UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE ODONTOLOGIA

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MICROFLORA BUCAL DE PACIENTES SUBMETIDOS À RADIOTERAPIA E QUIMIOTERAPIA

Porto Alegre
2018

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Trabalho de Conclusão de Curso apresentado ao Curso de Graduação em Odontologia da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul, como requisito parcial para obtenção do título de Cirurgiã-Dentista.

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Porto Alegre
2018

CIP - Catalogação na Publicação

Mincato Klaus, Natália
MICROFLORA BUCAL DE PACIENTES SUBMETIDOS À RADIOTERAPIA E QUIMIOTERAPIA / Natália Mincato Klaus. -- 2018.
26 f.
Orientador: Lina Naomi Hashizume.

Trabalho de conclusão de curso (Graduação) -- Universidade Federal do Rio Grande do Sul, Faculdade de Odontologia, Curso de Odontologia, Porto Alegre, BR-RS, 2018.

1. Radioterapia. 2. Quimioterapia. 3. Câncer. I. Hashizume, Lina Naomi, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Manifesto o meu agradecimento a todos os que, direta ou indiretamente, contribuíram para a realização deste Trabalho de Conclusão de Curso, fornecendo apoio cientifico e emocional para a sua concretização.

À minha orientadora e professora Lina Naomi Hashizume, pelo apoio e orientação constante ao longo de 3 dos meus 5 anos de curso. Mais do que aluna, me tornei amiga da professora, dividindo com ela muitos anseios que vivi no percurso da faculdade. Hoje, ainda com o desejo de me tornar professora que entrei na faculdade, vejo nela exemplo de competência, interesse, dedicação e paciência. Sem dúvida, ela é um grande motivo por eu sair da casa com vontade de voltar para prestar mestrado.

À minha colega e grande amiga Deise Kwiatkowski por dividir o árduo trabalho que a execução desse trabalho gerou. Obrigada pela atenção, paciência e disponibilidade de sempre. A Deise se tornou um exemplo de simpatia, profissionalismo e dedicação para mim.

À Luísa Mercado, técnica do Laboratório de Bioquímica e Microbiologia Bucal da UFRGS e amiga, que conheceu todos meus estados de ânimo e dividiu comigo incontáveis cafés. A presença confirmada dela no laboratório foi e sempre será um motivo de alegria.

Ao Maurício Moreira e à Ana Paula Dall'Onder, que mais que colegas se tornaram meus amigos. Foi ao lado deles que aprendi o quanto um mero experimento pode trazer alegrias e, às vezes, até frustrações. O Maurício foi um professor pra mim e é, hoje, um grande exemplo de cirurgião-dentista.

Às minhas colegas, mas, principalmente, amigas de faculdade Ana, Ananda, Débora, Emília, Julia, Lilian, Marina e Thaise, por dividirem comigo todos momentos da faculdade: provas, trabalhos, seminários, aulas, clínicas e estágios. A faculdade não seria a mesma sem elas ao meu lado.

À minha prima Luísa que, hoje mestre e doutoranda, foi sempre um modelo pra mim e corrigiu meu trabalho com muito capricho.

Finalmente, aos meus pais e irmão, pelo carinho de sempre e por compartilharem todos momentos – bons e ruins - ao longo da faculdade. Eles foram e sempre serão meus grandes apoiadores e dedico a eles cada vitória alcançada.

RESUMO

A radioterapia e a quimioterapia são duas das principais modalidades terapêuticas usadas no tratamento de neoplasias malignas. Ainda que esses métodos sejam muito efetivos, sabe-se que há importantes efeitos colaterais orais decorrentes do seu emprego, tais como: trismo, mucosite, cárie, hipossalivação, osteorradionecrose, perda de paladar e infecções secundárias. Estudos sugerem que a microflora bucal de pacientes submetidos à radioterapia e quimioterapia sofre alterações durante e após o tratamento, podendo, assim, vir a desempenhar um importante papel nessas complicações orais. Com o objetivo de determinar as possíveis modificações da microflora bucal decorrentes de quimioterapia e radioterapia, uma revisão sistemática foi conduzida. Quatro bases de dados (MEDLINE/Pubmed, LILACS, Cochrane e EMBASE) foram utilizadas para busca de estudos sobre o tema. Nenhuma restrição de idioma ou data de publicação foi aplicada. Critérios de exclusão rígidos tais como: comparação com grupo baseline (tempo pré-tratamento) e não utilização de antibiótico três meses antes e durante o estudo foram exigidos. A qualidade dos estudos incluídos foi avaliada baseada na escala de NEWCASTLE – OTTAWA modificada. A busca resultou em 189 publicações após eliminadas as duplicatas. Dessas, 156 foram excluídas, restando um total de 33 publicações. Ainda, 35 trabalhos foram selecionados a partir das referências dos artigos encontrados. Após leitura na íntegra, três estudos foram incluídos. Todas as publicações incluídas avaliaram a microflora do biofilme dentário de pessoas submetidas à radioterapia antes e ao longo de seu tratamento por métodos independentes de cultivo. A avaliação de qualidade dos artigos apontou que todos apresentam a mesma qualidade metodológica. Além disso, os estudos não diferiram em importantes aspectos, tais como: sítio, método e tempos de coleta da amostra. Como resultado, alterações da microflora bucal durante o tratamento radioterápico foram encontradas, indicando que a radiação estabelece um processo de desequilíbrio. Concluiu-se que ocorrem variações temporais da microflora bucal ao longo do tratamento radioterápico em adultos, no entanto essas alterações não são específicas. Além disso, uma associação negativa entre diversidade microbiana e dose de radiação foi observada.

Palavras-chave: Radioterapia. Quimioterapia. Câncer.

ABSTRACT

Radiotherapy and chemotherapy are two of the main therapeutic modalities used in cancer treatment. Although these methods are very effective, it is known that there are important oral side effects resulting from their use, such as: trismus, mucositis, caries, hyposalivation, osteorradionecrosis, loss of taste and secondary infections. Studies suggest that the oral microflora of patients submitted to radiotherapy and chemotherapy changes during and after treatment and may thus play an important role in oral complications. In order to determine the possible modifications of the oral microflora resulting from chemotherapy and radiotherapy, a systematic review was conducted. Four databases (MEDLINE/Pubmed, LILACS, Cochrane e EMBASE) were used to search for studies. No language restriction or publication date was applied. Rigorous exclusion criteria such as: comparison with baseline group (time pre-treatment) and non-use of antibiotic three months before and during the study were required. The quality of included studies was assessed based on the modified NEWCASTLE - OTTAWA scale. The search resulted in 189 publications after the duplicates were deleted. Of these, 156 were excluded, leaving a total of 33 publications. Still, 35 papers were identified from the reference lists of the articles found. After reading the full text of all selected articles, three studies were included. All included publications evaluated the oral microflora of the dental biofilm of persons undergoing radiotherapy before and throughout their treatment by independent culture methods. The quality assessment of the articles indicated that all present the same methodological quality. In addition, the studies did not differ in important aspects, such as: site, method and time of sampling. As a result, changes in oral microflora during radiotherapy treatment were found, indicating that the radiation establishes a process of imbalance. It was concluded that temporal variations of the oral microflora occur along the radiation therapy in adults, however these alterations are not specific. In addition, a negative association between microbial diversity and radiation dose was observed.

Keywords: Radiotherapy. Drug Therapy. Neoplasms. Microbiota. Mouth.

SUMÁRIO

1 **INTRODUÇÃO**..... 7

2 **REVISÃO DE LITERATURA**..... 8

2.1 CÂNCER..... 8

2.2 RADIOTERAPIA..... 8

2.3 QUIMIOTERAPIA..... 9

2.4 EFEITOS ADVERSOS ORAIS DA QUIMIOTERAPIA E RADIOTERAPIA..... 9

3 **OBJETIVO**..... 11

4 **ARTIGO CIENTÍFICO**..... 12

5 **CONSIDERAÇÕES FINAIS**..... 24

REFERÊNCIAS..... 25

1 INTRODUÇÃO

Câncer é o nome geral dado a um conjunto de mais de 100 doenças, as quais têm em comum o crescimento desordenado de células, que tendem a invadir tecidos e órgãos vizinhos (INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA, 2012). As três formas principais de tratamento dessa doença são: quimioterapia, radioterapia e cirurgia.

Destas modalidades terapêuticas, a radioterapia e a quimioterapia, apesar de sua importância e efetividade, determinam a ocorrência de efeitos colaterais consideráveis, os quais afetam expressivamente a qualidade de vida dos pacientes a elas submetidos (SAWADA; DIAS; ZAGO, 2006).

Os efeitos colaterais decorrentes da terapia por radiação estão relacionados com a dose desta, forma de administração, extensão e localização da área a ser irradiada, qualidade e poder de penetração da radiação e fatores individuais do paciente (ARISAWA et al., 2005). Por outro lado, os efeitos adversos da quimioterapia têm direta relação com o agente quimioterápico utilizado, dose utilizada e duração do tratamento (SCHEIN et al., 2006).

Dentre as complicações decorrentes da radioterapia de cabeça e pescoço e da quimioterapia, as complicações orais estão entre as mais devastadoras a curto e longo prazo. Isso porque esses efeitos adversos têm importante impacto sobre atividades cotidianas básicas como alimentação e comunicação, funções essas imprescindíveis não apenas pra saúde física, mas também mental, visto que tem grande importância para as relações sociais (ARISAWA et al., 2005).

São exemplos de complicações orais decorrentes dessas terapias: mucosite, hipossalivação, cáries, perda de paladar, infecções secundárias, osteorradionecrose e trismo (VIEIRA; LOPES, 2006). Dentre essas, vale destacar que a hipossalivação resulta em alterações na composição da saliva e do seu pH, mudanças essas que são seguidas de alterações na microflora bucal (MB) (VOLPATO et al., 2007).

Estudos atribuem às alterações da MB decorrentes da terapia antineoplásica papel importante no desenvolvimento de efeitos adversos do tratamento antineoplásico (STRINGER; LOGAN, 2015; TONG; GAO; DONG, 2003). Deste modo, dada a potencial influência da MB em complicações orais e a relevância clínica dessas complicações, torna-se fundamental identificar os possíveis efeitos da quimioterapia e radioterapia na MB.

2 REVISÃO DE LITERATURA

2.1 CÂNCER

Câncer é o nome geral dado a um conjunto de mais de 100 doenças, as quais têm em comum o crescimento desordenado de células, que tendem a invadir tecidos e órgãos vizinhos (INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA, 2012). Com base no documento World Cancer Report 2014 da International Agency for Research on Cancer, da Organização Mundial da Saúde, é inquestionável que o câncer é um problema de saúde pública, especialmente entre os países em desenvolvimento, onde é esperado que, nas próximas décadas, o impacto do câncer na população corresponda a 80% dos mais de 20 milhões de casos novos estimados para 2025 (INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA, 2015).

Os principais objetivos do tratamento do câncer são cura, prolongamento da vida útil e melhora da qualidade de vida do paciente. Para essa finalidade, existem três formas principais de tratamento antineoplásico: quimioterapia, radioterapia e cirurgia. Essas três modalidades terapêuticas podem ser usadas em conjunto, variando apenas quanto à suscetibilidade dos tumores a cada uma e à melhor sequência de sua administração (INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA, 2012). Dentre essas, a quimioterapia e a radioterapia determinam a ocorrência de efeitos adversos, os quais podem provocar mudanças bruscas em simples atividades do dia-a-dia (ARISAWA et al., 2005).

2.2 RADIOTERAPIA

A radioterapia é uma modalidade de tratamento de neoplasias malignas cujo agente terapêutico é a radiação ionizante, ou seja, aquela que promove ionização no meio onde incide, tornando-o eletricamente instável (JHAM; FREIRE, 2006). A radiação pode causar efeitos biológicos através de dois mecanismos. No mecanismo de ação indireta, a radiação rompe moléculas de água no processo conhecido por hidrólise. Essa quebra da molécula resulta na dissociação da água em seus dois elementos, H+ e OH-, sendo que este último reage com as bases de DNA, interferindo no processo de duplicação (DEL NERO et al., 2015; JHAM; FREIRE, 2006). No segundo mecanismo, de ação direta, a molécula de DNA é clivada, interferindo no processo de duplicação (JHAM; FREIRE, 2006).

Segundo Arisawa et al. (2005), os efeitos colaterais decorrentes da radioterapia dependem da dose da radiação, forma de administração, extensão e localização da área a ser irradiada, qualidade e poder de penetração da radiação e de fatores individuais do paciente. No estudo de Sawada, Dias e Zago (2006) a maioria dos pacientes apontaram que os efeitos que mais incomodaram e apareceram nas categorias "em demasia/o tempo todo", "muito/muitas vezes" e "um bocado/ algumas vezes" foram: boca seca, saliva pegajosa, dificuldade no paladar, pele ressecada, irritado, deprimido e triste, coceira na pele e dificuldade de engolir.

2.3 QUIMIOTERAPIA

Da mesma forma que a radioterapia, a função principal da terapia com quimioterápicos é eliminar as células que formam as neoplasias malignas (SCHEIN et al., 2006). Porém, células normais com alta taxa mitótica também são afetadas por essa intervenção, particularmente aquelas que estão na mucosa oral, gastrointestinal e no sistema hematopoiético. Este caráter não-seletivo da terapia pode estar associado ao aparecimento de diversas complicações (KREUGER et al., 2009).

Os efeitos adversos da quimioterapia dependem do agente quimioterápico utilizado, dosagem, número de ciclos de tratamento, idade do paciente e da coadministração de outras drogas neurotóxicas (KAMEO; SAWADA; SILVA, 2016; SCHEIN et al., 2006). No estudo de Schein et al. (2006), os principais efeitos colaterais relatados pelos pacientes durante o ciclo quimioterápico foram náusea, perda de apetite, vômito, distensão e dor abdominal, refluxo, diarreia, constipação, xerostomia e gosto amargo.

2.4 EFEITOS ADVERSOS ORAIS DA QUIMIOTERAPIA E RADIOTERAPIA

Como exposto acima, efeitos adversos orais ocorrem como consequência da quimioterapia e da radioterapia de cabeça e pescoço. Essas complicações se encontram entre as mais devastadoras a curto e longo prazo tendo em vista que a sua ocorrência, pela possibilidade de prejudicar a alimentação e a comunicação dos pacientes, acarreta prejuízos sociais e físicos, principalmente no que se refere ao estado nutricional dos pacientes (ARISAWA et al., 2005; SCHEIN et al., 2006).

As principais complicações orais advindas do tratamento antineoplásico são: mucosite, osteorradionecrose, trismo, cárie dentária, infecções secundárias, perda

de paladar e hipossalivação (KREUGER et al., 2009; MAIA, 2010; VIEIRA; LOPES, 2006). A redução de fluxo salivar decorrente de danos causados às glândulas salivares causa alterações na composição da saliva e do seu pH, que são seguidas de mudanças na MB (VOLPATO et al., 2007).

Stringer e Logan (2015) afirmam que a microbiota oral pode ter o potencial de exacerbar o dano à mucosa que ocorre como resultado do tratamento do câncer. Semelhantemente, Tong, Gao e Dong (2003) afirmam que uma das principais razões para a alta prevalência de cárie dentária em indivíduos submetidos a tratamento antineoplásico poderia ser a mudança de MB após a irradiação.

Verifica-se, portanto, que a MB pode desempenhar importante papel em complicações orais decorrentes do tratamento antineoplásico. Diante disso e levando em consideração a relevância clínica destas complicações, o conhecimento das modificações que ocorrem na MB de indivíduos submetidos à quimioterapia e radioterapia de cabeça e pescoço é de grande importância.

3 OBJETIVO

Conduzir uma revisão sistemática da literatura a respeito das possíveis modificações da microflora bucal ocasionadas pela radioterapia e quimioterapia em pacientes oncológicos submetidos a essas terapias.

4 ARTIGO CIENTÍFICO

MODIFICATIONS OF THE ORAL MICROFLORA OF PATIENTS SUBMITTED TO RADIOTHERAPY AND CHEMOTHERAPY - A SYSTEMATIC REVIEW

Abstract

Purpose This systematic review aimed to assess the literature to determine the modifications of the oral microflora associated to radiotherapy and chemotherapy in patients under these treatments.

Methods The electronic databases of MEDLINE/PubMed, EMBASE, Cochrane and LILACS were searched for studies describing microflora alterations of individuals submitted to radiotherapy and/or chemotherapy treatment. The methodological quality of the included studies was assessed and scored as proposed by the modified Newcastle–Ottawa Scale.

Results Our research returned 189 publications after duplicates were removed, of which 156 were excluded as off-topic. Thirty-five additional records were identified through hand search review of the retrieved articles references. A total of 68 articles were therefore eligible for full reading after screening of abstracts. Of these selected studies, only 3 were included in this review. All the included publications evaluated the supragingival biofilm microflora of subjects who were undergoing radiotherapy; independent culture methods were used before and throughout their treatment. The quality assessment showed a very similar methodological quality between studies. In addition, the studies did not differ in many important aspects, including sample site, collection methods and collection times. A modification of the oral microflora during radiotherapy was found in all studies, indicating the irradiation as a disequilibrium process.

Conclusions There are alterations and temporal variations across different time points in the oral microflora of adults patients submitted to radiotherapy, however, these modifications are not specifics. Also, a negative correlation between the microbial diversity and radiation dose was observed.

Keywords: Radiotherapy. Drug Therapy. Neoplasms. Microbiota. Mouth.

Introduction

Chemotherapy, radiation and surgery are the main ways of treating cancer. These three treatment modalities might be used in combination, varying only as to the sensitivity of tumors to each of them and the expected best outcome after its administration. Currently, only a few malignancies are treated with only one therapy modality [1].

The adverse effects of chemotherapy and radiotherapy can substantially change the patient's quality of life. These adverse effects might vary according to the chemotherapeutic agent used, the dosage, the number of treatment cycles, the patient age, and if other neurotoxic drugs are co-administered [2, 3]. Similarly, the side effects of radiation therapy may differ depending on the radiation dose, the manner of administration, the extent and location of the area to be irradiated, the quality and penetrating power of radiation and patients individual characteristics [4].

Chemotherapeutic drugs and radiation inhibit cell growth and maturation, disrupting the primary mucosal barrier of the mouth and throat. Oral mucositis, oral candidiasis, hyposalivation, trismus, dental caries and osteoradionecrosis are consequences of this mucosal damage [5]. The hypothesis that the oral microflora (OM) could exacerbate this mucosal damage that occurs as a result of cancer treatment by potentiating apoptosis and production of pro-inflammatory cytokines is discussed in the literature [6]. Also, it is possible that the OM is a major reason for the high prevalence of dental caries in individuals submitted to antineoplastic treatment [7]. However, there is neither a consensus regarding the role of OM modifications in oral complications, nor clarity on what are the changes that occur.

In view of the OM potential influence on oral complications of great clinical relevance and taking into account the high global prevalence of cancer, a systematic review was conducted aiming to identify modifications of OM associated with radiotherapy and chemotherapy.

Methods

A systematic review of human studies was undertaken to identify the changes in the OM of patients receiving radiotherapy and/or chemotherapy. Articles that evaluated the OM of patients undergoing radiotherapy and/or chemotherapy and describe the changes occurred were reviewed.

Review articles, clinical case reports, literature reviews, and other non-research articles were excluded from the review as well as studies with no baseline group (study group when no dose was received) and with no statistical analysis. Additionally, studies performed among subjects receiving antibiotics during therapy or within 3 months before the study were also excluded to reduce the effect that this confounding variable would have on the selection of OM.

A literature search was performed using MEDLINE/PubMed, EMBASE (Excerpta Medica Database), LILACS (Latin-American and Caribbean Literature on Health Sciences/Virtual Health Library) and Cochrane databases. In addition, the reference lists of all retrieved articles were searched to locate additional suitable studies. The search was completed using the terms “oral microflora”, “oral microbiota” and “oral microbiome” in combination with the terms “chemotherapy,” “radiotherapy,” and “chemoradiotherapy” in all databases. No restrictions were placed on the language and the search and the search was carried out until October/2017.

The study selection was completed in two phases. In phase 1, two authors (NMK and DK) independently reviewed titles and abstracts identified in all electronic databases and selected articles that appeared to meet the inclusion criteria. In phase 2, the same authors independently read the full text of all selected articles and excluded studies that did not meet the eligibility criteria. Disagreements between the two initial evaluators were solved by consensus. When they did not reach a consensus, a third reviewer (LNH) was involved to make a final decision.

Two reviewers (NMK and DK) scored the methodological quality of the included studies, which was performed as proposed by the modified Newcastle–Ottawa Scale [8]. Criteria were designated to each domain of internal validity, external validity, and statistical methods. Each aspect of the score list was given a ‘+’ sign for an informative description of the item at issue and a study design that met the quality standard.

Results

In phase 1 of the study selection, 291 records were identified across the four electronic databases: 177 from MEDLINE/PubMed, 106 from EMBASE and 8 from Cochrane. No study was found in LILACS. After the duplicate articles were removed, 189 records remained. Of these, 156 articles were excluded after comprehensive

evaluation of the titles and abstracts was completed, so the full texts of 33 articles were assessed. Additionally, 35 articles were selected from the reference lists of the identified studies.

A full-text review was conducted on the 68 articles retrieved in phase 1. This process led to the exclusion of 65 studies. Of these 65 studies, 3 studies were not found. Other excluded articles were not in accordance with the exclusion criteria. At the end, three articles [9–11] were selected. A flow chart detailing the process of identification, inclusion, and exclusion of studies is shown in Figure. 1.

Included studies

Only three studies fulfilled the entry criteria for the review. We have summarized details of the included studies in Table 1. All the included studies were conducted exclusively in people submitted to radiotherapy. No study conducted with subjects undergoing chemotherapy met the entry criteria. All the studies were performed in adults and were conducted in China.

The three studies are observational and analyzed the OM in supragingival biofilm of adults submitted to radiotherapy and evaluated changes occurred throughout the treatment. The population size of the two studies of Hu et al (2013) [10, 11] was the same. Both samples were composed by eight subjects that provided 56 plaque samples each. In the study of Shao et al (2011) [9] the population was composed by 25 subjects, resulting in 175 plaque samples.

The microbial samples were obtained by collecting plaque sample of the maxillary first molar in all studies. The samples were collected before and during radiotherapy in 7-day intervals in all studies. The method used for microflora analysis was the same for two of the studies [10, 11]. In these studies the V1– V3 hypervariable regions of bacterial 16S rRNA genes were amplified, followed by high-throughput pyrosequencing. On the other hand, the analysis was performed using denaturing gradient gel electrophoresis (DGGE) in the third study [9].

In the first included study, Hu et al (2013) investigated the compositional profiles and microbial shifts of oral microbiota during head-and-neck radiotherapy [10]. The study identified 140 different genera from samples of seven time points. Among all genera, 50% of all sequences were comprised of the top five genera, including *Streptococcus, Veillonella, Actinomyces, Capnocytophaga* and *Derxia.* The top 10 and top 30 genera constituted roughly 80% and 90% of total sequences,

respectively. These findings are in agreement with that indicates by Lorenz curve, that microbial communities were dominated by several major taxa and the genera evenness was low, even though the diversity was high.

The top three genera found in this study [10] at the time prior to treatment (when no dose had been received) were, in order of prevalence: *Neisseria*, *Streptococcus* and *Capnocytophaga*. However, the top three genera varied significantly across different time points during radiotherapy. In addition, 11 genera found in all subjects also varied in relative abundance during the course of radiotherapy. It indicated that there existed temporal variations in microbial communities through the course of treatment, which was also indicated by the cluster analysis of the study.

In the second study [11], Hu et al (2013) explored the dynamic core microbiome of oral microbiota in supragingival plaque of adults during the course of head-and-neck radiotherapy. Similarly to their first study [9], the study identified 140 different genera from samples of seven time points and the top 10 genera constituted roughly 80% of total sequences.

The most abundant genus at the time prior to treatment was *Neisseria*. However, at the time points 10 Gy and 20 Gy, 30 Gy and 40 Gy, and 50 Gy and 60 Gy, the most abundant genus was *Streptococcus, Veillonella and Actinomyces*, respectively. It shows that major genera varied significantly in relative abundance across different time points.

Only two genera (*Streptococcus and Actinomyces*) were present at all time points during the radiation treatment in all subjects. However, nine genera, which could be recognized as ‘‘potential common taxa’’ in the study, were identified in all subjects during radiotherapy but were absent at some time points for each subject. By contrast, six genera (*Streptococcus, Actinomyces, Capnocytophaga, Neisseria, Granulicatella, and Gemella*) were found at the time prior to treatment in all subjects and were designated as common at this time point.

Also at the time prior to treatment, the subjects had the largest number of operational taxonomic units (OTUs). Fewer OTUs were found in the later period (40 Gy, 50 Gy, 60 Gy) than in the early stage (10 Gy, 20 Gy, 30 Gy), and there was a negative correlation between the number of OTUs and dosage.

Finally, thirteen phyla were found in the oral microbiota at seven time points in this study. The top 4 phyla at the time prior to treatment and during the

radiotherapy (across six time points) were *Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria*, however they were present in different order of prevalence before and during the treatment. Those four phyla were found at all time points in all subjects and comprised the core microbiome.

Shao et al (2011) [9] evaluated the salivary flow rates from each of the major glands before, midterm, and after radiotherapy and the temporal variation of the plaque profile of patients with head and neck cancer treated with postoperative intensity-modulated radiotherapy (IMRT) or conventional radiotherapy (CRT). Both IMRT and CRT subjects suffered from hyposalivation. All samples showed changes in community constancy over time. There were no identical Denaturation Gradient Gel Electroforesis fingerprints, indicating that the microbiota changed during the course of the study. Different subjects had different bacteria genera that increased in their abundance. There was no specific modifications of biofilm microbiota during irradiation among individuals. Findings indicated irradiation as a disequilibrium (dysbiosis) process. The eubiosis of the mouth system was profoundly disrupted during this process.

Quality of studies

Eight quality assessment criteria for eligibility studies were established (Table 2). The criteria “clear description of objectives” and “clear description of outcome” were met by all the selected studies. Both the inclusion and exclusion criteria are specified in all articles. The criteria "calculation of sample size and study power", as well as the “drop-out rate” and “Examiners training and calibration” were not found in any of the reviewed studies. Hence, the studies were considered of a similar methodological quality.

Discussion

The aim of this study was to systematically review the literature to determine potential OM alterations caused by radiotherapy and chemotherapy. Only three articles were included in this review and all were conducted in adults submitted to radiotherapy. No studies conducted with patients undergoing chemotherapy were included.

The three studies included in the present review did not differ in many important aspects, including sample sites, collection methods and collection times.

This methodological homogeneity is probably explained by the fact that all studies were performed by the same Chinese research group, which may represent a limitation of the present study. Nevertheless, it reflects the coherent study design and methodological quality of the studies carried out by the group.

The small number of three articles included in this review was a consequence of rigorous exclusion criteria applied. However, it was fundamental to exclude confounding variables, which may interfere with the results. In all included studies, none of the patients received antibiotics during therapy or within 3 months before the study. This criterion resulted in the exclusion of several articles.

Another rigorous exclusion criterion was the absence of baseline samples to compare with samples during treatment. This was required because the OM of healthy individuals that could be used as a control group differs from that of individuals with cancer, even before treatment [12]. This makes the collection of samples at the pre-treatment time fundamental to increase the studies quality.

The population of all included studies in the current review was composed of adults. Therefore, the findings cannot be generalized to other populations such as the pediatric. At this point, it is important to consider that pediatric populations differ from adults in their resident OM, and likely in their response to chemotherapeutic regimens [13] which means that the results may be different for children.

The population size of two studies [10, 11] was composed by eight subjects, while the population of one study [9] was composed by 25 subjects. This small population size may be considered as a limitation of the present review, however, it is important to consider that the collections were performed at 7 time-points, which considerably increases the number of samples. In addition, the logistic and cost issues of the culture-independent methods used in all included studies might have made difficult to recruit and analyze data from a large sample.

The use of culture-independent methods in all included studies in this review is a positive point. Molecular techniques for bacterial identification have shown that bacterial diversity is severely underestimated in studies using culture-based techniques. Further, it is believed that only 50% of all oral bacteria have been cultivated [14].

Modifications of the OM during the radiation therapy was found in all included articles, indicating the irradiation as a disequilibrium process. This imbalance in the oral microbial ecosystem is probably a result of radiation-induced hyposalivation.

Corroborating that fact, Shao et al (2011) found that both IMRT and CRT subjects suffered from hyposalivation [9].

Further research using high quality methodologies is needed to clarify the OM modifications resulting from chemotherapy and radiotherapy and what is their role in oral health. Nevertheless, the present review has as merit the inclusion of articles with high quality methodologies, that used refined analysis methods and presented concordant results.

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Conclusion

There are modifications and temporal variations across different time points in the oral microflora of adults patients submitted to radiotherapy, however these modifications are not specific. In addition, there is a negative association between microbial diversity and radiation dose.

References

1. INCA (2011) ABC of cancer: basic approaches to the cancer control.
2. Kameo YK, Sawada NO, Silva GM (2016) Prevalence of peripheral neuropathy after chemotherapy in patients treated in a service of oncology: a retrospective review. Rev Saúde Com 12:566–574
3. Schein CF, Marques AR, Vargas CL, Kirsten VR (2006) Side effects of chemotherapy in hospitalized cancer patients. Disc. Scientia 7:101–107
4. Arisawa EAL, Silva CMOM, Cardoso CAC, Lemos NRP, Pinto MC (2005) Antitumor therapy: side effects in patients undergoing chemo-and radiotherapy. Rev Biociên 11:55–61
5. Ferretti GA, Raybould TP, Brown AT, Macdonald JS, Greenwood M, Maruyama Y, Geil J, Lillich TT, Ash RC (1990) Chlorhexidine prophylaxis for chemotherapy-and radiotherapy-induced stomatitis: a randomized double-blind trial. Oral Surg Oral Med Oral Pathol 69:331–338
6. Stringer AM, Logan RM (2015) The role of oral flora in the development of chemotherapy-induced oral mucositis. J Oral Pathol Med 44:81–87
7. Tong HC, Gao XJ, Dong XZ (2003) Non-Mutans Streptococci in Patients Receiving Radiotherapy in the Head and Neck Area. Caries Res 37:261–266
8. Wells GA, Shea B, O'connell D, Peterson J, Welch V, Losos M, Tugwell P. 2017 The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Ottawa Hosp Res Inst. Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed October 20, 2017
9. Shao ZY, Tang ZS, Yan C, Jiang YT, Ma R, Liu Z, Huang ZW (2011) Effects of Intensity-modulated Radiotherapy on Human Oral Microflora. J Radiat Res 52:834–839

10. Hu YJ, Wang Q, Jiang YT, Ma R, Xia WW, Tang ZS, Liu Z, Liang JP, Huang ZW (2013) Characterization of oral bacterial diversity of irradiated patients by high-throughput sequencing. Int J Oral Sci 5:21–25
11. Hu YJ, Shao ZY, Wang Q, Jiang YT, Ma R, Tang ZS, Liu Z, Liang JP, Huang ZW (2013) Exploring the Dynamic Core Microbiome of Plaque Microbiota during Head-and-Neck Radiotherapy Using Pyrosequencing. PLoS One 8:1–7
12. Xu Y, Teng F, Huang S, Lin Z, Yuan X, Zeng X, Yang F (2014) Changes of saliva microbiota in nasopharyngeal carcinoma patients under chemoradiation therapy. Arch Oral Biol 59:175–186
13. Vozza I, Caldarazzo V, Ottolenghi L (2015) Changes in microflora in dental plaque from cancer patients undergoing chemotherapy and the relationship of these changes with mucositis: A pilot study. Med Oral Patol Oral y Cir Bucal 20:259–266 .
14. Paster BJ, Boches SK, Galvin JL, Ericson E, Lau CN, Levanos VA, Dewhirst FE, Ericson RE, Sahasrabudhe A (2001) Bacterial Diversity in Human Subgingival Plaque. J Bacteriol 183:3770–3783

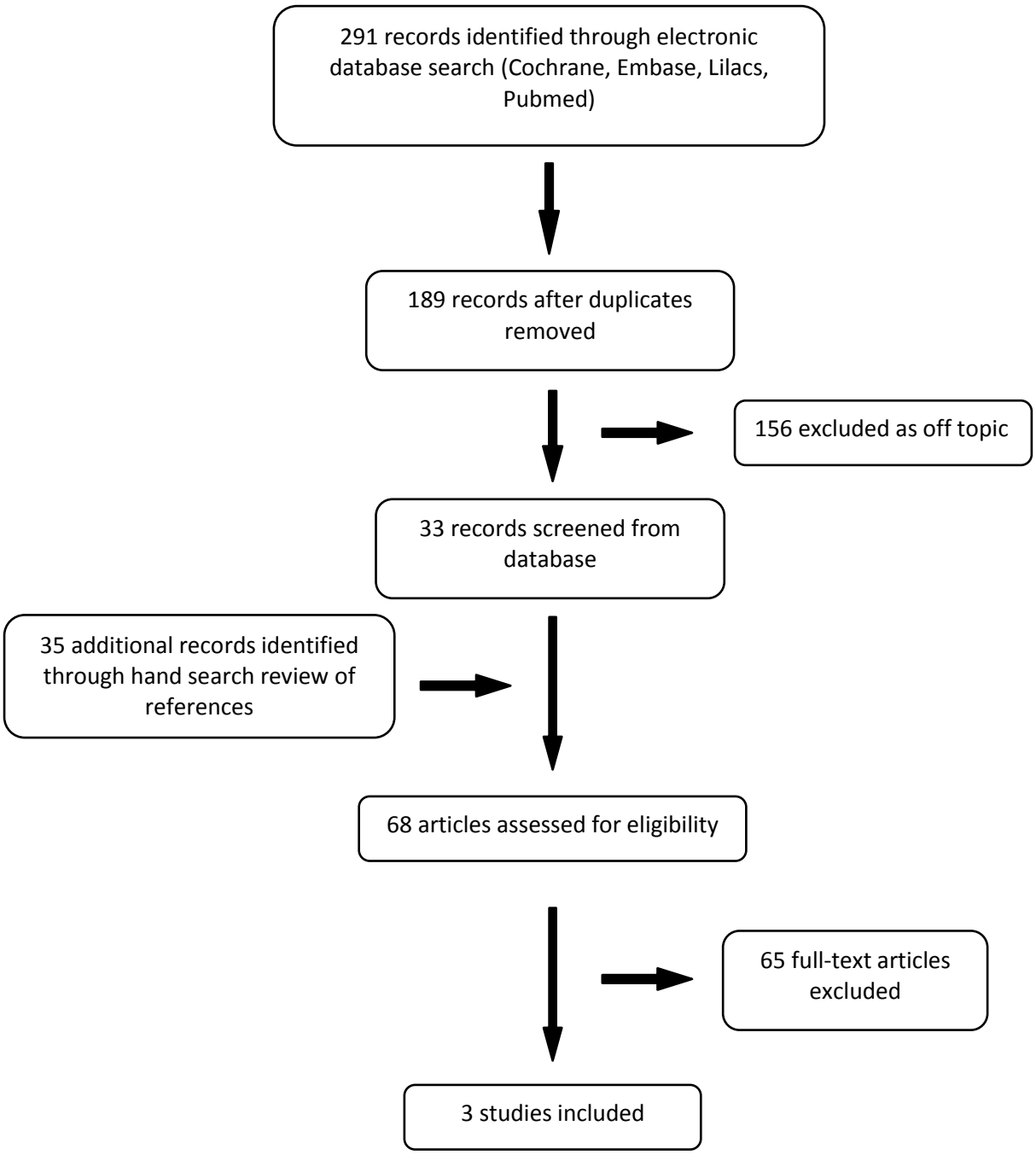

Figure. 1 Flow chart detailing the process of identification, inclusion, and exclusion of studies.

Table 1. Details of the included studies

Author, year	Country	Study design	Population size	Patients age	Intervetion/ Exposure	Sample	Times of sample collection	Methodology
Hu et al (2013) [10]	China	Observational	Eight subjects	The average age was about 45 years	Radiotherapy	Plaque samples from the maxillary first molar	Before and during radiotherapy at 7-day intervals (before, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy)	Pyrosequencing
Hu et al (2013) [11]	China	Observational	Eight subjects	Ranging from 26 to 70 years.	Radiotherapy	Supragingival plaque from the buccogingival surfaces of the maxillary first molar	Before and during radiotherapy at 7-day intervals (before, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy)	Pyrosequencing
Shao et al (2011) [9]	China	Observational	Twenty-five subjects	18 years of age or older	Conventional and Intensity-modulated radiotherapy	Plaque sample from the buccogingival surfaces of the upper first molar	Before and during radiotherapy at 7-day intervals (before, 10 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy and 60 Gy)	Denaturing gradient gel electrophoresis

Table 2. Quality assessment criteria for eligibility studies.

Author, year	Clear description of objectives	Clear description of outcome	Dropout rate	Inclusion criteria	Exclusion criteria	Sample size calculation and study's power	Outcome variability measures	Examiners training and calibration
Hu et al (2013) *[10]*	+	+	-	+	+	-	+	-
Hu et al (2013) *[11]*	+	+	-	+	+	-	+	-
Shao et al (2011) *[9]*	+	+	-	+	+	-	+	-

5 CONSIDERAÇÕES FINAIS

Dada a relevância clínica das complicações orais decorrentes da radioterapia e quimioterapia e a potencial influência de alterações da MB nestas complicações, a busca pelo conhecimento das alterações da MB decorrentes dessas modalidades terapêuticas e suas consequências são de grande importância.

A revisão sistemática de literatura conduzida com o objetivo de trazer evidências a respeito das alterações de MB ocorridas em pacientes submetidos à quimioterapia e radioterapia incluiu um número pequeno - de três artigos - e nenhum estudo incluído abordou pacientes submetidos à quimioterapia, limitando a possibilidade de se responder inteiramente à questão inicial que incluía também essa terapia. Apesar disso, considera-se que tenha sido de suma importância a aplicação de critérios de exclusão rígidos, os quais foram responsáveis pela exclusão de numerosos artigos, a fim de controlar ao máximo variáveis de confusão.

Ainda que as três publicações pertençam a um mesmo grupo de pesquisa e tenham sido realizadas apenas com indivíduos submetidos à radioterapia, elas apresentaram a mesma qualidade metodológica, utilizaram técnicas de análise microbiana refinadas e trouxeram resultados concordantes. Esse foi um ponto positivo para a revisão sistemática conduzida.

Concluiu-se através da condução da revisão sistemática, que o tratamento radioterápico determina a ocorrência de alterações da MB de adultos, o que indica que a radiação estabelece um processo de desequilíbrio. Observou-se, também, que ocorrem variações temporais da MB ao longo do tratamento radioterápico, as quais, no entanto, não são específicas. Além disso, uma correlação negativa entre diversidade microbiana e dose de radiação foi observada.

Mais estudos com alto rigor metodológico são necessários para obter-se um número maior de evidências científicas a respeito das modificações de MB decorrentes da exposição à irradiação e a agentes quimioterápicos.

REFERÊNCIAS

ANTUNIASSI, A.R. **Ocorrência e grau de severidade da mucosite bucal em relação ao fluxo salivar de pacientes sob quimioterapia.** 2005. 62f. Dissertação (Mestrado em Diagnóstico Bucal) - Faculdade de Odontologia, Universidade de São Paulo, São Paulo.

ARISAWA, E. A. L. et al. Efeitos colaterais da terapia antitumoral em pacientes submetidos à químio e à radioterapia. **Rev. Biociên.,** Taubaté, v. 11, no. 1–2, p. 55–61, jan./jun. 2005.

INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA. **ABC do câncer:** abordagens básicas para o controle do câncer. 2. ed. Rio de Janeiro, 2012.

INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA**.. Estimativa 2016:** Incidência de câncer no Brasil. Rio de Janeiro, 2015.

DEL NERO, R. A. et al. Transformação dos conhecimentos sobre a radiação ionizante para usuários da radioterapia do Hospital das Clínicas de Botucatu. In: **CONGRESSO DE EXTENSÃO UNIVERSITÁRIA DA UNESP**, 2015, Botucatu. Diálogos da extensão: do saber acadêmico à prática social. [S.l.]:UNESP, 2015.

JHAM, B. C.; FREIRE, A. R. DA S. Complicações bucais da radioterapia em cabeça e pescoço. **Rev. Bras. Otorrinolaringol.,** Rio de Janeiro, v. 72, no. 5, p. 30180–112, set./out. 2006.

KAMEO, Y. K.; SAWADA, N. O.; SILVA, G. M. Prevalência de neuropatia periférica pós quimioterapia em pacientes atendidos em um serviço de oncologia: uma análise retrospectiva. **Rev. Saúde. Com.,** Jequié, v. 12, no. 2, p. 566–574, 2016.

KREUGER, M. R. O. et al. Complicações orais em pacientes em tratamento quimioterápico na UNACON, no município de Itajaí/SC. **Rev. Faculdade. Odontol. Lins.,** Taubaté, v. 21, no. 1, p. 39–47, 2009.

LUIZ, A. C. et al. Alterações bucais e cuidados orais no paciente transplantado de medula óssea. **Rev. Bras. Hematol. Hemoter.**, São Paulo, v. 30, no. 6, p. 480–487, 2008.

MAIA, W. O. **Mucosite e complicações orais em pacientes sob tratamento quimioterápico e radioterápico.** 2010. 43 f. Trabalho de Conclusão de Curso (Especialização em Periodontia) - Faculdade de Odontologia, Universidade Federal de Minas Gerais, Belo Horizonte.

SAWADA, N. O.; DIAS, A. M.; ZAGO, M. M. F. O efeito da radioterapia sobre a qualidade de vida dos pacientes com câncer de cabeça e pescoço. **Rev. Bras. Cancerol.,** Rio de Janeiro, v. 52, no. 4, p. 323–329, 2006.

SCHEIN, C. F. et al. Efeitos colaterais da quimioterapia em pacientes oncológicos hospitalizados. **Disc. Scientia.**, Santa Maria, v. 7, no. 1, p. 101–107, 2006.

SHAO, Z. Y. et al. Effects of Intensity-modulated Radiotherapy on Human Oral Microflora**. J. Radiat. Res.,** Tokyo**,** v. 52, no. 6, p. 834–839, 2011.

STRINGER, A. M.; LOGAN, R. M. The role of oral flora in the development of chemotherapy-induced oral mucositis. **J. Oral Pathol. Med.,** Copenhagen, v. 44, no. 2, p. 81–87, 2015.

TONG, H. C.; GAO, X. J.; DONG, X. Z. Non-Mutans Streptococci in Patients Receiving Radiotherapy in the Head and Neck Area. **Caries Res**., Basel, v. 100081, no. 22, p. 261–266, 2003.

VIEIRA, A. C. F.; LOPES, F. F. Mucosite oral: efeito adverso da terapia antineoplásica. **Rev. Ciênc. Méd. Biol**., Salvador, v. 5, no. 3, p. 268–274, 2006.

VOLPATO, L. E. R. et al. Mucosite bucal radio e quimioinduzida. **Rev. Bras. Otorrinolaringol.,** Rio de Janeiro**,** v. 73, no. 4, p. 562–568, 2007.