio; Priscilla Barbosa Ferreira Soares; Paula Dechichi; Letícia de Souza Castro Filice; Paulo Sérgio Quagliatto; Flaviana Rocha
Radiation Oncology

Dear Dra. Rocha,

I am pleased to inform you that your manuscript "The effect of hyperbaric oxygen therapy on bone macroscopy, composition and biomechanical properties after ionizing radiation injury" (RAON-D-19-00642R2) has been accepted for publication in Radiation Oncology.

Before publication, our production team will check the format of your manuscript to ensure that it conforms to the standards of the journal. They will be in touch shortly to request any necessary changes, or to confirm that none are needed.

Articles in this journal may be held for a short period of time prior to publication. If you have any concerns please contact the journal.

Any final comments from our reviewers or editors can be found, below. Please quote your manuscript number, RAON-D-19-00642R2, when inquiring about this submission.

We look forward to publishing your manuscript and I do hope you will consider Radiation Oncology again in the future.

Best wishes,

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Comments (if any):

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The effect of hyperbaric oxygen therapy on bone macroscopy, composition and biomechanical properties after ionizing radiation injury

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Abstract

Background: Radiotherapy used in tumor treatment compromises vascularization of bone tissue. Hyperbaric oxygenation (HBO) increases oxygen availability and improves vascularization, minimizing the deleterious effects of ionizing radiation (IR). Therefore, the aim of this study was to evaluate HBO therapy effect on bone macroscopy, composition and biomechanical properties after IR damage.

Methods: Twenty male *Wistar* rats weighing 300 ± 20 g (10 weeks of age) were submitted to IR (30 Gy) to the left leg, where the right leg was not irradiated. After 30 days, ten animals were submitted to HBO therapy, which was performed daily for 1 week at 250 kPa for 90-min sessions. All animals were euthanized 37 days after irradiation and the tibia were separated into four groups ($n = 10$): from animals without HBO - right tibia Non-irradiated (nolRnoHBO) and left tibia Irradiated (IRnoHBO); and from animals with HBO - right tibiae Non-irradiated (noIRHBO) and left tibia Irradiated (IRHBO). The length (proximal-distal) and thickness (anteroposterior and mediolateral) of the tibiae were measured. Biomechanical analysis evaluated flexural strength and stiffness. Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) was used to calculate the amide I ratio, crystallinity index, and matrix to mineral ratios.

Results: In the macroscopic and ATR-FTIR analysis, the IRnoHBO showed lower values of length, thickness and amide I ratio, crystallinity index and matrix to mineral ratios compared to nolRnoHBO ($p < 0.03$). IRnoHBO showed no statistical difference compared to IRHBO for these analyses ($p > 0.05$). Biomechanics analysis showed that the IRnoHBO group had lower values of flexural strength and stiffness compared to nolRnoHBO and IRHBO groups ($p < 0.04$). In addition, the nolRHBO group showed higher value of flexural strength when compared to nolRnoHBO and IRHBO groups ($p < 0.02$).

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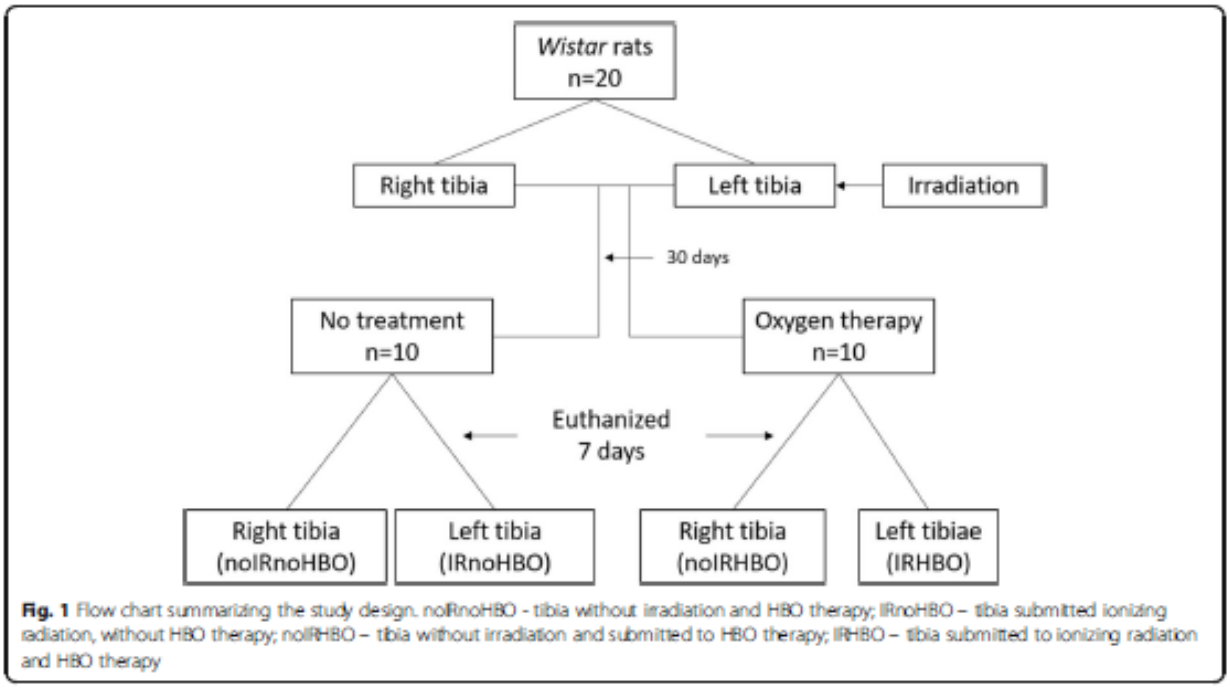

Fig. 1 Flow chart summarizing the study design. noIRnoHBO - tibia without irradiation and HBO therapy; IRnoHBO – tibia submitted ionizing radiation, without HBO therapy; noIRHBO – tibia without irradiation and submitted to HBO therapy; IRHBO – tibia submitted to ionizing radiation and HBO therapy

anteroposterior (Fig. 2b, c). The thickness was measured in the middle of the diaphysis. All measurements were taken using a digital pachymeter (Western @PRO DC-6*, São Paulo, Brazil).

Biomechanics analysis and attenuated Total reflectance (ATR)-Fourier transform infrared spectroscopy (FTIR) analysis

After the macroscopy analysis, the tibiae were analyzed in a three-point bending test until failure, using the universal-testing machine (EMIC DL 2000, EMIC Equipamentos e Sistemas de Ensaio Ltda, São José dos Pinhais, Brazil). Each specimen was positioned horizontally on the two holding fixtures with a distance of 16 mm on the machine, while the upper loading fixture applied the force to the middle of the diaphysis at a loading of 20 N at 1.0 mm/min displacement (Fig. 3a). Load and displacement data were recorded and subsequently, load vs. displacement curves were plotted. Evaluations were derived from data with flexural strength (N/mJ) and stiffness values (N/mm). The fractured tibiae (Fig. 3b) were maintained, after the mechanical test, in phosphate buffered saline until the attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) analysis.

The proximal fragment diaphysis was sectioned on the transversal axis with a diamond disk under constant irrigation to obtain three cortical cylindrical fragments. The bone fragments were dehydrated in ovens at 37 °C for one day, and an external cortical surface placed against the diamond crystal of the ATR-FTIR unit, pressed with a force gauge at a constant pressure to facilitate contact (Fig. 4a). Data were recorded and analyzed with OPUS 6.5 software (Bruker*, Ettlingen, Germany). The bone composition was analyzed using Fourier transform infrared spectroscopy (FTIR, Vertex 70 Bruker*, Ettlingen, Germany) equipped with an accessory that allowed for spectrum acquisitions in the attenuated reflectance (ATR) mode. The spectra were recorded in the range of $400 \pm 4.000\,cm^{-1}$ at a $4\,cm^{-1}$ resolution, and the mean from 32 scans per fragment analyzed was used. Vector normalization and baseline correction were performed across all spectra, and these were considered absorbance height ratios.

The spectra was further analyzed by calculating the following parameters: amide I band (collagen ratio between the mature pyridinoline crosslink peaks (PYR) ± 1660 $^{cm-1}$ and the immature crosslinking dihydroxynorleucine (DHLNL) - $1690\,cm^{-1}$); crystallinity Index (the intensity ratio of peaks 551 and $597\,cm^{-1}$ for $588\,cm^{-1}$); and matrix-to-mineral ratios of amide I + II/hydroxyapatite (HA) (M:MI) (the ratio between the integrated areas of amide I + II ($1520 \pm 1720\,cm^{-1}$) for HA ($916 \pm 1180\,cm^{-1}$)) and amide III + collagen/HA (M:MIII) (the ratio between the integrated areas of amide III ($1210 \pm 1270\,cm^{-1}$) with two collagen bands ($1269 \pm 1296\,cm^{-1}$ and $1180 \pm 1213\,cm^{-1}$) for HA ($916 \pm 1180\,cm^{-1}$) (Fig. 4b).

Statistical analysis

The data from all measured parameters were tested for normal distribution (Shapiro-Wilk) and the equality of

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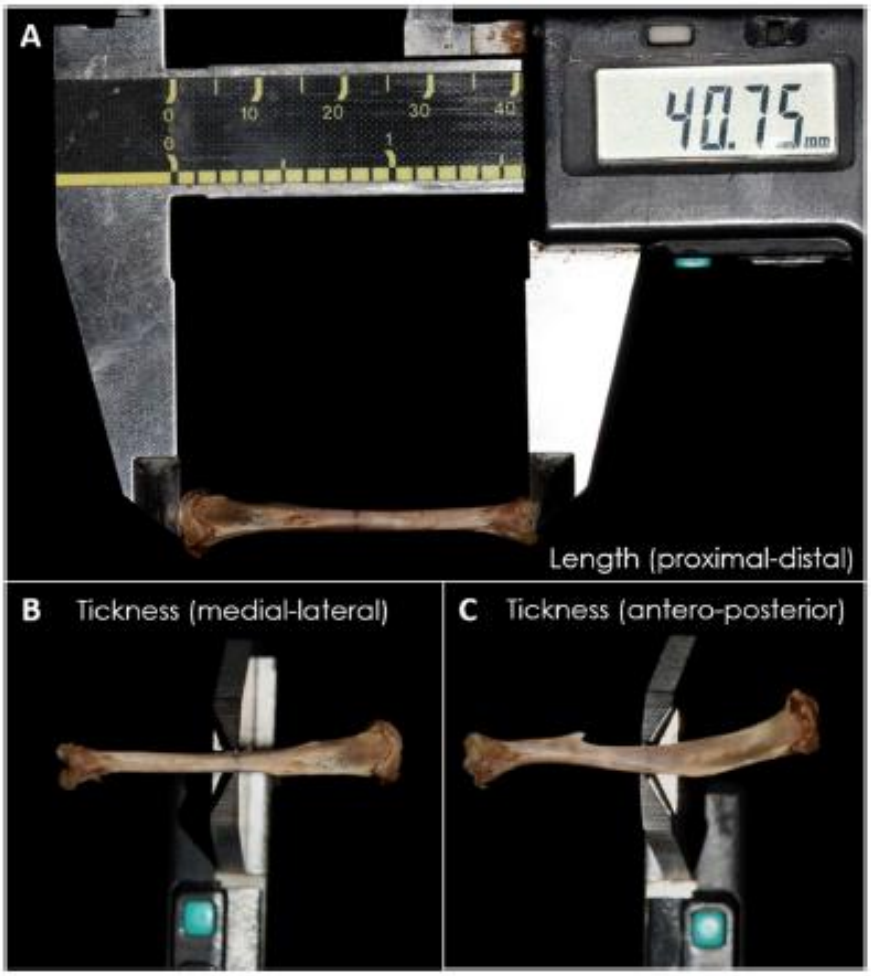

Fig. 2 Tibia macroscopic images showing the analysis of length (**a**) and thickness (**b** and **c**) measurement

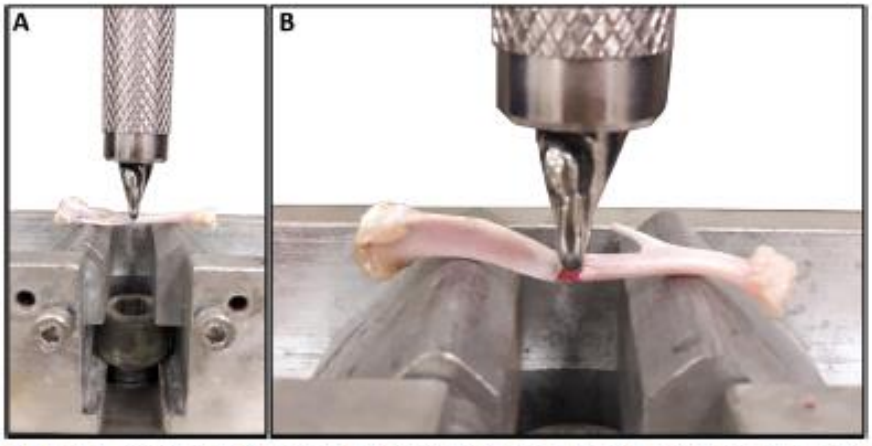

Fig. 3 Biomechanical analysis. **a** – The three-bending flexual test, showing the tibia positioned horizontally on the two holding fixtures, with a perpendicular load in middle of bone. **b** – Image showing the moment of bone fracture

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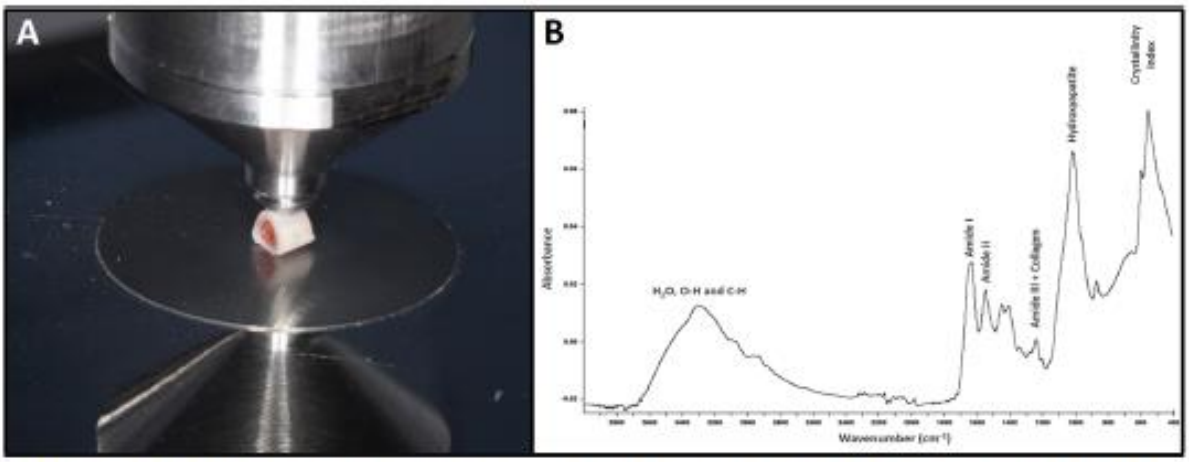

Fig. 4 Image of Attenuated Total Reflectance (ATR)-Fourier Transform Infrared Spectroscopy (FTIR) analysis. **a** - The external cortical surface of tibia pressed by a force gauge at ATR-FTIR diamond crystal. **b** – Characteristic bone spectrum obtained from the ATR-FTIR showing the region of the analyzed parameters, using the program OPUS 6.5

variances (Levene's test). The data submitted to the normality and equality of variance tests showed a parametric distribution and the results were expressed in mean and standard deviation. Thereby, the parametric test Two-way analysis of variance (ANOVA) was performed followed by the Tukey test. All tests employed a level of significance of $\alpha = 0.05$ and all-statistical analyses were carried out with Sigma Plot version 13.1 (Systat Software Inc., San Jose, CA, USA).

Results

The means and standard deviations of all parameter analyses are shown on Table 1. In the macroscopy analysis, the IRnoHBO and IRHBO groups showed lower length

Table 1 The means and standard deviation values of all parameters analysis

Tests/Groups	nolRnoHBO	nolRHBO	IRnoHBO	IRHBO
Length (proximal-distal)	37.67 ± 1.59	37.00 ± 0.95	35.30 ± 1.36	34.80 ± 1.22
	Aa	Aa	Ba	Ba
Thickness (anteroposterior)	3.00 ± 0.15	2.91 ± 0.30	2.81 ± 0.22	2.76 ± 0.23
	Aa	Aa	Ba	Ba
Thickness (medial-lateral)	2.29 ± 0.16	2.21 ± 0.18	2.15 ± 0.17	2.09 ± 0.14
	Aa	Aa	Ba	Ba
Flexural strength	58.60 ± 14.69	73.37 ± 23.86	45.66 ± 18.30	59.66 ± 19.46
	Ab	Aa	Bc	Bb
Stiffness	129.10 ± 19.45	120.75 ± 8.97	89.06 ± 13.96	106.00 ± 13.95
	Aa	Aa	Bb	Aa
Amide I ratio	2.56 ± 0.29	2.41 ± 0.44	2.04 ± 0.44	1.82 ± 0.34
	Aa	Aa	Bb	Bb
Crystallinity Index	2.96 ± 0.27	3.09 ± 0.41	2.65 ± 0.42	2.80 ± 0.31
	Aa	Aa	Bb	Bb
Amide I + II/ Hydroxyapatite	0.43 ± 0.14	0.36 ± 0.10	0.34 ± 0.06	0.29 ± 0.08
	Aa	Aa	Bb	Bb
Amide III + Collagen/ Hydroxyapatite	3.57 ± 1.31	4.75 ± 1.31	1.93 ± 0.42	1.53 ± 0.31
	Aa	Aa	Bb	Bb

Groups: Non-irradiated (nolRnoHBO) and Irradiated (IRnoHBO) - animals without HBO; and Non-irradiated (nolRHBO) and Irradiated (IRHBO) - animals' treatment with HBO. Different upper case letters within rows indicate significant differences for systemic condition factor (non-irradiated or irradiated); different lower case letters within rows indicate significant difference for HBO therapy factor (non-HBO or HBO therapy). Comparison performed by Tukey test ($p < 0.05$)

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values compared to noIRnoHBO and noIRHBO groups ($p < 0.01$). The thickness of the anteroposterior (AP) and medial-lateral (ML) parameters showed lower values in IRnoHBO and IRHBO compared to noIRnoHBO and noIRHBO groups, respectively (AP: $p < 0.03$ ML: $p < 0.02$). In addition, the IRnoHBO has no statistical difference in macroscopic analysis compared to IRHBO ($p > 0.05$).

The biomechanics analysis showed that the IRnoHBO group had lower value of flexural strength, when compared to the noIRnoHBO and IRHBO groups ($p < 0.04$). In addition, the noIRHBO group showed a higher value of flexural strength compared to noIRnoHBO and IRHBO groups ($p < 0.02$). The stiffness parameter showed that IRnoHBO had lower values compared to noIRnoHBO and IRHBO groups ($p < 0.03$), however, there is no statistical difference in noIRHBO, when compared to the IRHBO and noIRHBO groups ($p > 0.06$).

In the ATR-FTIR analysis, the IRnoHBO and IRHBO had lower values of collagen maturity, when compared to the noIRnoHBO and noIRHBO groups, respectively ($p < 0.03$). The crystallinity index showed that IRnoHBO and IRHBO had lower values, when compared to the noIRnoHBO and noIRHBO groups, respectively ($p < 0.04$). In addition, the organic/inorganic ratios (M:MI and M:MIII) showed that IRnoHBO and IRHBO had lower values compared to the noIRnoHBO and noIRHBO groups (M:MI: $p < 0.01$; M:MIII: $p < 0.02$).

Discussion

The present study showed that IR compromises bone growth, decreases mature/immature crosslinks ratio, changes morphology of HA crystals and collagen/HA ratio, decreasing flexural strength and stiffness in rat tibiae. HBO therapy improves flexural strength and stiffness parameters in irradiated tibia, showing no statistical difference with the noIRnoHBO group.

The 30 Gy used was based on previous studies, which showed that a single high dose of IR was similar to 50–70 Gy fractional radiotherapy received in most patients with carcinoma [15, 16]. Studies have shown that these doses can arrest cell cycle progression, allowing evaluations of the irradiation effects on bone tissue [7, 17]. HBO therapy has been widely used in situations where irradiation compromised microcirculation [2, 3], and the protocol accepted in animal studies involves the delivery of 100% oxygen at 150 to 300 kPa for 60 to 90 min, once daily [10, 11], as used in the present study. In addition, the period of 30 days after ionizing radiation is the time required for structural changes in bone tissue [7]; and the 7-day period of HBO therapy was used to evaluate the initial treatment response in bone compromised by IR.

Our results showed that in the macroscopy analysis, the irradiated groups (IRnoHBO and IRHBO) had lower length and thickness (anteroposterior and medial-lateral) values when compared to the non-irradiated groups (noIRnoHBO and noIRHBO). Macroscopic changes, such as growth arrest and/or angular deformity of the extremity or kyphosis and spine scoliosis, are frequently reported when the irradiation field includes the growth plate [5, 18]. IR compromises the endochondral ossification process through impairment of chondrocytes proliferation [19] in the serial cartilage zone. Moreover, IR damages small blood vessels in the ossification zone that blocks osteogenesis, thus preventing normal remodeling at the chondroosseous junction [12]. In addition, noIRHBO and IRHBO showed no significant difference in macroscopic analysis compared to noIRnoHBO and IRnoHBO, respectively. This suggests that irradiation, applied during the animal growth, significantly damages and impairs the ossification process [5]. In addition, HBO therapy did not show significant improvement, after the bone was compromised.

In the FTIR analyses, our results showed that IRnoHBO and IRHBO had lower values of collagen maturity, crystallinity index and organic/inorganic ratios, when compared to the noIRnoHBO and noIRHBO groups. Studies have shown that irradiation induces side chain decarboxylation of the collagen molecule, thus modifying the interaction or binding between the organic matrix and the HA mineral [6, 20]. This increases immature cross-links on collagen [21], changes the morphology HA crystals [22] and impairs the mineralization process [7]. The present study showed that the noIRHBO and IRHBO groups demonstrated no significant difference for the ATR-FTIR analysis, when compared to the noIRnoHBO and IRnoHBO groups, respectively. This methodology analyzes nanostructure changes, then our results suggest that the HBO therapy did not minimize the IR deleterious effects, however, some microstructural changes might have occurred, according to the findings in the biomechanical analysis.

In the biomechanics analysis, the IR groups showed lower values of flexural strength and bone stiffness, leading to greater susceptibility to fractures. The collagen arrangement and the interaction with apatite crystals are important for establishing mechanical and structural properties of bone [23]. The primary aspect of the irradiation-induced loss of fracture resistance could be due to the complete loss of plastic deformation (intrinsic toughness) after irradiation [20], induced by damaging collagen molecules [21]. In the present study, the IRHBO group showed no statistical difference of flexural strength and stiffness, when compared to the noIRnoHBO group. The study showed that HBO therapy holds the potential to increase intermolecular

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interactions (by hydrogen bonds) in the collagen, followed by induced cross-linking that stabilizes the fibrils [9]. Our study suggests that IR decreases the number of intermolecular interactions in collagen molecules and HBO therapy improves the quality of these remaining interactions. This is in agreement with previous reports that HBO therapy increases bone mechanical properties by increasing the organization of collagen fibers [24, 25]. However, further studies are required to clarify the molecular mechanisms underlying intermolecular interaction under HBO therapy conditions.

The morphologic and biomechanical alterations in bone induced by a high dose of IR are a major concern for surgeons who are considering rehabilitation in patients after therapeutic irradiation treatment. Studies suggests that, for people with irradiation tissue injury, HBO therapy is associated with an improved outcome, including cases with severe mandibular osteoradionecrosis [2, 3, 26]. However, other studies showed controversy regarding the clinical effectiveness of HBO therapy in bones compromised by radiotherapy [27–29]. Although, these studies have some bias, such as variations in radiation dosage, patients excluded on the basis of advanced osteoradionecrosis, HBO protocol, adjunctive therapy other than HBO, time between radiation and tooth extraction, method of extraction, and adjunctive therapy other than radiation. Although the results of the present animal study cannot be extrapolated to humans [30], but, serve to support new research in this area.

Conclusion

The results of the present study showed that ionizing radiation arrests bone development, as well as decreasing collagen maturation and the mineral deposition process, along with reducing the flexural strength and bone stiffness mechanical parameters. Moreover, HBO therapy was shown to minimize deleterious effects of irradiation on flexural strength and the bone stiffness analysis.

Abbreviations

IR: Ionizing radiation; HAP: Hydroxyapatite; HBO: Hyperbaric Oxygen; kPa: Kilopascal; ATR-FTIR: Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy; PYR: Pyridinoline; DHLNL: Dihydroxynorleucine; AI: Amide I band; CI: Crystallinity Index; MMI: Matrix-to-mineral ratio: Amide I+ II/Hydroxyapatite; MMII: Amide III +Collagen/HA

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Authors' contributions

L.H.F.J., P.H.J.O.L., P.B.F.S., P.D., L.S.C.F., P.S.Q. and F.S.R. participated in the acquisition and/or analysis of data. P.H.J.O.L., P.B.F.S., P.D. and F.S.R participated in the design and/or in the intepretation of the results. L.H.F.J., P.H.J.O.L., P.B.F.S., P.D., L.S.C.F., P.S.Q. and F.S.R participated in writing and/or revising the manuscript. The authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the research ethics committee, University of Uberlândia, Brazil.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests in association with this study.

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References

1. Phulpin B, Dolivet G, Marie PY, Poussier S, Huger S, Bravetti P, et al. Feasibility of treating irradiated bone with intramedullary delivered autologous mesenchymal stem cells. J Biomed Biotechnol. 2011;2011:560257.
2. Bennett MH, Feldmeier J, Hampson NB, Smee R, Milross C. Hyperbaric oxygen therapy for late radiation tissue injury. Cochrane Database Syst Rev. 2016;4:CD005005.
3. Gaviel H, Eviatar E, Abu ER. Hyperbaric oxygen therapy for maxillary bone radiation-induced injury: a 15-year single-center experience. Head Neck. 2017;39:275–8.
4. Margulies BS, Horton JA, Wang Y, Damron TA, Allen MJ. Effects of radiation therapy on chondrocytes in vitro. Calcif Tissue Int. 2006;78:302–13.
5. Rocha FS, Limirio PH, Zanetta-Barbosa D, Batista JD, Dechichi P. The effects of ionizing radiation on the growth plate in rat tibiae. Microsc Res Tech. 2016;79:1147–51.
6. Nyman JS, Reyes M, Wang X. Effect of ultrastructural changes on the toughness of bone. Micron. 2005;36:566–82.
7. Limirio P, Soares PBF, Emi ETP, Lopes CCA, Rocha FS, Batista JD, et al. Ionizing radiation and bone quality: time-dependent effects. Radiat Oncol. 2019;14:15.
8. Thom SR. Hyperbaric oxygen: its mechanisms and efficacy. Plast Reconstr Surg. 2011;127(Suppl 1):131S–41S.
9. Limirio P, da Rocha Junior HA, Morais RB, Hiraki KRN, Balbi APC, Soares PBF, et al. Influence of hyperbaric oxygen on biomechanics and structural bone matrix in type 1 diabetes mellitus rats. PLoS One. 2018;13:e0191694.

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

10. Rocha FS, Gomes Moura CC, Rocha Rodrigues DB, Zanetta-Barbosa D, Nakamura Hiraki KR, Dechichi P. Influence of hyperbaric oxygen on the initial stages of bone healing. Oral Surg Oral Med Oral Pathol Oral Radiol. 2015;120:581–7.
11. An H, Lee JT, Oh SE, Park KM, Hu KS, Kim S, et al. Adjunctive hyperbaric oxygen therapy for irradiated rat calvarial defects. J Periodontal Implant Sci. 2019;49:2–13.
12. Kitazono Hammell MT, Bunin N, Edgar JC, Jaramillo D. Paraphyseal changes on bone-age studies predict risk of delayed radiation-associated skeletal complications following total body irradiation. Pediatr Radiol. 2013;43:1152–8.
13. Meixel AJ, Hauswald H, Delorme S, Jobke B. From radiation osteitis to osteoradionecrosis: incidence and MR morphology of radiation-induced sacral pathologies following pelvic radiotherapy. Eur Radiol. 2018;28:3550–9.
14. Chronopoulos A, Zarra T, Ehrenfeld M, Otto S. Osteoradionecrosis of the jaws: definition, epidemiology, staging and clinical and radiological findings. A concise review. Int Dent J. 2018;68:22–30.
15. Ohmell LO, Branemark R, Nyman J, Nilsson P, Thomsen P. Effects of irradiation on the biomechanics of osseointegration. An experimental in vivo study in rats. Scand J Plast Reconstr Surg Hand Surg. 1997;31:281–93.
16. Sun HB, Gao XJ, Deng J, Li NY, Lu HJ. Progress of oral sequelae during head-neck radiotherapy. Chin J Dental Res. 2010;13:51–5.
17. Lerouxel E, Moreau A, Bouler JM, Giumelli B, Daculsi G, Weiss P, et al. Effects of high doses of ionising radiation on bone in rats: a new model for evaluation of bone engineering. Br J Oral Maxillofac Surg. 2009;47:602–7.
18. Pritchard MR, Horton JA, Keenawinna LS, Damron TA. Microarray analysis of irradiated growth plate zones following laser microdissection shows later importance of differentially expressed genes during radiorecovery. Cells Tissues Organs. 2010;192:240–9.
19. Damron TA, Horton JA, Naqvi A, Margulies B, Strauss J, Grant W, et al. Decreased proliferation precedes growth factor changes after physeal irradiation. Clin Orthop Relat Res. 2004;422:233–42.
20. Desmons S, Heger M, Delfosse C, Falgayrac G, Sarrazin T, Delattre C, et al. A preliminary investigation into the effects of X-ray radiation on superficial cranial vascularization. Calcif Tissue Int. 2009;84:379–87.
21. Cao X, Wu X, Frassica D, Yu B, Pang L, Xian L, et al. Irradiation induces bone injury by damaging bone marrow microenvironment for stem cells. Proc Natl Acad Sci U S A. 2011;108:1609–14.
22. Farlay D, Panczer G, Rey C, Delmas PD, Boivin G. Mineral maturity and crystallinity index are distinct characteristics of bone mineral. J Bone Miner Metab. 2010;28:433–45.
23. Bozkurt O, Bilgin MD, Evis Z, Pleshko N, Severcan F. Early alterations in bone characteristics of type I diabetic rat femur: a Fourier transform infrared (FT-IR) imaging study. Appl Spectrosc. 2016;70:2005–15.
24. Kawada S, Wada E, Matsuda R, Ishii N. Hyperbaric hyperoxia accelerates fracture healing in mice. PLoS One. 2013;8:e72603.
25. Yeh W, Lin SS, Yuan LJ, Lee KF, Lee MY, Ueng SW. Effects of hyperbaric oxygen treatment on tendon graft and tendon-bone integration in bone tunnel: biochemical and histological analysis in rabbits. J Orthop Res. 2007;25:636–45.
26. Costa DA, Costa TP, Netto EC, Joaquim N, Ventura I, Pratas AC, et al. New perspectives on the conservative management of osteoradionecrosis of the mandible: a literature review. Head Neck. 2016;38:1708–16.
27. Annane D, Depondt J, Aubert P, Villart M, Gehanno P, Gajdos P, et al. Hyperbaric oxygen therapy for radionecrosis of the jaw: a randomized, placebo-controlled, double-blind trial from the ORN96 study group. J Clin Oncol. 2004;22:4893–900.
28. Sultan A, Hanna GJ, Margalit DN, Chau N, Goguen LA, Marty FM, et al. The use of hyperbaric oxygen for the prevention and Management of Osteoradionecrosis of the jaw: a Dana-Farber/Brigham and Women's Cancer center multidisciplinary guideline. Oncologist. 2017;22:343–50.
29. Esposito M, Worthington HV. Interventions for replacing missing teeth: hyperbaric oxygen therapy for irradiated patients who require dental implants. Cochrane Database Syst Rev. 2013;30:9CD003603.
30. Nyberg J, Hertzman S, Svensson B, Johansson CB. Osseointegration of implants in irradiated bone with and without hyperbaric oxygen treatment: an experimental study in rat tibiae. Int J Oral Maxillofac Implants. 2013;28:739–46.

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Conclusões

A oxigenação hiperbárica promove uma hiperóxia, que resulta em maiores níveis de oxigênio no sangue e consequentemente nos tecidos, favorecendo o reparo. Tem sido utilizada como uma ferramenta auxiliar no tratamento da osteoradionecrose e da osteonecrose associada ao uso de medicamentos, bem como um adjuvante no processo de regeneração em grandes reconstruções ósseas.

A resposta celular à oxigenação hiperbárica mostra modulação do processo inflamatório e aceleração da formação óssea, além de aumento da angiogênese e do metabolismo celular. Considerando os dados da literatura apresentados nas revisões sistemáticas e os resultados obtidos do estudo laboratorial, fica claro que o uso de terapias adjuvantes como a oxigenação hiperbárica é positivo, reduzindo os efeitos deletérios da radiação ionizante e da OAM, otimizando a neoformação óssea, melhorando as propriedades mecânicas e bioquímicas do tecido ósseo.

Embora o uso da oxigenação hiperbárica tenha mostrado resultados positivos, há uma grande discrepância entre os protocolos aplicados, em função do tipo de paciente, condição clínica, entre outros fatores. Em relação à procedimentos de reconstrução óssea, essa heterogeneidade se deve aos diferentes tipos e tamanhos de enxertos, qualidade do leito receptor e envolvimento de complicações pós-operatórias.

Apesar das evidências atuais disponíveis, estudos futuros devem incluir mais ensaios clínicos randomizados para determinação da longevidade dos benefícios produzidos, definição de protocolos, diretrizes de tratamento e ampliação do conhecimento relacionado às terapias adjuvantes de regeneração do tecido ósseo.

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Referências*

1. Wei J, Ducy P. Co-dependence of bone and energy metabolisms. Arch Biochem Biophys [Internet]. 2010;503(1):35–40. Available from: http://dx.doi.org/10.1016/j.abb.2010.05.021

2. Clarke B. Normal Bone Anatomy and Physiology. Clin J Am Soc Nephrol [Internet]. 2008 Nov;3(Supplement 3):S131–9. Available from: http://cjasn.asnjournals.org/lookup/doi/10.2215/CJN.04151206

3. Chen J-H, Liu C, You L, Simmons CA. Boning up on Wolff's Law: Mechanical regulation of the cells that make and maintain bone. J Biomech [Internet]. 2010 Jan;43(1):108–18. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021929009005077

4. Florencio-Silva R, Sasso GR da S, Sasso-Cerri E, Simões MJ, Cerri PS. Biology of Bone Tissue: Structure, Function, and Factors That Influence Bone Cells. Biomed Res Int [Internet]. 2015;2015(6):1–17. Available from: http://www.hindawi.com/journals/bmri/2015/421746/

5. Teitelbaum SL. Bone Resorption by Osteoclasts. Science (80-) [Internet]. 2000 Sep 1;289(5484):1504–8. Available from: https://www.sciencemag.org/lookup/doi/10.1126/science.289.5484.1504

6. KJÆR M. Role of Extracellular Matrix in Adaptation of Tendon and Skeletal Muscle to Mechanical Loading. Physiol Rev [Internet]. 2004 Apr;84(2):649–98. Available from: https://www.physiology.org/doi/10.1152/physrev.00031.2003

7. Betti LV. Análises microscópica e radiográfica do reparo de defeitos confeccionados em fêmures de coelhos preenchidos com matriz óssea bovina medular em bloco ou cortical em micrograânulos [Internet]. [Bauru]: Universidade de São Paulo; 2004. Available from: http://www.teses.usp.br/teses/disponiveis/25/25138/tde-21032005-104116/

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

8. Hartmann C. Transcriptional networks controlling skeletal development. Curr Opin Genet Dev [Internet]. 2009 Oct;19(5):437–43. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0959437X09001415

9. Karsenty G, Kronenberg HM, Settembre C. Genetic Control of Bone Formation. Annu Rev Cell Dev Biol. 2009;25(1):629–48.

10. Currey JD, Pitchford JW, Baxter PD. Variability of the mechanical properties of bone, and its evolutionary consequences. J R Soc Interface [Internet]. 2007 Feb 22;4(12):127–35. Available from: https://royalsocietypublishing.org/doi/10.1098/rsif.2006.0166

11. Cole JH, Van Der Meulen MCH. Whole bone mechanics and bone quality. Clin Orthop Relat Res. 2011;469(8):2139–49.

12. Chappard D, Baslé MF, Legrand E, Audran M. New laboratory tools in the assessment of bone quality. Osteoporos Int [Internet]. 2011 Aug 24;22(8):2225–40. Available from: http://link.springer.com/10.1007/s00198-011-1573-6

13. Diniz JS, Dionísio VC, Nicolau RA, Pacheco MTT. Propriedades mecânicas do tecido ósseo: uma revisão bibliográfica. … Lat Am … [Internet]. 2005;1363–6. Available from: http://www.cpaqv.org/biomecanica/analise cinetica 11.pdf

14. Hart NH, Nimphius S, Rantalainen T, Ireland A, Siafarikas A, Newton RU. Mechanical basis of bone strength: Influence of bone material, bone structure and muscle action. J Musculoskelet Neuronal Interact. 2017;17(3):114–39.

15. Schmidt-Bleek K, Schell H, Schulz N, Hoff P, Perka C, Buttgereit F, et al. Inflammatory phase of bone healing initiates the regenerative healing cascade. Cell Tissue Res. 2012;347(3):567–73.

16. Midwood KS, Williams LV, Schwarzbauer JE. Tissue repair and the dynamics of the extracellular matrix. Int J Biochem Cell Biol [Internet]. 2004 Jun;36(6):1031–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1357272503004291

17. Schlundt C, El Khassawna T, Serra A, Dienelt A, Wendler S, Schell H, et al.

Macrophages in bone fracture healing: Their essential role in endochondral ossification. Bone [Internet]. 2018 Jan;106:78–89. Available from: https://linkinghub.elsevier.com/retrieve/pii/S8756328215003920

18. Pelissier PH, Masquelet AC, Bareille R, Pelissier SM, Amedee J. Induced membranes secrete growth factors including vascular and osteoinductive factors and could stimulate bone regeneration. J Orthop Res [Internet]. 2004 Jan;22(1):73–9. Available from: http://doi.wiley.com/10.1016/S0736-0266%2803%2900165-7

19. Croci AT, Camargo OP de, Bitar G, Pereira SLB, Moreira M, Freitas Jr. S de. Efeito do concentrado de plasma em falhas ósseas provocadas em fêmures de camundongos como estimulação de formação óssea: estudo experimental. Acta Ortopédica Bras [Internet]. 2003 Dec;11(4):230–9. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1413-78522003000400006&lng=pt&tlng=pt

20. Roschger P, Dempster DW, Zhou H, Paschalis EP, Silverberg SJ, Shane E, et al. New Observations on Bone Quality in Mild Primary Hyperparathyroidism as Determined by Quantitative Backscattered Electron Imaging. J Bone Miner Res [Internet]. 2007 Jan 29;22(5):717–23. Available from: http://doi.wiley.com/10.1359/jbmr.070120

21. Guadalupe-Grau A, Fuentes T, Guerra B, Calbet JAL. Exercise and Bone Mass in Adults. Sport Med [Internet]. 2009 May;39(6):439–68. Available from: http://link.springer.com/10.2165/00007256-200939060-00002

22. Reszka AA. Bisphosphonate Mechanisms of Action. In: Osteoporosis [Internet]. Totowa, NJ: Humana Press; 2010. p. 443–68. Available from: http://link.springer.com/10.1007/978-1-59745-459-9_19

23. Da Cunha SS, Sarmento VA, Ramalho LMP, De Freitas AC, De Almeida D, Tavares ME, et al. Effects of radiotherapy on bone tissue. Radiol Bras. 2007;40(3):189–92.

24. Damron TA, Margulies BS, Strauss JA, O'Hara K, Spadaro JA, Farnum CE.

Sequential histomorphometric analysis of the growth plate following irradiation with and without radioprotection. J Bone Jt Surg - Ser A. 2003;85(7):1302–13.

25. Paulino AC. Late effects of radiotherapy for pediatric extremity sarcomas. Int J Radiat Oncol [Internet]. 2004 Sep;60(1):265–74. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0360301604002408

26. Bandstra ER, Pecaut MJ, Anderson ER, Willey JS, De Carlo F, Stock SR, et al. Long-Term Dose Response of Trabecular Bone in Mice to Proton Radiation. Radiat Res [Internet]. 2008 Jun;169(6):607–14. Available from: http://www.bioone.org/doi/10.1667/RR1310.1

27. Oh D, Huh SJ, Nam H, Park W, Han Y, Lim DH, et al. Pelvic Insufficiency Fracture After Pelvic Radiotherapy for Cervical Cancer: Analysis of Risk Factors. Int J Radiat Oncol [Internet]. 2008 Mar;70(4):1183–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0360301607038680

28. Dhakal S, Chen J, McCance S, Rosier R, O'Keefe R, Constine LS. Bone Density Changes After Radiation for Extremity Sarcomas: Exploring the Etiology of Pathologic Fractures. Int J Radiat Oncol [Internet]. 2011 Jul;80(4):1158–63. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0360301610005304

29. Green DE, Adler BJ, Chan ME, Lennon JJ, Acerbo AS, Miller LM, et al. Altered Composition of Bone as Triggered by Irradiation Facilitates the Rapid Erosion of the Matrix by Both Cellular and Physicochemical Processes. Roeder RK, editor. PLoS One [Internet]. 2013 May 31;8(5):e64952. Available from: https://dx.plos.org/10.1371/journal.pone.0064952

30. Batista JD, Zanetta-Barbosa D, Cardoso S V., Dechichi P, Rocha FS, Pagnoncelli RM. Effect of low-level laser therapy on repair of the bone compromised by radiotherapy. Lasers Med Sci [Internet]. 2014 Nov 10;29(6):1913–8. Available from: http://link.springer.com/10.1007/s10103-014-1602-8

31. Muhonen A, Haaparanta M, Grönroos T, Bergman J, Knuuti J, Hinkka S, et al. Osteoblastic activity and neoangiogenesis in distracted bone of irradiated rabbit mandible with or without hyperbaric oxygen treatment. Int J Oral Maxillofac

Surg [Internet]. 2004 Mar;33(2):173–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0901502703904899

32. Michel G, Blery P, Pilet P, Guicheux J, Weiss P, Malard O, et al. Micro-CT Analysis of Radiation-Induced Osteopenia and Bone Hypovascularization in Rat. Calcif Tissue Int [Internet]. 2015 Jul 8;97(1):62–8. Available from: http://link.springer.com/10.1007/s00223-015-0010-9

33. Rabelo GD, Beletti ME, Dechichi P. Histological analysis of the alterations on cortical bone channels network after radiotherapy: A rabbit study. Microsc Res Tech [Internet]. 2010 Oct;73(11):1015–8. Available from: http://doi.wiley.com/10.1002/jemt.20826

34. Wernle JD, Damron TA, Allen MJ, Mann KA. Local irradiation alters bone morphology and increases bone fragility in a mouse model. J Biomech [Internet]. 2010 Oct;43(14):2738–46. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0021929010003532

35. Rahman N, Khan R, Badshah S. Effect of x-rays and gamma radiations on the bone mechanical properties: literature review. Cell Tissue Bank [Internet]. 2018 Dec 13;19(4):457–72. Available from: http://link.springer.com/10.1007/s10561-018-9736-8

36. Barth HD, Zimmermann EA, Schaible E, Tang SY, Alliston T, Ritchie RO. Characterization of the effects of x-ray irradiation on the hierarchical structure and mechanical properties of human cortical bone. Biomaterials [Internet]. 2011 Dec;32(34):8892–904. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0142961211009185

37. Willey JS, Lloyd SAJ, Robbins ME, Bourland JD, Smith-Sielicki H, Bowman LC, et al. Early Increase in Osteoclast Number in Mice after Whole-Body Irradiation with 2 Gy X Rays. Radiat Res [Internet]. 2008 Sep;170(3):388–92. Available from: http://www.bioone.org/doi/abs/10.1667/RR1388.1

38. Hopewell JW. Radiation-therapy effects on bone density. Med Pediatr Oncol [Internet]. 2003 Sep;41(3):208–11. Available from:

http://doi.wiley.com/10.1002/mpo.10338

39. Ruggiero SL, Dodson TB, Assael LA, Landesberg R, Marx RE, Mehrotra B. American Association of Oral and Maxillofacial Surgeons Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws—2009 Update. J Oral Maxillofac Surg [Internet]. 2009 May;67(5):2–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278239109001153

40. Rogers MJ, Crockett JC, Coxon FP, Mönkkönen J. Biochemical and molecular mechanisms of action of bisphosphonates. Bone [Internet]. 2011 Jul;49(1):34–41. Available from: https://linkinghub.elsevier.com/retrieve/pii/S8756328210020399

41. Maruotti N, Corrado A, Neve A, Cantatore FP. Bisphosphonates: effects on osteoblast. Eur J Clin Pharmacol [Internet]. 2012 Jul 9;68(7):1013–8. Available from: http://link.springer.com/10.1007/s00228-012-1216-7

42. Di Salvatore M, Orlandi A, Bagalà C, Quirino M, Cassano A, Astone A, et al. Anti-tumour and anti-angiogenetic effects of zoledronic acid on human non-small-cell lung cancer cell line. Cell Prolif [Internet]. 2011 Apr;44(2):139–46. Available from: http://doi.wiley.com/10.1111/j.1365-2184.2011.00745.x

43. Sharma D, Ivanovski S, Slevin M, Hamlet S, Pop TS, Brinzaniuc K, et al. Bisphosphonate-related osteonecrosis of jaw (BRONJ): diagnostic criteria and possible pathogenic mechanisms of an unexpected anti-angiogenic side effect. Vasc Cell [Internet]. 2013;5(1):1. Available from: http://vascularcell.com/index.php/vc/article/view/10.1186-2045-824X-5-1

44. McLeod NMH, Brennan PA, Ruggiero SL. Bisphosphonate osteonecrosis of the jaw: A historical and contemporary review. Surg [Internet]. 2012 Feb;10(1):36–42. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1479666X11001259

45. Migliorati CA, Siegel MA, Elting LS. Bisphosphonate-associated osteonecrosis: a long-term complication of bisphosphonate treatment. Lancet Oncol [Internet]. 2006 Jun;7(6):508–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1470204506707264

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

46. American Association of Oral and Maxillofacial Surgeons Position Paper on Bisphosphonate-Related Osteonecrosis of the Jaws. J Oral Maxillofac Surg [Internet]. 2007 Mar;65(3):369–76. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278239106019793

47. Silva ML, Tasso L, Azambuja AA, Figueiredo MA, Salum FG, da Silva VD, et al. Effect of hyperbaric oxygen therapy on tooth extraction sites in rats subjected to bisphosphonate therapy—histomorphometric and immunohistochemical analysis. Clin Oral Investig [Internet]. 2017 Jan 9;21(1):199–210. Available from: http://link.springer.com/10.1007/s00784-016-1778-3

48. Liu S, Lin T, Fu E, Hsia Y, Chiu H, Tu H, et al. Immediate hyperbaric oxygen after tooth extraction ameliorates bisphosphonate-related osteonecrotic lesion in rats. J Periodontol [Internet]. 2019 Dec;90(12):1449–56. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1002/JPER.18-0761

49. Tiwari S, Avinash A, Katiyar S, Aarthi Iyer A, Jain S. Dental applications of ozone therapy: A review of literature. Saudi J Dent Res [Internet]. 2017 Jan;8(1–2):105–11. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2352003516300260

50. Thom SR. Hyperbaric Oxygen: Its Mechanisms and Efficacy. Plast Reconstr Surg [Internet]. 2011 Jan;127:131S-141S. Available from: http://journals.lww.com/00006534-201101001-00020

51. Al Hadi H, Smerdon GR, Fox SW. Hyperbaric oxygen therapy accelerates osteoblast differentiation and promotes bone formation. J Dent [Internet]. 2015 Mar;43(3):382–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0300571214002802

52. Rocha FS, Gomes Moura CC, Rocha Rodrigues DB, Zanetta-Barbosa D, Nakamura Hiraki KR, Dechichi P. Influence of hyperbaric oxygen on the initial stages of bone healing. Oral Surg Oral Med Oral Pathol Oral Radiol [Internet]. 2015 Nov;120(5):581–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2212440315010780

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

53. Salgado CJ, Raju A, Licata L, Patel M, Rojavin Y, Wasielewski S, et al. Effects of hyperbaric oxygen therapy on an accelerated rate of mandibular distraction osteogenesis. J Plast Reconstr Aesthetic Surg [Internet]. 2009 Dec;62(12):1568–72. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1748681508008565

54. Jacobson AS, Buchbinder D, Hu K, Urken ML. Paradigm shifts in the management of osteoradionecrosis of the mandible. Oral Oncol [Internet]. 2010 Nov;46(11):795–801. Available from: https://linkinghub.elsevier.com/retrieve/pii/S136883751000254X

55. Kawada S, Wada E, Matsuda R, Ishii N. Hyperbaric Hyperoxia Accelerates Fracture Healing in Mice. Zadpoor AA, editor. PLoS One [Internet]. 2013 Aug 14;8(8):e72603. Available from: https://dx.plos.org/10.1371/journal.pone.0072603

56. Pedersen TO, Xing Z, Finne-Wistrand A, Hellem S, Mustafa K. Hyperbaric oxygen stimulates vascularization and bone formation in rat calvarial defects. Int J Oral Maxillofac Surg [Internet]. 2013 Jul;42(7):907–14. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0901502713000222

57. Sawai T, Niimi A, Takahashi H, Ueda M. Histologic study of the effect of hyperbaric oxygen therapy on autogenous free bone grafts. J Oral Maxillofac Surg [Internet]. 1996 Aug;54(8):975–81. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0278239196903961

58. Nilsson LP, Granström G. Changes of Serum Alkaline Phosphatase Following Mandibular Osteotomy in the Rat. J Dent Res [Internet]. 1987 Jun 9;66(6):1195–8. Available from: http://journals.sagepub.com/doi/10.1177/00220345870660062001

59. Fok TCO, Jan A, Peel SAF, Evans AW, Clokie CML, Sándor GKB. Hyperbaric oxygen results in increased vascular endothelial growth factor (VEGF) protein expression in rabbit calvarial critical-sized defects. Oral Surgery, Oral Med Oral Pathol Oral Radiol Endodontology [Internet]. 2008 Apr;105(4):417–22.

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

ANEXO

Universidade Federal de Uberlândia

– Comissão de Ética na Utilização de Animais –

Comissão de Ética na Utilização de Animais CEUA

CERTIFICADO

Certificamos que o protocolo para uso de animais em experimentação nº 028/12, sobre o projeto de pesquisa intitulado "Efeito da laserterapia e oxigenoterapia hiperbárica no reparo, microestrutura e resistência biomecânica do osso submetido à radiação ionizante.", sob a responsabilidade da **Profa. Dra. Paula Dechichi,** está de acordo com os princípios éticos na experimentação animal conforme regulamentações do Conselho Nacional de Controle e Experimentação Animal (CONCEA) e foi **APROVADO** pela Comissão de Ética na Utilização de Animais (CEUA) – UFU em reunião de **29 de Maio de 2012.**

(We certify that the protocol nº 028/12, about "Effect of lasertherapy and hyperbaric oxygenotherapy in bone repair, microstructure and resistance after ionizing radiation ', agrees with the ETHICAL PRINCIPLES ON ANIMAL RESEARCH as regulations of National Advice of Control and Animal Experimentation (CONCEA) and approved by Ethics Commission on Use of Animals (CEUA) – Federal University of Uberlândia in 29/05/2012).

Uberlândia, 04 de Junho de 2012.

Universidade Federal de Uberlândia

Profa. Dra. Ana Elizabeth Iannini Custódio
Vice Coordenadora *Pro Tempore* da Comissão de Ética
Na utilização de animais

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