Universidade Estadual de Londrina

RENAN CAPITANI CASAGRANDE

RADIODERMITE E ESTRESSE OXIDATIVO NO CÂNCER DE MAMA

Londrina
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Dissertação apresentada ao Programa de Pós-Graduação em Fisiopatologia Clínica e Laboratorial da Universidade Estadual de Londrina (UEL), como requisito parcial à obtenção do título de Mestre.

Orientadora: Profª. Drª. Alessandra Lourenço Cecchini Armani

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Londrina, 21 de julho de 2021.

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“Não tenhamos pressa, mas não percamos tempo’.

(José Saramago)

Casagrande, Renan Capitani. **Radiodermite e estresse oxidativo no câncer de mama**. 2021. 55 f. Dissertação (Mestrado em Fisiopatologia Clínica e Experimental) - Universidade Estadual de Londrina, Londrina, 2021.

RESUMO

O câncer de mama é a neoplasia maligna mais frequente em mulheres no Brasil e no mundo, com exceção dos cânceres de pele não-melanomas. Dentre as três principais modalidades de tratamento, a radioterapia é utilizada como tratamento adjuvante com intenção curativa em cerca de metade dos casos, tendo como principal efeito colateral agudo a radiodermite. Tanto a própria patologia como os tratamentos realizados podem influenciar no status redox. O objetivo deste estudo foi caracterizar os graus de radiodermite e os padrões de estresse oxidativo apresentados por pacientes com câncer de mama submetidas a tratamento radioterápico adjuvante. Pacientes com indicação de tratamento (n=50) foram convidadas a participar desta pesquisa. Após serem esclarecidas sobre o projeto e assinarem o TCLE (termo de consentimento livre e esclarecido), responderam a um questionário e foram submetidas a exame clínico e à coleta de sangue para análise de marcadores de estresse oxidativo. As coletas foram feitas em três fases distintas do tratamento: M1 (dia do início), M2 (metade das sessões), M3 (dia do término da radioterapia). Foram realizadas, com a amostra sanguínea, técnicas para a quantificação de lipoperóxidos de membrana pela técnica de quimiluminescência (QL) com tert-butil-hidroperoxil, quantificação de malondialdeído (MDA) por cromatografia líquida de alta performance e a quantificação espectrofotométrica de glutationa reduzida (GSH) e oxidada (GSSG). Avaliou-se ainda a atividade das enzimas superóxido dismutase (SOD) e catalase (CAT) por espectrofotometria. As análises sanguíneas de estresse oxidativo foram comparadas com um grupo controle (n=30). Não houve aumento dos níveis marcadores de estresse oxidativo ao longo do tratamento no grupo de fracionamento convencional (FC). Redução no níveis de QL (peroxidação lipídica por quimioluminescência, CAT (catalase), GSH (glutationa reduzida) e GSSG (glutatina oxidada) e aumento de MDA (malondialdeído) foram observados no grupo FC em relação ao controle. Pacientes tratadas com esquema hipofracionado (HF) apresentaram menores graus de radiodermite e de outros efeitos colaterais avaliados. Tratamentos com esquemas de dose diferentes, período de realização da coleta e tratamentos prévios poderiam interferir nos níveis destes marcadores. Não foi possível estabelecer uma relação entre o aumento destes marcadores e a radiodermite. Novos estudos com número maior de participantes que receberam tratamento com esquema hipofracionado poderiam elucidar melhor uma possível relação do estresse oxidativo com a radiodermite.

Palavras chave: radioterapia; câncer de mama; radiodermite; estresse oxidativo.

Casagrande, Renan Capitani. **Radiodermitis and oxidative stress in breast cancer**. 2021. 55 p. Masters (Dissertation in Clinical and Experimental Pathophysiology) - Universidade Estadual de Londrina, Londrina, 2021.

ABSTRACT

Breast cancer is the most frequent malignant neoplasm in women in Brazil and in the world, with the exception of non-melanoma skin cancers. Among the three main treatment modalities, radiotherapy is used as an adjuvant treatment with curative intent in about half of the cases, with radiodermatitis as the main acute side effect. Both the pathology itself and the treatments performed can lead to redox status. The aim of the study was to characterize the degrees of radiodermatitis and the patterns of oxidative stress due to breast cancer patients undergoing adjuvant radiotherapy treatment. Patients with indication for treatment (n = 50) were invited to participate in this research. After being informed about the project and signing the informed consent form (term of informed consent), they answered a questionnaire and underwent clinical examination and blood collection for analysis of oxidative stress markers. Collections were performed in three distinct phases of treatment: M1 (starting day), M2 (half the preparations), M3 (ending radiotherapy day). Techniques for the quantification of membrane lipoperoxides by the chemiluminescence (QL) technique with tert-butyl-hydroperoxyl, quantification of malondialdehyde (MDA) by high-performance liquid chromatography and the spectrophotometric quantification of reduced glutathione (GSH) were performed with the blood sample) and oxidized (GSSG). The activity of the enzymes superoxide dismutase (SOD) and catalase (CAT) is also analyzed by spectrophotometry. Blood analyzes of oxidative stress were compared with a control group (n = 30). There was no increase in oxidative stress levels during treatment in the conventional fractionation (FC) group. Decreased levels of QL (lipid peroxidation by chemiluminescence, CAT (catalase), GSH (reduced glutathione) and GSSG (oxidized glutatin) and increased MDA (malondialdehyde) were observed in the CF group compared to the control. Patients treated with a hypofractionated regimen (HF) lower degrees of radiodermatitis and other associated effects Treatments with different dose regimens, period of collection and previous treatments may interfere with marker levels. New studies with a larger number of participants who received treatment with a hypofractionated regimen elucidate better a possible relationship of oxidative stress with radiodermatitis.

Key words: radiotherapy; breast cancer; radiodermatitis; oxidative stress.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

CAT	Catalase
DVH	Dose-volume histogram
ERO	Espécies reativas de oxigênio
FC	Fracionamento convencional
EO	Estresse Oxidativo
EORTC	European Organisation for Research and Treatment of Cancer
GC	Grupo controle
GSH	Glutationa deduzida
GSSG	Glutationa oxidada
HER 2	Receptor tipo 2 do fator de crescimento epidérmico
HF	Hipofracionamento
HT	Hormonioterapia
MDA	Malondiádeído
NMM	Neoplasia maligna da mama
QL	Lipoperoxidação por quimioluminescência
QT	Quimioterapia
RDT	Radioterapia
RDM	Radiodermite
RTOG	Radiation Therapy Oncology Group
SOD	Superóxido dismutase

SUMÁRIO

INTRODUÇÃO

As neoplasias malignas da mama (NMM) são as mais comuns entre as mulheres no Brasil e no mundo, depois do câncer de pele não-melanoma. O risco de desenvolvimento é de 12% ao longo da vida (HOWLADER et al., 2020). No Brasil, a estimativa de casos novos foi de 59.700 em 2018 e a mortalidade de aproxidamente 25% (INCA, 2019). São fatores de risco conhecidos para o desenvolvimento destas neoplasias a menarca precoce, nuliparidade e terapias de reposição hormonal, além de desordens genéticas como as mutações BRCA 1 e 2 (SUN et al., 2017).

A classificação atual subdivide as NMM em carcinomas usuais "não especiais" (anteriomente classificados como ductais invasivos), que representam cerca de 85% do total e os "especiais": lobular, mucinoso, cribriforme, papilar entre outros (SINN, KREIPE, 2013). Após a confirmação histopatógica, a decisão sobre o tratamento inicial irá depender do estádio, perfil imunoistoquímico, idade, entre outros.

No passado, a mastectomia radical descrita inicialmente por Halsted (1894) era o tratamento cirúrgico inicial de escolha para o tratamento das NMM. Atualmente é um procedimento incomum, devido à alta morbidade e aos resultados bastante satisfatórios de técnicas menos invasivas (FRANCHESCINI et al., 2015). A cirurgia é utilizada como tratamento inicial em estádios iniciais, presença de indicadores de bom prognóstico e em pacientes com contra-indicação à quimioterapia (RIEDEL, HENNINGS, HUGIS, 2017). A opção pela cirurgia radical (mastectomia) ou conservadora (nodulectomia, setorectomia ou quadrantectomia) é complexa e envolve diversos fatores como perfil imunoistoquímico/genético e possibilidade de radioterapia adjuvante (SUNA et al., 2017). Deve, portanto, ser avaliada de maneira multidisciplinar.

Nos últimos anos a quimioterapia (QT) neoadjuvante tornou-se mais importante devido aos resultados favoráveis obtidos, apresentando vantagens interessantes como a possibilidade de realização de cirurgia conservadora em casos inicialmente avançados (ASAOKA et al., 2020). A escolha por determinado tratamento de quimioterapia e/ou hormonioterapia é individualizada, dependente dos resultados observados na imunoistoquímica (KORDE et al., 2021).

Pacientes com indicação de hormonioterapia (HT) recebem, comumente, tratamento com antagonistas dos receptores de estrogênio ou inibidores da aromatase. Os quimioterápicos mais utilizados são a doxorrubicina, ciclofosfamida e paclitaxel. Além dos tratamentos hormonal e quimioterápico, pacientes com superexpressão do receptor tipo 2 do fator de crescimento epidérmico (ErbB2 ou HER 2) podem receber terapias com anticorpo monoclonal humanizado recombinante ou, ainda, terapias alvo específicas (WANG, SU, 2019).

A radioterapia (RDT) como modalidade de tratamento para NMM pode ser utilizada tanto com intuito curativo como em situações de doença metastática, por exemplo para alívio de sintomas (DARBY et al., 2011; NAKAMURA et al., 2018). Na situação de adjuvância, o tratamento é sequencial aos anteriormente citados e envolve um planejamento técnico minucioso antes do início das sessões. Após consulta inicial e avaliação da indicação de radioterapia pelo rádio-oncologista, inicia-se a fase de preparação para o tratamento.

Durante a simulação pré-tratamento, as pacientes são posicionadas com utilização de acessórios como o vacfix, rampas de mama e/ou outros métodos de imobilização, a critério da equipe, conforme Fig 1. (KALYANI RADIOTHERAPY SPECIALTY INDIA, 2021) e Fig 2. (XIANG, et al., 2018).

Figura 1. Rampa com inclinação e apoio para os braços e pescoço utilizada para imobilização, simulação e tratamento de pacientes tratados com radioterapia (KALYANI RADIOTHERAPY SPECIALTY INDIA, 2021).

Figura 2. Posicionamento de paciente do sexo feminino com os braços elevados e pescoço virado para o lado oposto ao tratado, na rampa de tratamento. Utlização de máscara termoplástica para melhora da reprodutibilidade. (XIANG, et al., 2018).

Marcadores rádio-opacos podem ser utilizados em casos selecionados como pontos de referência externos na aquisição da tomografia computadorizada para facilitar o delineamento da mama. Clipes cirúrgicos podem servir de referência para o delineamento das áreas de maior risco, conforme observado na Fig. 3 (SUNG et al., 2016).

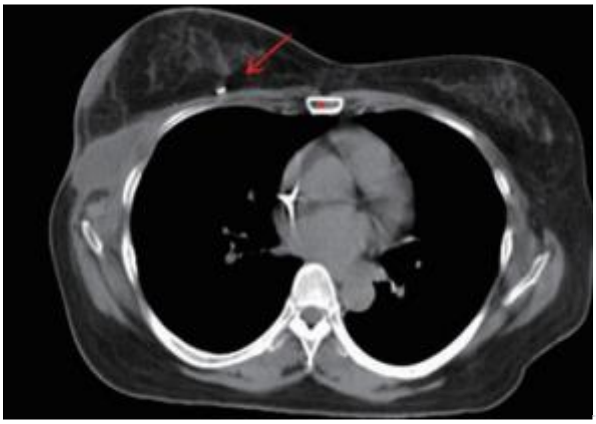

Figura 3. A seta vermelha demonstra a região do clipe cirúrgico (SUNG et al., 2016).

Para delineamento das áreas de tratamento e dos orgãos de risco que precisam ser protegidos (coração, pulmões, tireróide, entre outros), referências como Radiation Therapy Oncology Group (RTOG) (GEE et al, 2019) e European

Organisation for Research and Treatment of Cancer (EORTC) (SHAITELMAN et al, 2015) são habitualmente utilizadas. A Fig. 4 (HAUSSMANN et al., 2020) demonstra a dose de radiação mais elevada nas regiões cervical, axilar e mama esquerda (locais de tratamento) e menor dose em pulmão esquerdo e coração (orgaõs a serem protegidos).

Dois esquemas distintos de dose têm sido mais utilizados na radioterapia adjuvante: fracionamento convencional (FC), com 180 a 200 cGy (centiGreys, *1 Gy= 1J/kg) em 25 a 28 fracões diárias ou hipofracionamento (HF) com 265 a 267cGy em 15 sessões ou 16 frações diárias, 5 vezes por semana. Doses de reforço no local de maior risco para recidiva local (*boost*) podem ser feitas nos mesmos esquemas anteriomente citados nos casos de cirurgia conservadora ou carcinoma inflamatório (acrescentando-se 3 a 5 frações) (FREITAS et al., 2018).

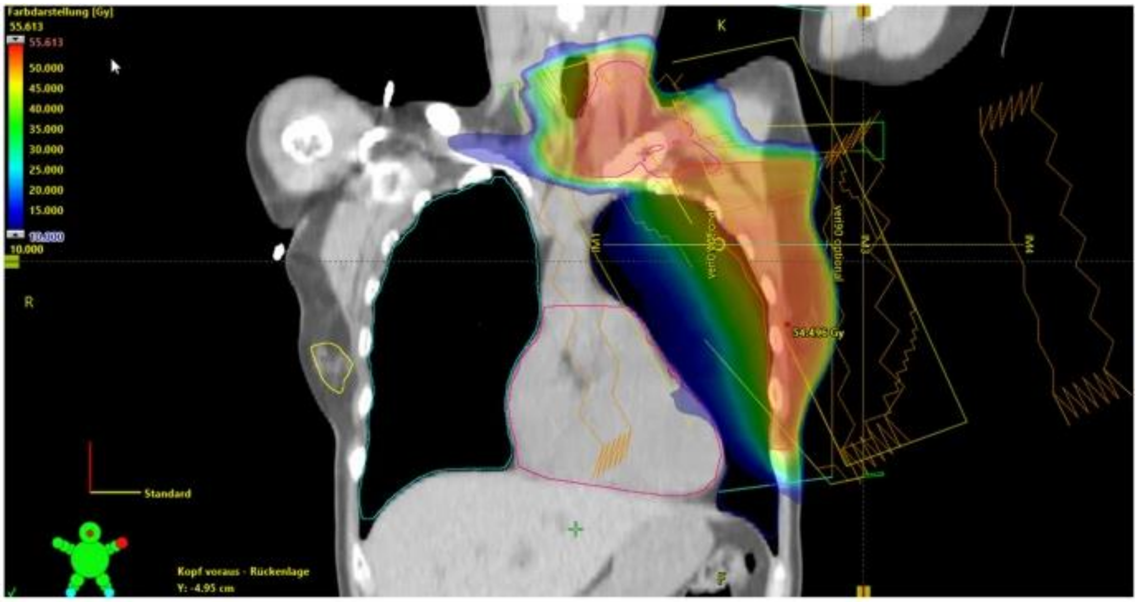

Figura 4: Plano de tratamento para paciente do sexo feminino submetida a radioterapia em mama esquerda e região nodal com técnica “ sliding window” em 4 campos. A dose de prescrição foi de 50,4 Gy em 28 frações. (HAUSSMANN et al., 2020).

Avaliações de histogramas dose-volumes (DVHs), restrições e prescrições de doses podem ser baseadas no protocolo RTOG 1005 (ARBOR, FREEDMAN, ARTHUR, 2013). As coberturas mínimas aceitas são de D90 > 90% (90% do volume alvo deve receber dose superior a 90% da dose de prescrição) e a porcentagem máxima de dose pontual estabelecida em 112% nos casos FC e de 120 % com o HF.

Diferentes técnicas de planejamento podem ser utilizadas para o planejamento e isso pode proporcionar diferentes distribuções de doses, conforme observado por Hu e cols (2020) na Fig 5. Técnicas mais avançadas como o *volumetric modulated arc therapy* (VMAT) proporcionam melhor conformidade e homogeneidade da dose. No dia de início do tratamento e , pelo menos, semanalmente, radiografias ortogonais são realizadas para conferência de posicionamento. Correções são realizadas de acordo com o protocolo estabelecido pelo próprio serviço.

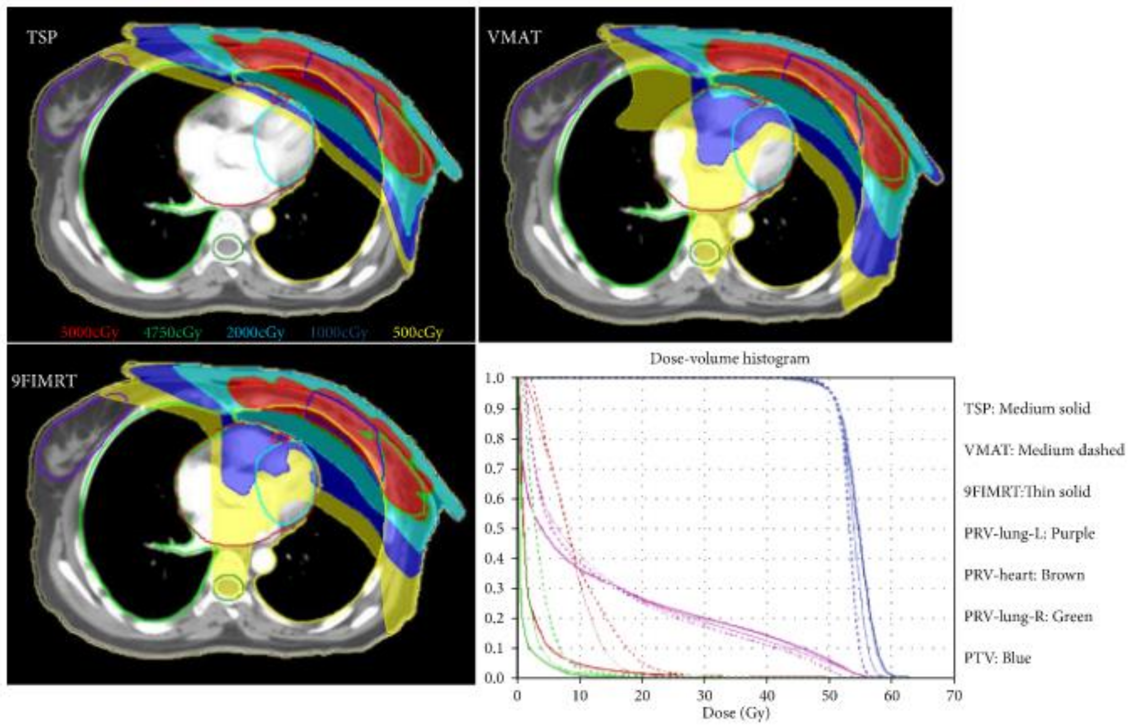

Figura 5. Distribuição das isodoses e DVH demonstrando diferenças entre as técnicas avaliadas (HU, J et al., 2020).

O objetivo do tratamento radioterápico é a eliminação de células tumorais por meio, principalmente, do dano ao DNA (HALL, GIACCIA, 2019). A ação de radicais livres (principalmente o radical hidroxila, ·OH) provenientes da interação de elétrons livres (secundários ao efeito Compton e fotoelétrico) com as moléculas de água, causa dano indireto ao DNA tumoral, sendo este o principal mecanismo de morte celular (SOTO et al, 2015). Estudos demonstraram que a radiação aumenta os níveis de estresse oxidativo e de lesão tumoral de maneira ampla, gerando também repercurssões à distância: efeito abscopal (efeitos que ocorrem distantes ao local irradiado) e *bystander* (efeitos que ocorrem adjacente ao local irradiado), por meio da disfunção de mecanismos celulares como ativação de fatores de transcrição, expressão de genes, metabolismo oxidativo e respostas inflamatórias (AZZAM, JAY-GERIN, PAIN, 2012; MEHER et al., 2018).

O status redox tem importância diagnóstica e prognóstica na terapia oncológica, onde observa-se baixos níveis de antioxidantes e altos níveis de estresse oxidativo em pacientes portadores de neoplasias malignas (SU et al., 2015). Níveis elevados de ERO (espécies reativas de oxigênio) estão também relacionados com a progressão tumoral (SIEFRIED et al., 2007). Na tentativa de manter o equilíbrio entre a formação e a degradação/neutralização de ERO, o sistema antioxidante (glutationa, superóxido dismutase (SOD), catalase (CAT), vitamina C, vitamina E, uréia, albumina) atua por diversos mecanismos para evitar o estresse oxidativo. Entretanto, estudos demonstram a quebra desta homeostase tanto pela neoplasia como por tratamentos (KHANZODE et al, 2004, TAS et al., 2005).

Pacientes submetidas ao tratamento radioterápico por NMM podem desenvolver efeitos colaterais como reações cutâneas, fadiga, adnamia, e odinofagia. (VELIKOVA et al., 2018; TARRASCH el al., 2018). A reação inflamatória cutânea (radiodermite) é o principal paraefeito, presente em mais de 95% das pacientes ao longo do tratamento (RYAN et al.,2012).

A radiodermite (RDM) é o processo inflamatório/reacional que ocorre na pele e tecido subcutâneo submetidos à radiação ionizante (JOLIEN, LAUBACH, 2018). A fase aguda, que se inicia algumas horas após a sessão e se estende por até 90 dias, pode provocar desconforto de maneira significativa (HYMES, STROM, FIPE, 2006). Com a continuidade do tratamento, o grau de radiodermite, assim como os sintomas locais, tendem a se intensificarem, o que pode levar a interrupção temporária do tratamento tanto pelo desconforto exacerbado como por complicações locais (infecção, entre outras). De acordo com a classificação do RTOG (COX, STETZ, PAJAK, 1995), os graus de radiodermite variam de 0 (ausência de alterações na pele) até 4 (efeitos como ulceração hemorrágica ou necrose). Observa-se na Fig. 6 (LEVENTHAL, YOUNG, 2015) área marcada com triângulo (A) apresentando radiodermite grau 0 (ausência de alterações significativas). As áreas com asterisco (em A, B e C) são classificadas como grau 1, onde ocorrem eritema, ressecamento e descamação seca. A seta (B e C) indica áreas com reações mais intensas como eritema mais brilhoso e descamação úmida em local de dobra cutânea, classificadas como radiodermite grau 2

O processo inflamatório agudo observado durante as sessões de radioterapia pode provocar aumento da temperatura local, dor, prurido, parestesias ou até infecção. O aumento da temperatura da mama irradiada em relação à mama não irradiada está relacionada ao aumento do risco de RDM grau 2 ou de grau superior (MAILLOT, LEDUC, ATALLAH, 2018).

Figura 6. A, B e C demonstram diferentes reações cutâneas em uma paciente que recebeu radioterapia com 50 Gy. Eritema com descamação seca (A) ocorrem na radiodermite grau 1, assinalados com *. Reações mais intensas ocorreram em axila (B) e sulco infra mamário(C). As setas indicam as áreas com descamação úmida classificadas como radiodermite grau 2. (LEVENTHAL, YOUNG, 2015.)

Existem poucas informações sobre a possível influência do status redox em efeitos colaterais como a RDM. Maior compreensão dessas informações poderiam contribuir para que intervenções (como por exemplo o uso de antioxidantes) buscando equilibrar o excesso de ROS pudessem ser realizadas, a fim de reduzir a intensidade desses paraefeitos, evitando-se interrupções, as quais podem impactar negativamente no resultado do tratamento.

Esse estudo, portanto, busca compreender por meio de análise de marcadores relacionados ao estresse oxidativo se níveis mais elevados de ROS ou outras variáveis como o fenotipo cutâneo estariam relacionados a maior intensidade de efeitos colaterais como a RDM.

2 OBJETIVO GERAL

Caracterizar a radiodermite e o estresse oxidativo em pacientes submetidas à radioterapia para tratamento de neoplasia maligna da mama.

2.1 Objetivos Específicos

- Avaliar a capacidade antioxidante enzimática e marcardores estresse oxidativo no sangue de pacientes em 3 períodos durante o tratamento com radioterapia.
- Caracterizar o perfil da população do estudo.
- Relacionar os diferentes graus de radiodermite com as variáveis analisadas.
- Comparar os esquemas de fracionamento convencional com o hipofracionado em relação ao estresse oxidativo e graus de radiodermite.

3 ARTIGO PARA PUBLICAÇÃO

O presente trabalho originou um artigo científico que será incluído nesta Dissertação como Anexo. Todas as análises experimentais contidas foram realizadas na Universidade Estadual de Londrina, nos laboratórios de Patologia Experimental. O trabalho será submetido para publicação em revista científica “Pathology & Oncology Research”.

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ANEXO: Radiodermitis and oxidative stress in breast cancer

RADIODERMITIS AND OXIDATIVE STRESS IN BREAST CANCER

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ABSTRACT

Introduction and objective: Radiotherapy is used as an adjuvant treatment for breast cancer, being radiodermatitis the main acute side effect. Oxidative stress is present as well as in the course of the disease as part of the treatment. The aim of this study was to characterize the degrees of radiodermatitis and the patterns of oxidative stress presented by patients with breast cancer undergoing conventional fractionation (CF) and hypofracionated (HF) adjuvant radiotherapy treatment. **Methods:** The participants (n=50) answered a demographic questionnaire and underwent clinical examination and blood collection for analysis of oxidative stress markers. Blood collection were made in three distinct phases of the treatment: M1 (first day), M2 (half the sessions), M3 (ending radiotherapy day). Lipid peroxidation (CL) and malondialdehyde (MDA), antioxidant enzymes quantification of superoxide dismutase (SOD), catalase (CAT), and reduced (GSH) and oxidized glutathione (GSSG) were measured. A control group (n=30) from a database was used as normal reference. **Results**: SOD levels in the CF group were higher compared to the control. However, the all markers studied showed lower values when compared to control, except the MDA, which was higher before start, but decreased significantly during the course of the treatment. There was no increase in markers related to oxidative stress in the CF group during treatment. HF patients had lower intensity of discomfort at the irradiated site, as well as less fatigue and lower score of radiodermatitis than CF. **Conclusion**: The patients presented low levels of oxidative stress markers and were kept low during the course of the treatment. Hypofractionated treatment provide less clinical side effects than Conventional Fractionation.

Keywords: Radiotherapy, Breast cancer, Radiodermatitis, Oxidative stress.

Introduction

Malignant breast neoplasms (MBN) are the most common among women in Brazil and worldwide, after non-melanoma skin cancer, with a development risk of 12% throughout life [1].

Radiotherapy (RDT) is a treatment modality for MBN that can be used for both curative or in situations of metastatic disease purposes [2,3]. In the adjuvant situation, the treatment is sequential to chemotherapy or can starts with hormone therapy and involves a detailed technical planning before the beginning of the sessions.

Two distinct dose regimens have been most used in adjuvant radiotherapy: conventional fractionation (CF), with 180 to 200 cGy (centiGreys, *1 Gy= 1J/kg) for 25 to 28 daily fractions or hypofractionation (HF) with 265 to 267cGy for 15 sessions or 16 daily fractions, 5 times a week. Booster doses at the highest risk site for local recurrence can be performed using the same regimens mentioned above in cases of conservative surgery or inflammatory carcinoma (adding 3 to 5 fractions) [4].

Both the radiotherapy treatment and the disease itself can interfere with the production of ROS (reactive oxygen species) [5]. The redox status has diagnostic and prognostic importance in cancer therapy, where low levels of antioxidants and high levels of oxidative stress are observed in patients with malignant neoplasms [6]. High levels of ROS are also related to tumor progression [7]. In an attempt to maintain the balance between ROS formation and degradation/neutralization, the antioxidant system (glutathione, superoxide dismutase (SOD), catalase (CAT), vitamin C, vitamin E, urea, albumin) acts through several mechanisms to avoid oxidative stress [8,9].

Patients undergoing radiotherapy treatment by MBN may develop side effects such as skin reactions, fatigue, prostration, and odynophagy [10,11]. The cutaneous inflammatory reaction (radiodermatitis) is the main side effect, present in more than 95% of patients during treatment [12].

Radiodermatitis (RDM) is the inflammatory/reactional process that occurs in the skin and subcutaneous tissue subjected to ionizing radiation [13]. The acute phase, which starts a few hours after the session and extends for up to 90 days, can cause significant discomfort [14]. With continued treatment, the degree of radiodermatitis, as well as local symptoms, tend to intensify, which can lead to temporary interruption of treatment due to both exacerbated discomfort and complications such as local infection.

There is little information about the redox status on patients presenting side effects such as RDM. Better understanding of this scenario could contribute to the search for new strategies in order to improve the effects of radiodermatitis, based on the findings of oxidative stress.

Thus, the aim of this study is to evaluate the redox profile of patients diagnosed with breast cancer who undergo radiotherapy and who have different levels of radiodermatitis and skin discomfort during treatment.

Methods and Materials

Patients referred to the Oncology and Radiotherapy Center of Londrina with an indication for radiotherapy were invited to participate in the project. This study was approved by the Ethics in Research Committee involving human beings of the State University of Londrina (CEP/UEL), under register number CAAE: 16280119.9.0000.5231. The inclusion criteria were patients with malignant breast cancer confirmed by histopathological analysis with indication for radiotherapy treatment. Thus, participants attended a medical consultation for initial care were instructed that blood tests would be collected at the same time for blood counts, which is part of the usual protocol. All the invitations to participate in this study was made between 8/15/2019 and 7/30/2020. Fifty patients accepted the invitation were submitted to the socio-demographic questionnaire. Blood collections were previously scheduled to coincide with the daily routine of radiotherapy sessions. The blood samples were collected in M1 (the day of the start of treatment), M2 (when half of the total sessions had been performed) and M3 (at the end of the sessions). Physical examination and evaluation of clinical parameters, including radiodermatitis (according to Radiation Therapy Oncology Group - RTOG classification) were also performed in M2 and M3 by the physician, being considered the grade 0 for the M1 [15]. The intensity of fatigue (characterized by muscle weakness, indisposition) was assessed by asking the patient directly on a scale created by the examiner, which used values from 0 (absence of symptoms) to 10 (extremely intense symptoms). The level of discomfort due to pain (characterized by burning or burning sensation on the skin) was assessed by the VAS scale (Visual Analog Scale), which uses a scale from 0 (no symptoms) to 10 (extremely intense and continuous symptoms) in M2 and M3 [16].

Participants were routinely instructed not to receive solar radiation directly at the irradiated site during the treatment, not to wear a bra or tight clothing in the breast region, not to use topical drugs or any non-specific products for radiotherapy in the irradiated site, as well as carefully remove such products before treatment sessions. They were also instructed to avoid exposure to heat from ovens, stoves or during the bath and to use compresses with cold chamomile tea after the sessions.

The control group consisted of 31 female patients, who were not known to have chronic diseases or malignant neoplasms, from a database of the Molecular Pathology Laboratory of the State University of Londrina.

Blood Samples

Blood was obtained by venipuncture and collected in heparin tubes to obtain plasma and erythrocytes and in a dry tube to obtain serum. Plasma and blood serum samples were centrifuged, separated and stored at -20ºC. The erythrocytes were washed three times with 1mL of 0.9% sodium chloride and centrifuged three times for 5min at 4 °C before storage in Alsever buffer at 4 °C, for a maximum of two weeks after collection.

Oxidative Stress Parameters

Lipid peroxidation was evaluated by chemiluminescence (CL) induced by t-butyl hydroperoxide [17]. Erythrocytes were diluted in ice-cold 10 mM monobasic phosphate buffer, pH 7.4 (0.9% NaCl). The chemiluminescent reaction was initiated by the addition of tert-butyl and curves were obtained on a Lumat3 LB9508 luminometer (Berthold Technologies GmbH & Co.KG).

Malondialdehyde (MDA) analysis was determined in blood plasma by high performance liquid chromatography (HPLC) on an LC-20AT® HPLC system (Shimadzu, Kyoto, Japan), as described by Victorino et al (2013). Readings were taken for 11 min (535nm), at a flow rate of 0.8mL/min at 35°C; results were expressed in nM of MDA.

The activity of the antioxidant enzyme CAT was determined as described by Aebi (1984) and modified by Panis et al., in 2012 [18]. This technique is based on the decomposition of hydrogen peroxide by catalase, directly related to its absorption at 240nm. Absorbance disappearance kinetics were monitored by UV-1650 PC® UV-vis spectrophotometer (Shimadzu, Kyoto, Japan). The results were expressed as absorbance decay rate per mg of protein.

The activity of the SOD enzyme in was determined at 420nm according to the method of Marklund and Marklund (1974) [19]. This test is based on the inhibition of pyrogallol auto-oxidation in aqueous solution. SOD inhibits the auto-oxidation of pyrogallol by catalyzing the dismutation of the superoxide anion to hydrogen peroxide. This oxidation is accompanied by the appearance of a yellow color in the reaction medium, monitored at 420nm for 5 minutes. The amount of SOD capable of inhibiting by 50% the oxidation of pyrogallol is defined as a unit of enzymatic activity (U). Final results were expressed in U per gram of protein.

Treatment Regimens

Patients were treated with the IMRT (intensity modulated radiotherapy) forward technique with opposing tangent fields. The CF group received a dose of 5040 cGy in 28 daily fractions of 180 cGy and HF group received 4005 cGy in 15 daily fractions of 267 cGy (both five times per week). Boost was performed for patients with conservative surgery and under 70 years of age in the previously cited regimens.

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 6.0 (GraphPad Software, 8 San Diego, CA), Microsoft Office Excel 2007 and

OriginLab software 8.0. The normality test used was the D'Agostin & Pearson. For parametric data, (α=0.05), the analysis of variance was One-Way ANOVA, with Tukey test as post-hoc, 11 multiple comparisons, and the results were expressed as mean ± standard error of the mean (SEM). For non-parametric data, (α=0.1), the analysis of variance was Kruskal-Wallis, with Dunn's test as post-hoc, multiple comparisons, and the results were expressed as minimum and maximum in box-plot. For the lipoperoxidation curves, the Two-Way ANOVA was used as analysis of variance, with Tukey's test as post-hoc and the results were expressed with only the mean. Differences were considered statistically significant when p value <0.05.

Results

Table 1. shows the profile of the studied groups. Fifty patients who completed the proposed treatment and who underwent 3 blood collections were evaluated; 26 patients had their collections interrupted due to the pandemic and were not analyzed. Of the patients included in the study, 41 (82%) received treatment with CF and 9 (18%) HF. The mean age of participants submitted to CF (52.8 ± 10.8) was lower compared to the group that received HF treatment (70.1 ± 10.4; t: 4.39, p<0.01) and to the control (61.4±7.7, p<0.01).

First-degree familial history for NMM was observed in 20% of the total number of patients treated and only 4% of patients continued to smoke during treatment. All patients were instructed to quit the smoking habit not only because of the known harms, but also because of the reduction in the oxygen effect in the treatment. Six patients were former smokers.

Patients were classified in stages from 0 to 3 according to the latest edition of the TNM (2017). The distribution of patients who received treatment between stages 0 to 3 was: 16%, 42%, 26% and 16%, respectively. In the CF group, 80% of the patients belonged to the initial stages (0 to 2) while in the HF group, this was observed in 100% of the patients.

Hormonal therapy was performed in 70% (35/50) of patients and approximately 50% received chemotherapy before radiotherapy. In the CF group, 56% of patients received chemotherapy vs 33% in the HF group. However, hormonal therapy was more used in the HF group (100% vs 61%). The performance of chemotherapy was related to the presence of more advanced stages and use hormonal treatment to less advanced stages.

The Fitzpatrick classification was used to assess skin phototypes. The scale varies gradually from 1 (white skin, very sensitive to the sun) to 6 (black skin, insensitive to the sun). Classification 2 was observed in 60% of patients in the CF group vs 78% in the HF group.

The tumor location in the left upper lateral (outer) quadrant was more present in both groups: 29% in CF vs 44% in HF and 21% of patients in the CF group had neoplasms located in the medial (internal) quadrants. In the HF group, this percentage is 11%.

Regarding the immunohistochemical profile, luminals A and B were observed, respectively, in 24% and 39% of patients in the CF group and in 33% for each luminal in the HF group ($p<0.7$). These results are used to define cancer therapy. Patients with absence of hormone receptors and HER 2 overexpression (triple negative or “basal like”) have a worse prognosis and accounted for 21% in the CF group and 11% in the HF group ($p<0.05$).

Table 1. Demographic and oncological data of the studied population

	CF (n=41)	**HF (n=9)**	**Control(n=30)**
Age (average/median)	52/51*	71/75	61/61
BMI (average/median)	25	25	25/26
Family History (first grade)	7	1	-
Smoking	1	1	2
Hormone therapy	26	9	-
Chemotherapy	23	3	-
Stage			
0	7	1	
I	16	5	-
II	10	3	-
III	8	0	-
Cutaneous phenotype (Fitzpatrick)			
1	0	1	2
2	25	7	14
3	15	1	11
4	1	0	3

Location			
Left breast	23	5	-
Upper Left Outer Quadrant	12	4	-
Right breast	18	4	-
Immunohistochemistry			
Luminal A	10	3	-
Luminal B	16	3	-
Luminal Hybrid	3	1	-
HER 2	3	1	-
Basal like	9	1	-
Histology/Carcinoma			
" In situ"	5	1	-
Non-special Invasive	33	8	-
Lobular	2	0	-
Papillary	1	0	-
Degree of differentiation			
Grade 1	3	1	-
Grade 2	29	8	-
Grade 3	9	0	-
Surgery			
Conservative	32	9	-
Mastectomy	9	0	-

CF: Conventional fractionation; HF: Hypofractionated; *p<0.01: age difference between HR and HF and control groups

Non-special invasive carcinoma of the breast was present in 82% of the biopsy samples of the study patients (80% in CF and 88% in the HF group). The degree of cell differentiation was analyzed by the tumor degree, which ranged from 1 to 3, with degree 2 being the most prevalent in both groups (70% and 88% for the CF and HF groups). All patients in the HF group underwent conservative surgery, while in the CF group, mastectomy occurred in 21% of cases.

The intensity of discomfort and fatigue at times M2 and M3 are illustrated in Figures 1 and 2. Patients treated with a hypofractionated regimen had lower intensity of discomfort at the irradiated site compared to the CF group (M2: 0.89 vs 2.57 and M3: 2.55 vs 4.04; $p<0.01$), with values expressed as mean. Regarding fatigue, there was also lower intensity in the HF group (M2: 0.66 vs 2 and M3: 2.1 vs 4.82; $p<0.01$).

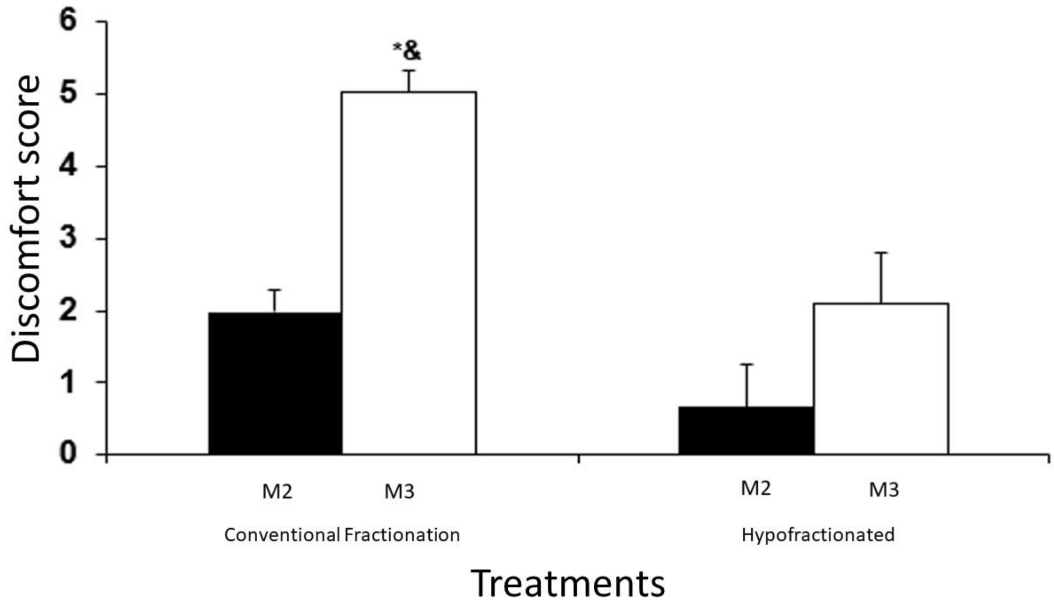

Figure 1. Discomfort intensity in the CF (conventional fractionation; n=41) and hypofractionated treatment (HF; n=9) groups at times M2 and M3. *: difference between M2 and M3 p<0.001; &: difference between groups p<0.001.

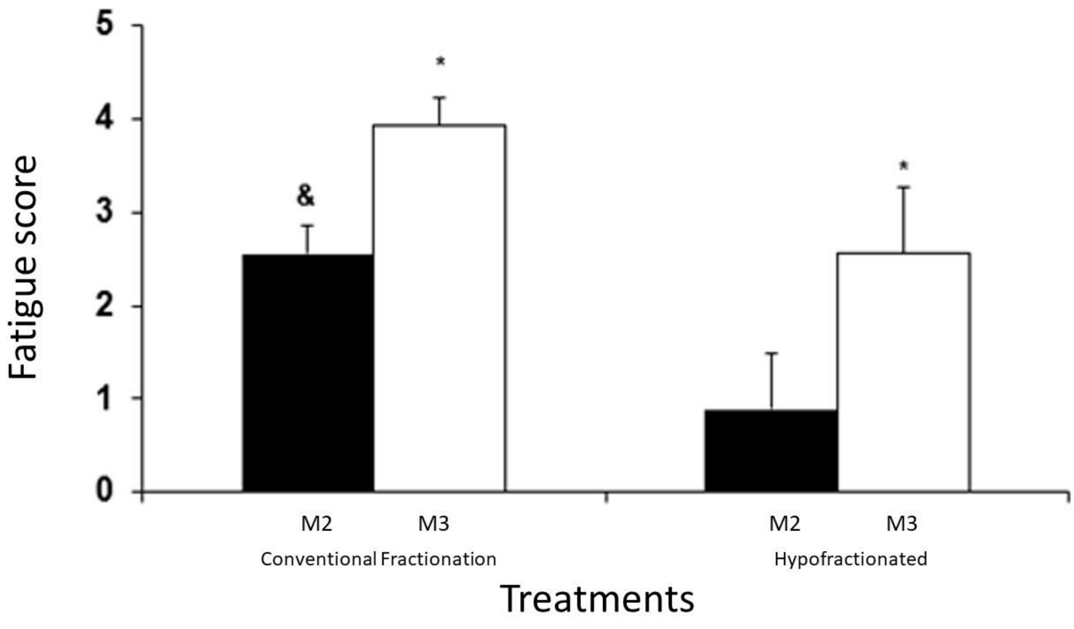

Figure 2. Intensity of fatigue in groups CF (conventional fractionation; n=41) and HF (hypofractionated treatment; n=9) at times M2 and M3. *: difference between M2 and M3 p<0.001; &: difference between groups p<0.05.

The incidence of more advanced radiodermatitis was higher in the group of patients who received conventional treatment, as shown in Table 2. Almost half (44%) of the patients in the HF did not have radiodermatitis throughout the treatment (grade 0) and no patient had a reaction advanced acute cutaneous (grades 3 or 4). Grade 2 radiodermatitis were higher in the CF group (53.5% vs 11%, p<0.01).

Table 2. Degree of Radiodermatitis according to the RTOG Scale

	Grade 0	Grade 1	Grade 2	Grade 3	N
Conventional fractionation(CF) Fitzpatrick	0	16(39%)	22(53.5%)	7(3%)	41
2	-	10	14	1	25
3	-	5	8	2	15
4	-	1	-	-	1
Hypofractionation (HF) Fitzpatrick	4(44.5%)	3(33%)	1(11%)	-	9
1	1	-	-	-	1
2	3	2	2	-	7
3	-	1	-	-	1

The values obtained from markers related to oxidative stress can be seen in Figure 3. GSH levels were lower in the CF group compared to the control (M1: 540.58±26.52, M2: 579.49±28.27, M3: 563.62±34,13 vs 688.02±35.91; p<0.05), but there was no variation throughout the treatment. For the GSSG the values were lower in the CF group compared to the control (M1: 97.67±11.27, M2: 109.17±7.80, M3: 105.43±7.77 vs 401.19±67.32; p<0.05). Again, a non-significant variation of values was observed throughout the treatment. A similar situation was observed in the GSSG/GSH ratio and in the CAT activity (M1: 14.21±4.47, M2: 13.19±2.49, M3: 13.69±3.58 vs 34.3±6.4; p<0.01).

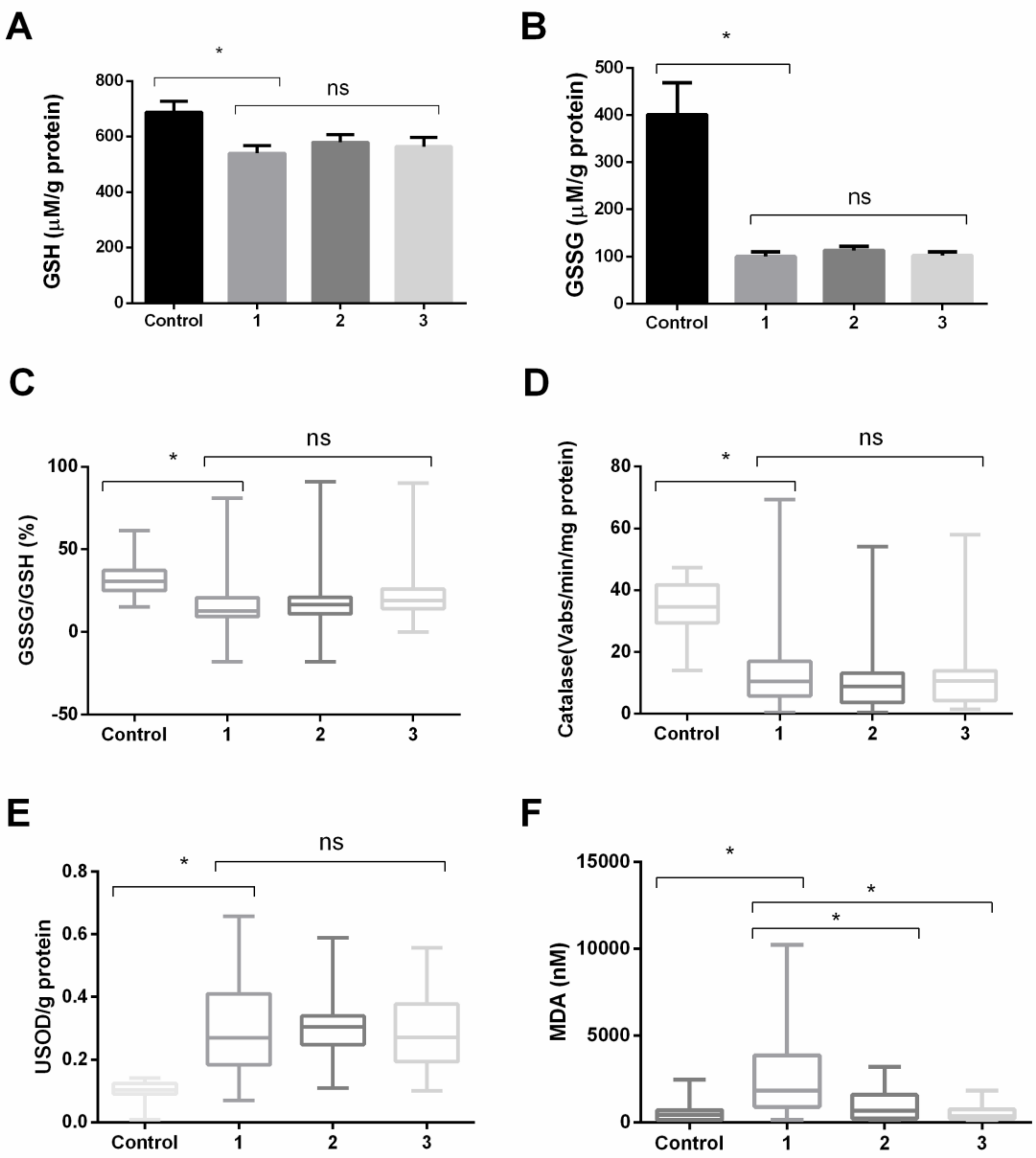

Figure 3. Values obtained from markers related to oxidative stress in the control group and in the group that received conventional fractionation (FC) before the beginning (1), in the middle (2) and at the end (3) of the radiation treatment. A, B, C, D, E and F express the values of GSH, GSSH, GSSH/GSH, CAT, SOD and MDA, respectively. (*p<0.05).

Unlike what was observed in the previously described markers, SOD activity was significantly higher in the CF group compared to the control (M1: 0.26±0.02, M2: 0.29±0.01, M3: 0.26±0.01 vs 0.12±0.02; p<0.05). There was no significant variation over the course of treatment.

High MDA values were observed in the CF group compared to the control. This difference was greater in M1 (2311.21±682.20 vs 506.14±161.43; p<0.01). There was variation in MDA levels throughout the treatment, to values more similar to those observed in the control group.

Finally, the lipid peroxidation (CL) levels were evaluated by the ascending velocity of the luminescence curve, peak and area over the curve, as observed in Figure 4. These levels were lower in the CF group compared to the control when evaluated by the peak and area over the curve. curve. There was no difference in speed between the groups (Control: 112.03±32.22 vs CF: M1: 62.09±22.46, M2: 62.96±9.54 M3: 60.63±13, 43; p: 0.156) or significant change in the values in M1, M2 or M3.

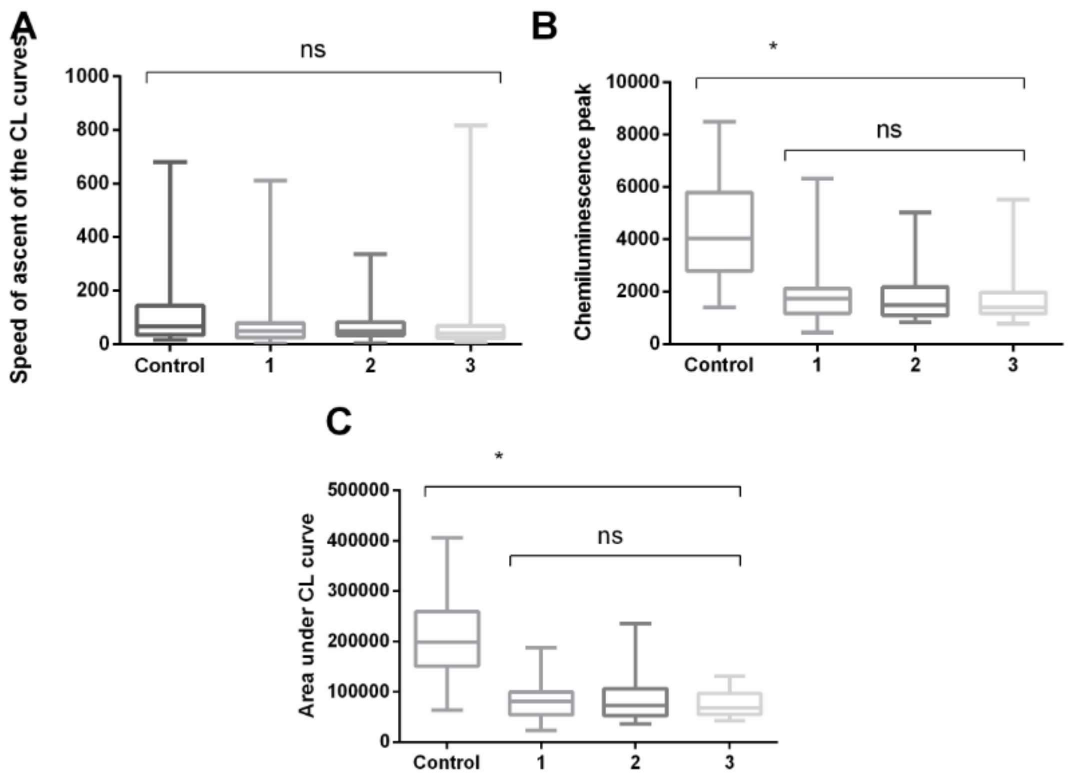

Figure 4. Evaluation of membrane peroxidation by means of the chemiluminescence ascending curve velocity (A), peak; (B) and area under the curve (C) of the studied groups compared to the control group (Control) and the group that received conventional fractionation (FC) before the beginning (1), in the middle (2) and at the end(3) of radiation treatment.

Table 3. shows the values of markers related to oxidative stress in the control, FC and HF groups. No significant differences were observed between these values in the CF and HF groups, except for SOD in M3 (0.26 vs 0.43) p < 0.05.

Table 3. Markers related to oxidative stress

	Control(n=31)	CF (n=41)	HF (n=9)	p value
		Mean/Standard Deviation		
QL (lipoperoxidation by chemiluminescence)				
M 1	112.03/32.22	62.9/22.46	60.32/18.47	0.740
M 2		62.96/9.54	48.32/13.53	0.569
M 3		60.63/13.43	63.01/8.49	0.306
Catalase (CAT)				
M 1	34.3/6.41	14.21/4.47	15.21/2.90	0.926
M 2		13.49/1.49	11.93/0.64	0.321
M 3		13.23/3.58	13.02/1.04	0.596
Superoxide Dismutase (SOD)				
M 1	0.12/0.02	0.28/0.02	0.31/0.07	0.293
M 2		0.29/0.01	0.27/0.04	0.416
M 3		0.26/0.01	0.43/0.06	**0.012**
Malondialdehyde (MDA)				
M 1	506.14/161.43	2311.21/682.20	2398.14/329.99	0.464
M 2		979.61/313.77	695.55/58.83	0.989
M 3		651.40/261.57	1014.42/66.94	0.481
Reduced glutathione (GSH)				
M 1	688.02/35.91	540.58/26,52	492.60/50,42	0.661
M 2		579.49/28,27	567.07/61,82	0.708
M 3		563.62/34,13	649.14/109.02	0.282
Oxidized glutathione (GSSG)				
M 1	401.19/67.32	97.67/11.27	98.95/20.03	0.596
M 2		109.17/7.80	91.72/14,79	0.102
M 3		105.43/7.77	112.93/19.17	0.388
Ratio GSSG/GSH				
M 1	0.31/0.04	0.19/0.03	0.23/0.02	0.244
M 2		0.21/0.06	0.15/0.03	0.163
M 3		0.22/0.05	0.21/0.02	0.805

Discussion

This study aimed to evaluate markers related to oxidative stress at the beginning, in the middle of the sessions and at the end of treatment in patients undergoing adjuvant radiotherapy for breast malignancy (NMM), as well as changes in the skin and in the patients' well-being during the treatment. Based on these data, we sought to analyze the variations of these markers at different stages of treatment in an attempt to characterize the changes that occurred both systemically and through the clinical analysis of possible complications in the face of CF or HF treatment.

The mean age of 51 years in the CF group was lower than that observed in North American data (62 years) [20] and in Brazil (56 years) [21]. However, the mean age in the HF group was higher (75 years). This difference can be explained by the selection of more advanced ages for the HF group. This will be discussed below.

In this study, the percentage of luminal A, luminal B, triple negative and HER2-enriched was 26, 46, 20 and 8%. Similarly, Simona et al., 2017 observed for these subgroups percentage of 30 to 40%, 20 a 30%, 15 to 20% and 12 to 20%, respectively [22]. According to the American Cancer Society, luminal A accounts for 73% of breast cancer cases, luminal B for 11%, triple negative for 12% and HER2-enriched for about 4%. Higher percentage of luminal B patients could be explained by the lower mean age presented in the study.

Most treated patients were in early stages (84%) of breast cancer progression. Most also had light skin tone (96%) (Fitzpatrick 2 and 3). This could be explained by the fact that the service only offers private treatment, which differs from the profile of patients treated by the public service. According to INCA data from 2019, only 66% of patients in the public network were diagnosed in early stages (I and II) and IBGE data from 2018 reported that 34% of the population of Paraná (Brazil) is made up of blacks or browns. The little variation observed in the cutaneous phenotypes did not allow this variable to be correlated with the degree of radiodermatitis.

The left breast was the most affected in both the CF and HF groups (56% for both). Despite the greater technical difficulty in protecting the cardiac area in left breast neoplasms, the percentage of neoplasms in the medial quadrants was only 22% in the CF group and 11% in the HF group. According to Kroman et al., (2013) [23], patients with tumors in different locations in the upper lateral quadrant have a lower survival rate.

This study included only patients who received adjuvant radiotherapy with curative intent. Patients with advanced-stage neoplasms were initially treated with neoadjuvant chemotherapy and then underwent surgery. In cases of initial disease, surgery was the treatment initially used. After completion of the last treatment (surgical or chemotherapy), radiotherapy was started within 60 days. According to Flores-Balcázar (2018) [24], beginnings beyond this period could negatively impact disease-specific survival.

Although more advanced treatment techniques such as VMAT (volumetric modulated arc therapy) have provided a reduction in the intensity of toxicity, radiodermatitis (RDM) can still negatively interfere in the course of radiotherapy, both due to discomfort/pain and the occurrence of complications as an infection, which can lead to temporary interruption and reduced effectiveness of the treatment [25,26].

Radiotherapy treatment using the CF method was performed in 82% of the patients in the study. In this group, markers related to oxidative stress remained stable during treatment at times M1, M2 and M3, with the exception of MDA, which showed decreasing values. This stability may suggest that daily doses of 180 to 200 cGy/day in a restricted area of the body are not capable of causing significant changes in systemic redox status. Compensatory mechanisms seem to work effectively in neutralizing ROS until the end of treatment in this group, since enzymatic and non-enzymatic antioxidant patterns remain unaltered.

The age of the patients is a factor that could influence the response mechanisms to oxidative stress [27], suggesting a lower redox homestasis capacity at older ages, greater than 60. The patients in this study who underwent treatment with HR had a mean age of 52 years and the mean in the control group was 61 years. Therefore, this factor would have little influence on the results.

Studies that evaluated markers of oxidative stress in patients undergoing radiotherapy showed no significant changes in MDA levels by the TRABS technique in the middle or at the end of treatment in relation to the beginning of the same in both the CF and HF groups [28]. Khoshbin et al., (2015) [29] used conventional fractionation and also did not observe differences in these levels at the end of radiotherapy performed after chemotherapy. In our analysis, we observed very high initial MDA values in relation to the control group, in accordance with data recently described by Youssef (2019) [30], suggesting a possible interference of the surgical and chemotherapy treatments used previously. It is known that chemotherapy can modulate inflammatory and oxidative parameters [31]; therefore, it is reasonable to accept that, depending on the patient's previous treatment, the redox and inflammatory status may be altered. Therefore, it was observed that throughout the radiotherapy treatment, the MDA levels were decreasing, which reinforces this hypothesis.

Initially high MDA levels were also observed by Arjmandi et al., (2015) [32] in a study that used radiotherapy with conventional fractionation (180 to 200cGy/day). They also observed the maintenance of CAT levels throughout treatment. In our study, we corroborate both results.

The systemic values of GSH, GSSG were lower in the CF group compared to the control, as oxidative stress secondary to previous treatments and the disease itself lead to GSH consumption. This was also observed in other neoplasms such as thyroid cancer [33].

Lower levels of antioxidants such as GSH can lead to consequences such as increased mutation rate, tumor progression, increased angiogenesis, activation of tumor growth promoters and therapeutic resistance by adaptation to oxidative stress [34]. This reduction in the antioxidant system and the consequent increase in ROS observed in CF compared to the control group could interfere with the intensity of side effects presented by patients undergoing radiotherapy.

SOD levels were higher in CF compared to control, suggesting an attempt to metabolize ROS as superoxide anions and hydrogen peroxides. This enzyme is, therefore, part of the antioxidant defense system and seems to continue to act for a long time during the entire radiotherapy [35].

The restricted number of participants in the HF may have interfered in the evaluation of the data obtained. Despite this fact, there was a different behavior of the levels of these markers in the HF group in relation to the CF. After a fall in M2 of CL CAT, SOD and MDA, in M3 or values they started to rise, suggesting an increase in oxidative stress in this group at the end of treatment. Although these results are merely speculative due to the reduced number of participants which impact in the statistical treatment, it opens up a new possibility of investigation, since the treatment with HF will be more and more frequent. Clinical findings indicate that less advanced levels of radiodermatitis were observed in patients who received HF treatment, in addition to cost/effectiveness and logistical advantages, even more important in the current pandemic scenario. These results were also observed in other studies such as START B [36], which showed lower rates of acute skin reaction (HR vs HF: 1.2 vs 0.3%), as well as no difference in appearance of the breast at 2 and 5 years after treatment. Lesser intensity of fatigue and discomfort were also reported in the hypofractionated group, as demonstrated in other studies [37,38].

The main studies that evaluated the efficacy and safety of hypofractionated treatment included patients over the age of 50 years, diseases at earlier stages and better prognostic factors [39,40,41]. Therefore, patients undergoing hypofractionated treatment in this study had more advanced mean/median age, only conservative surgeries, less use of chemotherapy and more use of hormonal treatments. They were, therefore, numerically less expressive, since HF was indicated only for a select group of patients. However, this scenario has been changing more quickly, as there are advantages to using HF radiotherapy, with results similar to CF radiotherapy.

Despite the evolution of linear accelerators, radiodermatitis is still the main side effect in the treatment of NMM with radiotherapy and, therefore, remains a major challenge during the course of treatment. Although HF is being used more and more due to the more mature results recently presented and the demonstration of non-inferiority, conventional fractionation is still commonly used, for example, in situations with breast implants [42].

This study characterizes the patients who are referred to a private health service to continue the treatment of breast cancer, through the use of radiotherapy. The oxidative stress indexes measured through membrane oxidation products, as well as the enzymatic and non-enzymatic antioxidants were different from the control group, however without difference during the course of the treatment. The relationship between radiodermatitis presented in patients and oxidative stress cannot be established, with the need to increase the number of participants and the inclusion of a group with active cancer, prior to surgical removal/chemotherapy for comparison.

Previous treatments such as surgery/ chemotherapy, radiation doses used, among others, may be determining factors in the development of radiodermatitis. More studies are needed to elucidate the true role of each of these variables to establish a better response to radiotherapy and thus improve the quality of life of patients undergoing this treatment.

Conclusion

The mechanisms involved in oxidative stress homeostasis are complex. Patients who received treatment with CF did not show significant variation in the levels of markers related to oxidative stress over the course of treatment.

More studies are needed to assess changes related to oxidative stress in patients with malignant breast cancer undergoing radiotherapy.

However, our results show that the hypofractionated treatment provides a better quality of life for patients with less fatigue and discomfort, over the conventional fractionation. The relationship of these findings with oxidative stress cannot be established with the number of participants in this study nor with the statistical treatment used. Knowing that there are many confounding factors, it is suggested that there is an increase in the number of participants to verify the real participation of oxidative stress during radiotherapy treatment.

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APÊNDICE A

Questionário Sóciodemográfico

			Data:____/____/______
Nome/ Número de identificação:			
Idade:	**Sexo:** Fem ☐ Masc ☐		**Paciente** ☐
Fitzpatrick: Tipo I ☐ Tipo II ☐ Tipo III ☐ Tipo IV ☐ Tipo V ☐	**Tabagismo:** Sim ☐ Parou ☐ Não ☐	**Altura:** **Peso:**	**Localização da Lesão:** Mama direita ☐ Mama esquerda ☐ Quadrante:
Consumo de bebida alcoólica / frequência (doses/semana):	**Ocupação:** **Procedência:** **Alguma restrição de dieta?:**		
Apresenta algum tipo de alergia?			
Medicamentos de uso contínuo? Quais? Utiliza há quanto tempo?			
Já apresentou outros tipos de cânceres? Sim ☐ Qual (is)? Não ☐			
Pratica/ Praticava atividade física? Sim ☐ Qual (is)? Por quanto tempo? Frequência? Não ☐			
Histórico familiar de Câncer?			

APÊNDICE B

Questionário informações clínicas

Data:____/____/______

Nome/ Número de identificação:			
Número da sessão atual/total:	**Dose da radioterapia (acumulada):**		**Fase da coleta:**
Grau da radiodermite:	**Score de Fadiga:**	**Score de desconforto:**	**Prurido:**
Utilização de sutiã:	**Mutações:**		
Utilização de tópico:			
Utilização de compressas com camomila:	**HT:**		
Histopatológico:			
Estádio do tumor:	**Perfil imuno-histoquímico:**		
Procedimentos anteriores à radioterapia:	**QT:**	**Tipo de cirurgia:**	
Esquema de fracionamento:			

APÊNDICE C

Termo de Consentimento Livre e Esclarecido

"FATORES RELACIONADOS À RADIODERMITE E AO ESTRESSE OXIDATIVO NA NEOPLASIA MALIGNA DA MAMA."

Prezado(a) Senhor(a):

Gostaríamos de convidá-la para participar da pesquisa: "FATORES RELACIONADOS À RADIODERMITE E AO ESTRESSE OXIDATIVO NO CÂNCER DE MAMA" no **"**Centro de Oncologia e Radioterapia de Londrina". O objetivo da pesquisa é compreender os efeitos inflamatórios, oxidativos e imunológicos durante a radioterapia da mama, avaliar os fatores de risco para a ocorrência de radiodermite e correlacionar esses efeitos com possíveis fatores de risco para sua ocorrência em graus mais avançados.

Sua participação é muito importante e ela se daria da seguinte forma: parte do sangue que já é obtido rotineiramente durante as sessões de radioterapia será separado e encaminhado ao laboratório de pesquisas da UEL, onde seria analisado. Você ainda responderá um questionário breve sobre aspectos sociodemográficos e de hábitos de vida. Esclarecemos que sua participação é totalmente voluntária, podendo você: recusar-se a participar, ou mesmo desistir a qualquer momento, sem que isto acarrete qualquer ônus ou prejuízo à sua pessoa ou ao seu tratamento. Esclarecemos, também, que suas informações serão utilizadas para os fins desta pesquisa e para pesquisas futuras e serão tratadas com o mais absoluto sigilo e confidencialidade, de modo a preservar a sua identidade. Após as análises, as amostras serão armazenadas no laboratório de genética molecular e imunologia da universidade estadual de Londrina para realização de futuras pesquisas (mas o senhor será consultado novamente sobre sua participação nestes estudos futuros).

Esclarecemos ainda, que você não pagará e nem será remunerado(a) por sua participação. Garantimos, no entanto, que todas as despesas decorrentes da pesquisa serão ressarcidas, quando devidas e decorrentes especificamente de sua participação.

Os benefícios esperados são indiretos, pois esperamos que este estudo possa fornecer ferramentas necessárias para correlacionar radiodermite no tratamento do câncer de mama com marcadores tumorais e com outras variáveis.

Quanto aos riscos, estes são mínimos, e são decorrentes da coleta de sangue (que pode trazer algum desconforto, contudo será realizada por profissional altamente capacitado). Contudo ressaltamos que caso sinta algum desconforto, o senhor(a) será prontamente atendido pelos membros da equipe do projeto.

Caso você tenha dúvidas ou necessite de maiores esclarecimentos poderá nos contatar Renan Capitani Casagrande (43-988577957), Profa. Dra. Alessandra L. Cecchini Armani (43-99912-1992), **,** ou procurar o Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina, situado junto ao LABESC – Laboratório Escola, no Campus Universitário, telefone 3371-5455, e-mail: cep268@uel.br.

Este termo deverá ser preenchido em duas vias de igual teor, sendo uma delas devidamente preenchida, assinada e entregue à você.

Londrina, ___ de ________de 201_.

Pesquisador Responsável RG:________________________

____ (NOME POR EXTENSO DO PARTICIPANTE DA PESQUISA), tendo sido devidamente esclarecido sobre os procedimentos da pesquisa, concordo em participar **voluntariamente** da pesquisa descrita acima.

Assinatura (ou impressão dactiloscópica):______________________________

Data:____________________

APÊNDICE D

Declaração de Concordância dos Serviços Envolvidos e/ou de Instituição Co-Participante

Londrina, 15 de julho de 2019

Ilmo. Sr. Prof. Dr. Osvaldo Coelho Pereira Neto
Coordenador do CEP/UEL

Senhor Coordenador,

Declaramos que nós do setor de Radioterapia do Centro de Oncologia e Radioterapia de Londrina, estamos de acordo com a condução do projeto de pesquisa "**FATORES RELACIONADOS À RADIODERMITE E AO ESTRESSE OXIDATIVO NO CÂNCER DE MAMA**", sob a responsabilidade de Renan Capitani Casagrande nas nossas dependências tão logo o projeto seja aprovado pelo Comitê de Ética em Pesquisa Envolvendo seres Humanos da Universidade Estadual de Londrina, até o seu final em 31/08/2021.

Estamos cientes que as unidades de análise da pesquisa serão os pacientes portadores de neoplasia maligna da mama que serão submetidos a radioterapia, bem como de que o presente trabalho deve seguir a Resolução 466/2012 do CNS e complementares.

Atenciosamente,

Responsável pelo Serviço
Dr. Miguel Gabriel Neto