

**Epidemiological and microbiological aspects of oral mucositis in head and neck cancer patients during radiotherapy**

**Aspectos epidemiológicos e microbiológicos da mucosite oral em pacientes com câncer na região de cabeça e pescoço durante a radioterapia**

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**ABSTRACT**

This cross-sectional study evaluated epidemiological and nutritional aspects as well as the microbial groups isolated from head and neck cancer patients, in the presence of oral mucositis. Three samplings were performed at baseline (jugal mucosa), on the 20<sup>th</sup> radiotherapy session and on the last day of treatment, over oral mucositis lesions, if present. The sample was composed of 22 patients, mostly men (81.8%) and with a median age of 58.1(±12.7) years, 95% of them had concomitant smoking and drinking habits and 40.9% were edentulous. Body mass index values decreased during treatment and were statistically significant over time. During the study, there was a decline in Gram-positive cocci and an increase in Gram-negative rods. The recovery of yeast species increased along the study period. Isolation of multidrug-resistant microorganisms (44.3%) and those related to nosocomial infections (50.0%) was observed. Our findings suggested that there was a reduction in the indigenous oral microbiota and an increase in opportunistic species. The understanding of the microbial content of oral mucositis lesions as well as the clinical and nutritional aspects of head and neck cancer patients may contribute to a better oriented treatment.

**Keywords:** Oral mucositis, Radiation therapy, Oral microbiota, Opportunistic microorganisms, Head and neck cancer.

**RESUMO**

Este estudo transversal avaliou aspectos epidemiológicos e nutricionais, bem como os grupos microbianos isolados de pacientes com câncer de cabeça e pescoço, na presença de mucosite oral. Três coletas foram realizadas, no início do estudo (mucosa jugal), na 20<sup>a</sup> sessão de radioterapia e no último dia de tratamento, nas lesões da mucosite oral, quando presentes. A amostra foi composta por 22 pacientes, a maioria homens (81,8%) e com média de idade de 58,1 (±12,7) anos, 95% deles apresentavam hábito de tabagismo e etilismo concomitantes e 40,9% eram edêntulos. Os valores do índice de massa corporal diminuíram durante o tratamento e foram estatisticamente significativos ao longo do tempo. Durante o estudo, houve um declínio nos cocos Gram-positivos e um aumento nos bastonetes Gram-negativos. A recuperação de espécies de leveduras aumentou ao longo do estudo. Observou-se isolamento de microrganismos multirresistentes (44,3%) e relacionados a infecções nosocomiais (50,0%). Nossos resultados sugerem que houve uma redução na microbiota oral indígena e um aumento nas espécies bacterianas oportunistas. A compreensão do conteúdo microbiano das lesões da mucosite oral, bem como dos aspectos clínicos e nutricionais dos pacientes com câncer de cabeça e pescoço, pode contribuir para um tratamento mais direcionado.

**Palavras-chave:** Mucosite oral, Radioterapia, Microbiota oral, Microrganismos oportunistas, Câncer de cabeça e pescoço.

## 1 INTRODUCTION

Head and neck cancer (HNC) treatment depends on many aspects including anatomical site, histological type, differentiation and staging grade, age, and patients' general health status. Treatment could include surgery, radiation therapy (RT), and chemotherapy (CT), alone or in combination<sup>1</sup>. Radiation treatment is measured in Gray units (Gy)<sup>1,2</sup>. Radiation-induced oral mucositis (OM) is a common debilitating side effect for HNC patients and is associated with painful ulceration of oral mucosa, compromising food intake<sup>3,4</sup>. The presence of OM lesions could lead to the need of enteral or parenteral nutritional support, hospitalization, and to treatment interruption, leading to a reduced response to tumor therapy<sup>3,4,5</sup>. The OM severity scored by the World Health Organization (WHO) includes five grades: grade 0, absence of oral lesions; grade 1, pain and erythema; grade 2, presence of ulceration; grade 3, ulceration and the use of liquid diet; and grade 4, lack of food intake<sup>6</sup>. The OM lesions are induced by RT and CT or both therapies<sup>4</sup>. The body mass index (BMI) has been associated as a risk factor for OM<sup>4,5,7</sup>. According to Saito<sup>7</sup>, poor nutritional status could reduce mucosal repair interfering with cell migration and renovation. In spite of phase II studies discussing new interventions to treat oral mucositis, such as the use of antimicrobials, antioxidants and inflammatory mediators, patients still receive symptomatic and palliative treatment<sup>8</sup>. Ulcerative oral mucositis can become a gateway for opportunistic microorganisms<sup>4</sup>. We hypothesized if OM lesions could be infected by these microbial agents, worsening the wound and also if BMI could enhance the OM severity. The present study proposes an evaluation of oral microbiota along RT treatment associating these findings with OM lesions. Despite that, the association of some clinical and epidemiological aspects could shed some light on this topic and contribute to more effective therapies.

## 2 MATERIAL AND METHODS

### 2.1 STUDY DESIGN AND PARTICIPANTS

This cross-sectional observational study was performed from December 20<sup>th</sup>, 2017, to July 15<sup>th</sup>, 2018, at the Muriaé Hospital of Cancer, Minas Gerais State, Brazil. The institution is a philanthropic hospital, mostly assisting patients from the Brazilian Unified Health System (SUS). The convenience sample<sup>9</sup> was composed of 22 patients and the inclusion criteria were as follows: age of 18-90 years; diagnosis of HNC as the primary site; those who were about to initiate RT, associated or not to CT or to surgical removal of the tumor during the study period; patients who did not use antimicrobials 2 weeks prior to RT<sup>10</sup>. The exclusion criteria comprised the presence of non-controlled cardiovascular diseases, diabetes mellitus, severe lung, kidney, and liver diseases, and systemic immunological disorders, diagnosed before antineoplastic therapy; patients with motor and psychiatric illnesses. All data were obtained from the patients included during the first visit. Patients who could not appropriately

comprehend the research purpose and those who did not sign or understand the informed consent form; those who were unable to open the mouth to receive oral examination prior to RT; and the impossibility of oral feeding before treatment.

## 2.2 SETTING AND DATA COLLECTION

Relevant information, such as hospitalization, antimicrobial use, body mass index, RT dose and protocol, and the chemotherapy agents were obtained from medical records, with permission. Radiation dose fractionation used in this study was conventional, and hypofractionated RT and the chemotherapy agents were cisplatin and fluorouracil (5FU). During a dental visit, an interview was conducted with all patients. Demographic questions included age, gender, ethnicity, marital status, household income<sup>11</sup>, level of education, and profession. Medical and dental history questions comprised cigarette and alcohol consumption, previous diseases, the use of dental prostheses, patients' dental care, including brushing and flossing, the use of a chemical plaque control agent, and visiting a dentist. To identify the nutritional status, Body Mass Index (BMI) was calculated dividing the weight in kilograms (kg) by the height in meters (m). The measures of weight and height were also obtained by accessing the health records.

## 2.3 MICROBIOLOGICAL SAMPLING

Three samples were collected during the study period, using a sterile swab moistened with Stuart medium: at baseline (before RT), on the 20<sup>th</sup> daily dose of RT, and on the last day of RT. The first visit comprised oral and dental examination<sup>12</sup>, the questionnaire, and microbiological sampling preceded by a mouthwash with sterile saline for 1 min; at the second and third consultations, only oral and dental exams and microbiological sampling were performed. Dental examination was conducted by a dentist who collected information about dental loss, number of present teeth, presence of dentures and dental decay and oral hygiene habits. Due to the patients' general health condition or to the availability to perform sampling, this interval could be of  $\pm 5$  days. Prior to RT, the site of the sampling was the jugal mucosa. A 2 x 2 cm area between the labial commissure and the retromolar trigone was swabbed (five movements over the entire surface, bottom-to-top and from base up). The second and third samplings were performed in the same site if OM was not clinically observed. When OM lesions were present, the specimen was collected swabbing with the same five movements, over the entire surface of the larger observed lesion. The sampling technique did not exceed patients' pain limits and was performed as deep as possible, without the use of topical anesthesia. The OM lesions were classified from 0 to 4, according to WHO<sup>6</sup>.

## 2.4 LABORATORY PROCEDURES

After sampling, swabs were inserted inside sterile Stuart's transport medium tubes and directed to the hospital laboratory, accompanied by a request form containing patient details: name, anatomical site, date, and the time of sampling. Vitek2<sup>®</sup> Compact (Biomerieux, Marcy l'Etoile, France) was used for automated identification (GN ID card, GP ID card and YST20 card) and performance of the antimicrobial susceptibility tests (AST-N239 and AST-585 cards). Sabouraud dextrose agar containing 2% chloramphenicol and Mycosel agar tubes<sup>13</sup> were used to culture and isolate yeast strains. Yeast growth was observed after 7 days of incubation and weekly for up to 30 days. MacConkey agar plates and chocolate agar plates containing 5% sheep blood were used to culture and isolate bacterial strains. The streaked plates of MacConkey agar were incubated at 37°C for 24 to 48 h. The plates containing chocolate agar were incubated in microaerophile conditions for the same period of time. Isolates exhibiting different and similar colony morphologies (up to three of each morphotype) were selected from the spread plates. After growth of pure cultures, Gram-staining was performed prior to identifying and establishing the susceptibility profile using Vitek2<sup>®</sup>. Susceptibility tests followed the *Clinical and Laboratory Standards Institute* breakpoints and protocols.

## 2.5 STATISTICAL METHODS

Data were collected in an Excel spreadsheet (*Microsoft Office*<sup>®</sup>, version 365), and descriptive analyses were performed. To evaluate the strength and direction of the correlation between quantitative variables (discrete and continuous), Pearson's correlation coefficient (r) was used. The level of statistical significance (p) was also evaluated, and p values of  $\leq 0.05$  were considered significant. Statistical analyses were performed using the STATA statistics software, version 12.0 (STATA Corp., TX, USA).

## 2.6 ETHICAL APPROVAL

Ethical approval was previously obtained from the Research Ethics Committees of the participating hospital and the Federal University of Minas Gerais (CAAE: 77980217.0.0000.5149). The study was conducted according to the international guidelines of Helsinki Declaration. Written informed consent was obtained from all patients prior to enrolling.

# 3 RESULTS

## 3.1 PARTICIPANTS AND DESCRIPTIVE DATA

Epidemiological characteristics and behavioral data of the patients included are shown in Table 1. Body mass index (BMI) values of the patients submitted to three samplings are presented in Figure

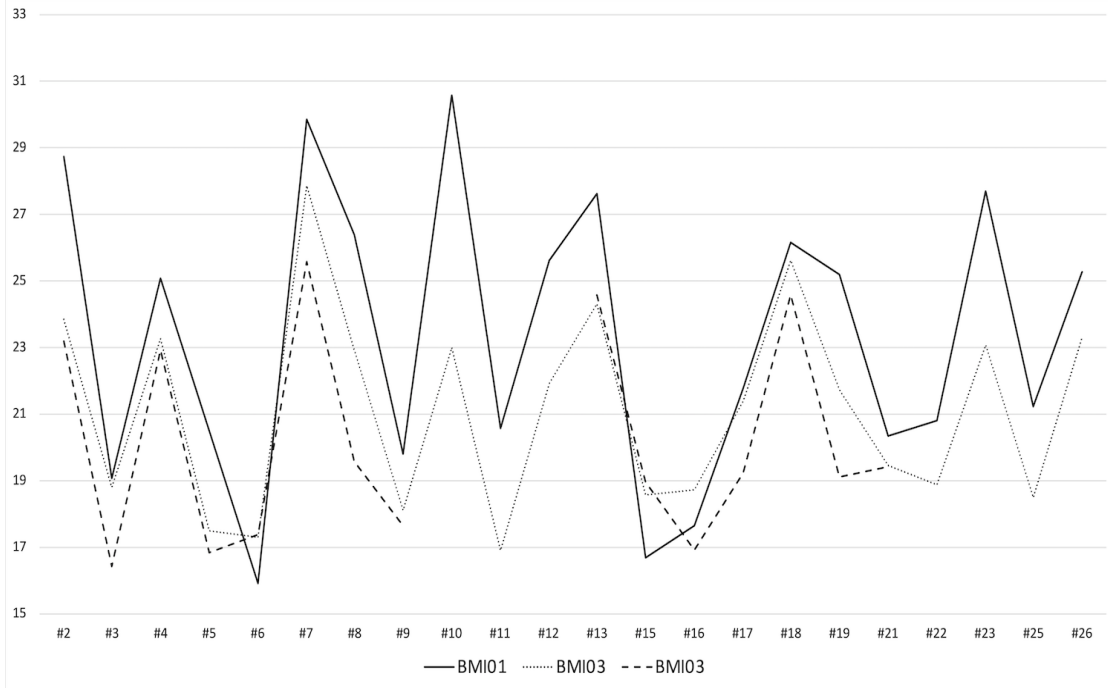
1. Among those patients with remaining teeth, 84.6% had no incisors and/or molars. The number of the present teeth was of 8(±10) per patient and is included on Table 2 which describes all dental characteristics. Pearson’s correlation demonstrated a strong negative correlation between existing teeth and age ( $r = -0.62$  and  $p < 0.01$ ), suggesting that with aging, the number of remaining teeth diminished. Pearson’s correlation also demonstrated a strong positive correlation among BMI values between the 1<sup>st</sup> and the 2<sup>nd</sup> samplings ( $r = 0.83$ ;  $p < 0.01$ ) and between the 2<sup>nd</sup> and 3<sup>rd</sup> samplings ( $r = 0.93$ ;  $p < 0.01$ ). The HNC patients who received palliative RT had their final result of OM classification registered during the second visit due to the equivalence of RT doses. Only 17 patients received the conventional therapy and were sampled three times. During the 2<sup>nd</sup> and the 3<sup>rd</sup> samplings of patients receiving RT and CT, the most prevailing OM lesion was grade III (Table 3).

Table 1 – Characteristics of the patients diagnosed with HNC, considering sociodemographic, and alcohol and smoking habits.

<i>Sociodemographic/Habit Characteristics</i>		<i>Values n (%)</i>
Gender	Male	18 (81.8)
	Female	4 (18.2)
Age (Mean ± SD)		58.1 y (±12.7)
Ethnicity	White	13 (59.1)
	Brown	6 (27.3)
	Black	3 (13.6)
Occupation	Farmer	12 (54.6)
	Retired	3 (13.7)
	Other	7 (13.7)
Marital status	Married	12 (54.5)
	Single	4 (18.2)
	Other	6 (27.3)
Household income	Minimum wage	13 (59.1)
	No income	5 (22.7)
	Other	4 (18.2)
Level of education	Illiterate	9 (41.0)
	Fundamental Incomplete	9 (41.0)
	Other	4 (18.0)
Alcohol use	Yes	20 (90.1)
	No	2 (9.1)
Smoking status	Ex-smoker	16 (72.6)
	Never	3 (13.7)
	Present smoker	3 (13.7)

Legend: SD: standard deviation, y: years.

Figure 1: Body mass index values of the patients with head and neck cancer, considering the three measures.



Legend: BMI01- Body mass index first sampling; BMI02 - Body mass index second sampling; BMI03- Body mass index third sampling; #- patient number; patients #10, 11, 12, 22, 25, and 26 did not receive the third measure.

Table 2- Characteristics of the patients diagnosed with HNC, considering the dental status.

Dental status		Values n (%)
Number of teeth (Mean ± SD)		8 (±10)
Dental brushing		22 (100.0)
No antiseptics use		20 (90.9)
Total edentulous	Yes	9 (40.9)
	No	13 (59.1)
Dental flossing	Yes	3 (23.1)
	No	10 (76.9)
Complete dentures	Superior	3 (33.3)
	Superior + inferior	3 (33.3)

Legend: SD: standard deviation.



Table 3 - Characteristics of the patients diagnosed with HNC, considering the treatment and the oral mucositis lesions classification.

<i>HNC/Treatment Characteristics</i>		<i>Values n (%)</i>
Tumor site	Oral cavity	14 (63.6)
	Other	8 (36.4)
Tumor staging	IV	18 (81.8)
	III	3 (13.7)
	Other	1 (4.5)
	RT + CT	13 (59.1)
Treatment	RT + CT + Surgery	5 (22.7)
	RT	3 (13.7)
	RT + Surgery	1 (4.5)
#RT sessions	39	11 (50.0)
	35	6 (27.3)
	15	5 (22.7)
Total RT dose (Gy)	70.2	11 (50.0)
	63.0	6 (27.3)
	37.5	5 (22.7)
	Yes	17 (77.3)
Palliative RT	No	5 (22.7)
	Grade (2 <sup>nd</sup> sampling <sup>1</sup> )	
OM classification	III	10 (45.4)
	II	5 (22.7)
	I	3 (13.7)
	0	3 (13.7)
	IV	1 (4.5)
	Grade (3 <sup>rd</sup> sampling <sup>2</sup> )	
	III	8 (47.1)
	II	5 (29.4)
	I	3 (17.6)
0	1 (5.9)	

Legend: SD: standard deviation; RT: radiation therapy; CT: chemotherapy; OM: oral mucositis; HCN: Head and neck cancer; <sup>1</sup>: values and frequency included patients who received complete RT and palliative RT; <sup>2</sup>: values and frequency included only patients who received complete RT.

### 3.2 MAIN RESULTS

The results of the three microbial samplings are shown in Table 4. In the first sampling, Gram-positive cocci (CGP) prevailed (68.2%), followed by Gram-negative rods (GNR) (22.7%) and the “mixed microbial population” (9.1%). The last result was given when an abundant growth of several bacterial morphotypes was observed, restraining bacterial count. Yeasts were recovered from 18.2% of the patients. The second sampling also showed the dominance of GPC (40.9%), succeeded by GNR (31.8%) and the “mixed microbial population” (27.3%); yeasts were also observed (22.7%). The third sampling detected an equivalent recovery of GPC and GNR (47.1% each), followed by the “mixed microbial population” (5.8%). Yeasts were recovered from 35.2% of the patients. Hospitalizations due to fever, vomiting and nausea corresponded to 31.8%, during seven days circa, and almost all those patients received intravenous antimicrobials. For one patient, who received no antimicrobial prescription, hospitalization occurred due to vomiting, nausea and dehydration. Two types of β-



lactamases, AmpC and extended-spectrum  $\beta$ -lactamase (ESBL), and clindamycin resistance (D-test induction) were detected in 22.7% of the strains (n = 5). At the first sampling, one strain, identified as *Enterobacter cloacae* complex recovered from the jugal mucosa, was ESBL-positive (patient #16, who had been previously submitted to surgical intervention around 1 month before sampling). At the second sampling, two strains, identified as *Streptococcus oralis* (patient #23) and *Klebsiella pneumoniae* (patient #25), were D-test positive and ESBL-positive, respectively. These species were associated with OM lesions grade III and II, respectively. During the last sampling, another two strains of coagulase-negative *Staphylococcus* (patient #2; OM grade II) and *Serratia marcescens* (patient #5; OM grade III) were D-test and AmpC positives, respectively. At the first sampling, Gram positive cocci were tested against 13 antimicrobials and 22.6% of the tests was resistant. Gram-negative rods were tested against 23 antimicrobials and resistance was detected in 19.2% of the tests. The second sampling demonstrated a slight increase on the resistant percent of the tests for Gram-positive cocci (23.9%) against 17 antimicrobials and for Gram-negative rods (21%) against 26. The result of the susceptible tests at the third sampling exhibited another increase for both Gram-positive cocci (35.7%) and Gram-negative rods (36.4%), either tested against 20 antimicrobials. Multidrug-resistant bacteria were observed in 44.2% of the samplings. In half of the patients, microorganisms that might be related to nosocomial infections were recovered. From the second sampling, a total of six strains were recovered: two strains of *Klebsiella pneumoniae* (patients #16 and #25; both associated to OM grade II); one *Staphylococcus aureus* (patient #13); one *Enterobacter cloacae* (patient #3; OM grade III); one *Pseudomonas aeruginosa* (patient #16; OM grade II), and one *Candida albicans* (patient #22; OM grade III). Only in patient #10, who presented OM grade IV, the species *Acinetobacter ursingii* was recovered. From the third sampling, seven microbial strains, namely two *Enterobacter cloacae* (patients #8 and #23), one *Pseudomonas* spp. (patient #6), one *Serratia marcescens* (patient #5), one *Candida albicans* (patient #18), all associated to OM lesions grade III, one *Pseudomonas aeruginosa* (patient #19), and one *Klebsiella pneumoniae* (patient #16), both related to OM lesions grade II, were isolated. Table 5 exhibited the results of microbial strains identification and the OM grade, during the second and third samplings.

Table 4 - Microbial strains recovered from patients with head and neck cancer at the Muriaé Hospital of Cancer, considering the first, second, and third samplings

Microbial Strains				
1 <sup>st</sup> Sampling <sup>a</sup>	Site	2 <sup>nd</sup> Sampling	Site	3 <sup>rd</sup> Sampling
<i>Streptococcus mitis</i>	P	MC	C	CoNS
<i>Streptococcus viridans</i>	T	<i>Enterobacter cloacae</i>	G	<i>S. malthophilia</i>
<i>S. viridans/ C. albicans</i>	C	MC	T	CoNS
<i>Streptococcus oralis</i>	C	<i>Pasteurella canis/</i> <i>Stephanoascus Ciferri</i>	C	<i>Serratia marcescens</i> AMPC/ <i>Stephanoascus</i> <i>ciferri</i>
<i>S. mitis</i>	T	<i>S. oralis</i>	T	<i>Pseudomonas</i> spp.
<i>Streptococcus</i> <i>pseudoporcinus</i>	C	<i>S. oralis</i>	C	<i>Pasteurella</i> <i>pneumotropica</i>
<i>Streptococcus oralis</i>	P	<i>Raoutella ornithinolytica</i>	T	<i>Enterobacter cloacae</i>
MC	C	<i>Streptococcus sanguinis</i>	C	<i>S. sanguinis</i>
<i>Enterobacter cloacae</i>	L	<i>Candida parapsilosis /</i> <i>Acinetobacter ursingii/</i>		Not performed <sup>b</sup>
MC / <i>Cryptococcus laurentii</i>	C	MC		Not performed <sup>b</sup>
<i>Klebsiella pneumoniae</i>	P	<i>Leuconostoc mesenteroides</i>		Not performed <sup>b</sup>
<i>Streptococcus viridans</i>	C	<i>Staphylococcus aureus</i>	T	MC / <i>Candida famata</i>
<i>S. sanguinis</i>	C	<i>S. oralis</i>	C	<i>S. mitis</i>
<i>Enterobacter cloacae</i> complex ESBL/ <i>C. albicans</i>	G	<i>Klebsiella pneumoniae/</i> <i>Cryptococcus laurentii</i>	C	<i>Klebsiella</i> <i>pneumoniae/C. albicans</i>
<i>Streptococcus thoralensis/</i> <i>Candida glabrata</i>	C	<i>Streptococcus thoralensis</i>	C	<i>Kocuria rósea/Candida</i> <i>norvegensis</i> <i>Gemella</i> <i>morbilorum/C.</i> <i>albicans</i>
<i>K. pneumoniae</i>	C	MC	T	
<i>Streptococcus</i> <i>pseudoporcinus</i>	C	<i>Pseudomonas aeruginosa</i>	C	<i>P. aeruginosa</i>
<i>Kocuria rosea</i>	C	MC / <i>Candida tropicalis</i>	C	<i>Streptococcus</i> <i>vestibularis/ C.</i> <i>tropicalis</i>
<i>S. mitis</i>	G	<i>Gemella haemolysans/</i> <i>Candida albicans</i>		Not performed <sup>b</sup>
<i>Leuconostoc mesenteroides</i>	C	<i>S. oralis</i>	C	<i>Enterobacter cloacae</i> complex
<i>K. pneumoniae</i>	L	<i>K. pneumoniae</i> ESBL	L	<i>Granulicatella elegans</i>
<i>Streptococcus</i> spp.	C	MC		Not performed <sup>b</sup>

<sup>a</sup>The site of first sampling was internal cheek lining for all patients; <sup>b</sup>Patients who received palliative RT did not undergo the third sampling; #: number. C: Cheek; G: Gum; L: lips; P: palate; T: tongue. MC: 'Mixed Culture'. CoNS: Coagulase-negative staphylococci.

Table 5 - Microbial strains isolated from patients with head and neck cancer at the Muriaé Hospital of Cancer, considering the grade of OM lesions at the second, and third samplings.

Second sampling			Third sampling		
Bacteria	Fungi	OM	Bacteria	Fungi	OM
Mixed culture	NR	II	CoNS	NR	II
<i>Enterobacter cloacae</i>	NR	III	<i>Stenotrophomonas maltophilia</i>	NR	III
Mixed culture	NR	I	CoNS	NR	II
<i>Pasteurella canis</i>	<i>Stephanoascus ciferri</i>	III	<i>Serratia marcescens</i>	<i>S. Ciferri</i>	III
<i>Streptococcus oralis</i>	NR	III	<i>Pseudomonas spp</i>	NR	III
<i>S. oralis</i>	NR	I	<i>Pasteurella pneumotropica</i>	NR	I
<i>Raoutella ornithinolytica</i>	NR	III	<i>E. cloacae</i>	NR	II
<i>Streptococcus sanguinis</i>	NR	0	<i>S. sanguinis</i>	NR	0
<i>Acinetobacter ursingii</i>	<i>Candida parapsilosis</i>	IV		NR	
Mixed culture	NR	III		NR	
<i>Leuconostoc mesenteroides</i>	NR	III		NR	
<i>Staphylococcus aureus</i>	NR	0 <sup>a</sup>	Mixed culture	<i>Candida famata</i>	II
<i>S. oralis</i>	NR	0	<i>Streptococcus mitis</i>	NR	I
<i>Klebsiella pneumoniae</i>	<i>Cryptococcus laurentii</i>	II	<i>K. pneumoniae</i>	<i>C. albicans</i>	II
<i>Streptococcus thoralensis</i>	NR	III	<i>Kocuria rosea</i>	<i>Candida norvegensis</i>	II
Mixed culture	NR	II	<i>Gemella morbillorum</i>	<i>C. albicans</i>	III
<i>Pseudomonas aeruginosa</i>	NR	II	<i>Pseudomonas aeruginosa</i>	NR	II
Mixed culture	<i>Candida tropicalis</i>	I	<i>Streptococcus vestibularis</i>	<i>C. tropicalis</i>	I
<i>Gemella haemolysans</i>	<i>C. albicans</i>	III		NR	
<i>S. oralis</i>	NR	III	<i>Enterobacter cloacae</i> complex	NR	III
<i>K. pneumoniae</i>	NR	II	<i>Granulicatella elegans</i>	NR	III
Mixed culture	NR	III		NR	

Legend: CoNS – coagulase negative *Staphylococci*; OM: oral mucositis lesions grades (according to WHO<sup>8</sup>); NR: Not recovered.

## 4 DISCUSSION

### 4.1 EPIDEMIOLOGICAL CHARACTERISTICS AND BEHAVIORAL DATA

The epidemiological profile of HNC patients in underdevelopment countries is well established and discussed in other studies<sup>14,15</sup>. Despite the knowledge of the majority of men in their sixties, the

presence of smoking and drinking habits, the diagnose is still performed in advanced stages and related to high mortality rates<sup>16,17</sup>. Similar to our findings, the study of Stanford-Moore et al.<sup>17</sup> showed a stronger relationship of smoking and alcohol use among HNC patients of lower socioeconomic status. In spite of the similarities between studies, it is important to highlight that the profile of a Brazilian public hospital attending to the Unified Health System may be a bias of our results. Symptoms such as dysphagia, dysgeusia and nausea caused by HNC therapy could result in poor nutrient intake and, consequently, a low BMI values. Some authors<sup>4,7,18</sup> mentioned that BMI could be a risk factor to develop OM lesions since a poor nutritional status affecting mucosal repair. However, the emergence and development of OM lesions can have a severe impact on patient's ability to eat and drink diminishing the BMI. So, it was possible to observe that the emergence of OM lesions could be influenced by the decrease of BMI.

#### 4.2 ORAL HEALTH HABITS AND DENTAL STATUS

Almståhl et al.<sup>14</sup> encountered a medium of present teeth of 25 ( $\pm 5$ ), so differing from our findings. The prevalence of complete or partially edentulous patients could indicate a poor oral health for economic reasons, lack of access to dental health care, fear of dental treatment, or even an option to prevent or treat dental pain<sup>19</sup>. It is important to emphasize that dental caries is one of the most important cause of dental loss. Dental caries is a multifactorial infectious disease which depend on various factors including the time<sup>20</sup>. Considering the place and the country of the studied population, the prevailing age group (58 years), the poor oral health detected and the partial or total tooth loss found herein were quite expected. In contrast, patients who regularly attend to the dentist are more careful about their oral hygiene so helping to prevent or even eliminating the possibility to be exposed to carcinogens<sup>21</sup>, once poor oral hygiene status has been related to cancer<sup>17</sup>.

#### 4.3 HEAD AND NECK CANCER ASPECTS AND TREATMENT CHARACTERISTICS

The most frequent histological type in this study was squamous cells carcinoma (95.5%); a similar percentage (96.2%) was also observed by Bragante et al.<sup>22</sup>. The study of Costa and Silva et al.<sup>23</sup> demonstrated that 60% of the patients had the tumor stages III and IV, numbers inferior to those encountered in the present study. Our numbers also differ from those encountered by Almståhl et al.<sup>14</sup>, who detected that 42% of their patients were stage IV. The authors<sup>14</sup> showed that the tonsils (58%) were the most frequent site, differently from the present study, where the oral cavity prevailed (63.6%). In this study (59.1%), as in others<sup>22,23</sup>, the combination of RT and CT was the most prescribed treatment. Despite improvements in treating malignant tumors, mortality rates of HNC cancer did not significantly decreased in recent decades<sup>16</sup>. The possible explanation for this scenario could be the late

diagnosis as demonstrated in our study and by other authors<sup>14,22,23</sup>. Additionally, according to Svider et al.<sup>16</sup>, in recent years HNC have had a lesser degree of financial investment compared to other malignant diseases.

#### 4.4 ORAL MUCOSITIS INFORMATION

The prevailing OM lesions were grade III (68.1%), and the majority of the patients (66.7%) received RT/CT had OM grade III. According to Barasch and Peterson<sup>3</sup> and Curra et al.,<sup>24</sup> antimetabolite (5FU) and alkylating agents (cisplatin) increase the incidence and the severity of OM lesions. Rodriguez-Caballero et al.<sup>25</sup> also reported that the use of RT and CT simultaneously result in longer and more severe lesions. Araújo et al.<sup>26</sup> demonstrated that OM lesions grade III and IV prevailed. Almståhl et al.<sup>14</sup> also found these two grades in 61% of the patients with tumor stages III and IV. This finding corroborates with the present study, where OM lesions grade III and IV were observed in 71.4% of the patients staging III and IV. Along the RT treatment, the grade of OM lesions also increased, confirming that the induced OM lesions depend on a cumulative dose<sup>27</sup>.

#### 4.5 MICROBIOLOGICAL INFORMATION

Here, comparing the two first samplings and the second with the third sampling, we observed a decrease in Gram-positive cocci (mainly *Streptococcus* and *Staphylococcus*) and an increase in Gram-negative rods and yeasts. Conversely, Soni et al.<sup>28</sup> found a preponderance of Gram-negative rods in 63.6% of their patients submitted to RT and CT. Among this group, the most prevalent genera were *Pseudomonas* (32.3%) and *Klebsiella* (29.4%), almost the same genera as those recovered in the present study (*Klebsiella* - 30%, *Enterobacter* - 25%, and *Pseudomonas* - 15%). These numbers are in contrast to those encountered by Yamashita et al.<sup>29</sup>, who found a predominance of *Staphylococcus*, *Pseudomonas aeruginosa*, and *Candida*. According to Sonalika et al.<sup>30</sup>, isolation of *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and the genus *Candida*, could aggravate OM lesions. Soni et al.<sup>28</sup> observed that the genus *Candida* was isolated during RT/CT treatment (63.6%) and the species *Candida tropicalis* predominated. In the present study, the genus was also more frequently recovered during RT/CT (35.2%), but the prevailing species was *Candida albicans* (22.7%). All of the mentioned studies suggested that during RT, Gram-negative bacteria prevail<sup>28,29,30</sup>. The criterium for multidrug resistance was resistance to at least one agent in three or more different antimicrobial categories<sup>13</sup> and represented 44.3% of the isolated strains. Besides the presence of multidrug profile strains, with time, the percentage of resistant tests increased. Microorganisms that could be related to nosocomial infections were recovered from half of the sampled patients. Cancer patients are submitted to diagnostic and treatment procedures that increase hospital stay, consequently

expanding the exposition to pathogen colonization, including multidrug-resistant ones<sup>31</sup> Kamath et al.<sup>32</sup> showed a decrease in the oropharyngeal microbiota in HNC patients receiving RT when compared to the control group. Despite the differences among the compared studies, in the majority, microbial recovery occurred inside OM lesions grades III and IV, highlighting the isolation of opportunistic pathogens<sup>28,29,30</sup>.

Oncological patients may present changes in salivary flow and in the saliva viscosity which added to dysphagia could lead to swallowing changes<sup>33</sup>. Consequently, patients could start using some utensils such as towels to collect their saliva. In the present study, it was observed that these towels were not properly sanitized and also deposited by the patient in several places, such as chairs, shoulder and even the floor. This may be a source of contamination of OM lesions.

#### 4.6 LIMITATIONS

The present study has some limitations: obligate anaerobes were not investigated due to the lack of infrastructure. Sample collection, transportation and maintenance require some equipments and resources that are not available to a Brazilian public hospital. More robust statistical analyses were not performed owing to the small sample size. Some included patients used antimicrobials during the study period. Despite these limitations, this observational study precisely described the characteristics of the studied population. More studies with a bigger sample should be necessary to overcome these mentioned points.

#### 5 CONCLUSION

Despite that the epidemiological and behavioral profile of HNC have been well discussed in the literature, our findings reinforced the need to improve oral health promotion measures once that late diagnosis still prevails, especially in under development countries. Changings in oral microbiota profile during RT treatment, with a prevalence of Gram-negative bacteria have been reported in other studies and herein. We emphasize the relevance to understand the microbial profile of OM lesions once they may represent a gateway for opportunistic bacteria. As HNC patients are immunocompromised and MDR bacteria were recovered inside OM lesions, we believe that studies on this subject are capital and will contribute to understand and prevent the severity of oral mucositis. We also believe that a larger sample size in new studies may offer more information concerning HNC and result in good clinical practices.

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