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Assessment of Subgingival Biofilm and its Relationship with the Status of Tumors in a Population with Breast Cancer

Evaluación del Biopelícula Subgingival y su Relación con el Estado de los Tumores en una Población con Cáncer de Mama

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ABSTRACT: This study aimed to compare the subgingival microbiota of subjects with and without breast cancer (BC). Patients with BC (Group 1; n= 50) and without BC (Group 2; n=50) with periodontitis (A) and without periodontitis (B). The study was conducted in two phases (P1 and P2). One biofilm sample was collected from each subject and analyzed by DNA-DNA Hybridization (Checkerboard DNA-DNA). The relative abundance of the subgingival microbiota differed between the Case and Control groups. However, some species were higher in patients in the Case than in Control subjects and differed between the groups in both phases. Composition of the subgingival microbial community according to the Socransky complex was related to periodontal disease, followed by clinical attachment of level (CAL≥4mm), age, and tooth loss, which were found to be abundant in Cases when compared with controls. Patients with Tumor Grade II and III had a higher prevalence of tooth loss and CAL≥4mm. It was concluded that in individuals with BC, the sub-gingival microbiota exhibited atypical changes, but they developed periodontal disease.

KEY WORDS: subgingival microbiota, periodontal disease, breast tumors, DNA-DNA hybridization, dental research.

INTRODUCTION

Breast cancer (BC) is a highly heterogeneous disease due to its morphological aspects, biologic profile, and different clinical conditions (Viale, 2012), so the classification of the disease is according to its histological (Elston & Ellis, 1991), immunohistochemical and molecular characteristics (Dowsett *et al*., 2011). Many studies have associated cancer with periodontal disease (PD) (Michaud *et al*., 2007; Arora *et al*., 2010). More specifically, others have evaluated the correlation between BC and PD. The authors demonstrated a higher incidence of cancer in patients who had PD and had lost some molar teeth from the jaw (Söder *et al*., 2011).

This could be justified by the fact that periodontitis or PD, a disorder that affects millions of people each year (Darveau, 2010), is considered a polymicrobial inflammatory disease, characterized by a synergistic and dysbiotic oral microbiota. In this case, a plethora of microorganisms display distinct roles from their classic ones and cause disease-provoking microbiota (Carrouel *et al*., 2016) that eventually lead to the loss of tooth-supporting tissues and ultimately, loss of teeth (Kinane *et al*., 2017).

BC and DP correlate regarding the oral microbiota in triggering cancerous mechanisms at the

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cellular level, such as inflammation that contributes to the development and progression of malignant tumors (Michaud *et al*., 2008; Meyer *et al*., 2008). Indeed, it has been shown that changes in the oral microbiota play a role in cancer development (Mantovani *et al*., 2008). Moreover, studies have demonstrated that there could be a large number of subgingival pathogens and that these could influence the progression of PD, thus fomenting the strong correlation between the oral microbiota and BC (Parhi *et al*., 2020).

The main objective of this study was to describe the subgingival microbiota obtained from samples of biofilm from periodontal sites of the studied population, according to the microbiota complexes (red, orange, yellow, purple, green, blue or Actinomyces species and other species) described by Socransky *et al*. (1994). The biofilm samples were analyzed by the checkerboard DNA-DNA hybridization technique. Also, tooth loss and clinical attachment level (CAL; the region between the cemento-enamel junction and the most apical portion of the periodontal in a coronal direction) were evaluated as clinical parameters. They were, then, associated with the histological grade of the tumor (I, II, and III) by the Nottingham morphological system of BC in both biopsied tissue and cells (Elston & Ellis, 1991). Herein, we demonstrate differences in the subgingival microbiota of individuals with and without BC.

MATERIAL AND METHOD

Study design. This was an explorative study of the human oral subgingival microbiota run in biological samples collected from volunteers with BC, who were either exposed to PD or not. The patients were initially evaluated using a semi-structured questionnaire for obtaining socio-demographic information, medical history, and BC treatment plan. The study has been reported in compliance with the STROBE Statement guidelines (Von Elm *et al*., 2007) and was by the Health Sciences Center Ethics Committee of the Federal University of Espirito Santo (UFES) under de protocol number 88131918.6.000.5060 with its registration in the Brazilian Clinical Trials Registry number 11346 (https: ensaiosclinicos.gov.br). The written term of free and informed consent was obtained from all patients enrolled in the study between 2018 and 2019.

Data collection. After recruitment, patients who met the following inclusion criteria were enrolled in the study: confirmed diagnosis of BC, no other proven systemic diseases, had not undergone periodontal therapy for the last 6 months, and did not have any antibiotic therapy and anti-inflammatory drugs. The reasons for exclusion criteria were: edentulousness, comorbidity beyond BC, over 70 years old, inconclusive report, pregnancy, lactation, and refuse to sign an informed consent form (n=159). The study population consisted of 100 female participants (30-69 years old) either diagnosed with BC (Group 1 [n=50]) or without BC (Group 2 [n=50]). The participants were classified into 2 subgroups, according to their periodontal health status: 1A: 24 with periodontitis; 1B: 26 without periodontitis; 2A: 30 with periodontitis; 2B: 20 without periodontitis.

This longitudinal study was run in phases one and two (P1 e P2), respectively, following intervals of 90 days between the phases, and basic periodontal therapy was performed twice on days 1 and 14. Diagnostic criteria for BC were mainly based on the diagnostic guidelines of the Anatomical Pathology Service of University Hospital (UFES) by the American Society of Clinical Oncology (ASCO) (2010) and American College of Pathologists (CAP) Consensus 2010. The diagnosis of PD was established by the American Periodontology Academy criteria. All participants underwent a dental examination at the Department of Clinical Dentistry, Institute of Dentistry, Federal University of Espirito Santo. The study population was submitted to a dental examination for a comprehensive assessment of the oral soft and hard tissues, including a periodontal examination. CAL was measured in millimeters using a periodontal probe (UNC 15, Hu-Friedy, Chicago, IL) at six sites of all existing teeth. The presence of periodontitis was defined with probing depth (PD) $\geq$4.0mm and PD $\leq$3mm without periodontitis. However, only one periodontal site of any tooth in the maxilla or jaw was selected for biofilm collection. If no teeth or dental implants were present in the quadrant, no sample was collected. The quadrant to be sampled was isolated with cotton rolls/ pads and a saliva ejector. A supragingival plaque was carefully removed at the sampling site selected using a sterile curette, leaving the subgingival dental biofilm undisturbed.

The biofilm was collected using individual sterile mini-Gracey curettes. A total of one hundred biofilm samples were evaluated. The samples were immediately placed in separate Eppendorf tubes containing 150 ul of TE (10 mM Tris-HCl, 1 mM EDTA, pH 7.6) and 100 ul of 0.5 M NaOH was added to each tube, so, the final solution of 250 ul stored at -20 ºC

until further processing. The biofilm samples were evaluated for their content of 40 bacterial species, by checkerboard DNA-DNA hybridization, as previously described (Socransky *et al*., 1994). The clinical data and subgingival biofilm samples were collected from each patient in P1 and P2. The procedure was repeated in all sites selected. Basic periodontal treatments were performed, such as supra and subgingival root scraping, smoothing, and polishing for biofilm control, using straight sterile Gracey curettes 5-6, 7-8 or 11-12 or 13-14, smoothing and low-speed polishing in a crown-down direction, mouthwashes with 0.12 % (w/v) chlorhexidine and topical fluoride application in both phases. Patients were given oral hygiene instructions and asked to avoid drinking water and eating food for at least 40 minutes before the intervention).

Microbiological Examination

DNA-DNA Hybridization (Checkerboard DNA-DNA). The biofilm samples of each group were transferred to Eppendorf tubes containing 100 mL of TE buffer (10 mM Tris-HCl, 1 mM EDTA [pH 7.6]), with 100 mL of 0.5 M NaOH and 800 µl of ammonium 5M to neutralized were added. The tubes containing the samples and the final solution de 1000 ml were boiled for 10 min. The samples were analyzed individually for the presence and quantity of the 40 bacterial species, using the DNA-DNA hybridization technique. Briefly, upon lysis of the samples, the DNA was plated onto a nylon membrane using a Minislot device (Immunetics). After DNA attachment to the membrane, it was placed in a Miniblotter 45 (Immunetics). Digoxigenin labeled with DNA probes of the entire genome for the subgingival species used were hybridized to individual lanes of Miniblotter 45. After hybridization, the membranes were washed, and DNA probes were detected using a specific antibody to digoxigenin conjugated phosphatase alkaline. The signals were detected using the AttoPhos substrate (Amersham Life Sciences), and the results were obtained using Typhoon Trio Plus (Molecular Dynamics). Two channels in each run contained the standards with 10^5 and 10^6 bacterial cells of each species.

Signals obtained with the Typhoon Trio were converted to absolute counts, by comparison with the patterns on the same membrane. Failure to detect a signal was recorded as zero. Counts below the method detection limit ($1x10^4$ bacterial cells) were considered zero to calculate the mean counts of respective bacterial species. They were recorded as: 0=not detected; 1= $<10^5$ cells; 2= ~10^5 cells; 3=105-10^6 cells; 4=~10^6 cells; 5= >10^6 cells. The sensitivity of this assay was adjusted to allow the detection of 10^4 bacterial cells of a given species by adjusting the concentration of each DNA probe (Mestnik *et al*., 2010).

Statistical Data Analysis. Age and periodontal clinical parameters (tooth loss and CAL), histological tumor grade of BC, and subgingival microbiota data were extracted and analyzed from a database maintained prospectively. Values for tooth and CAL (≥4 mm) as dichotomous predictors were chosen based on the previously published literature. The Wilcoxon test was used to compare the quantity of microbiota >105 between phases 1 and 2. The Spearman correlation test was used to associate CAL ≥4 mm, age, and tooth loss with subgingival microbiota. The Simple Poisson regression with the robust standard error was used to associate the CAL≥4 mm and the tooth loss with histological tumor grade. The associations were considered statistically with p≤0.05 and a confidence interval of 95 %. Statistical analysis was performed using IBM SPSS Statistics version 24 and STATA version 14.0.

RESULTS

Clinical features of the study population. Age ranges were between 30-39 years old (14 % in G1, 18 % in G2), between 40-50 years old (26 % in G1, 40 % in G2), between 51-59 years old (32 % in G1, 26 % in G2) and between 60-69 years old (28 % in G1, 16 % in G2). In Phase 1, tooth loss in Group 1B was (76.09 %), and CAL≥4mm in Group 1A (60.4 %). In Phase 2, tooth loss in Group 2A was (55.32 %), in Group 1B (54.17 %), and CAL>4mm (72.94 %) in Group 1A.

Subgingival microbiota diversity by Socransky complex

Bacterial count description. The 40 bacterial species studied here were grouped into the complexes defined by Socransky *et al*. (1994). The percentages of each of these complexes per site were analyzed (Figs. 1 and 2). When compared to group 1A, group 1B displayed complexes with higher proportions of bacteria in almost all evaluations except the bacterial species *Parvimonas micra*, *Actinomyces naeslundi* I, *Actinomyces israelli*, *Actinomyces oris*, *Eubacterium saburreum*, *Propionybacterium acnes* and *Gemella morbillorum*, *Prevotella melaninogenica* and *Leptotrichia buccalis* that were lower in phase 2 than in phase 1 (Fig. 1).

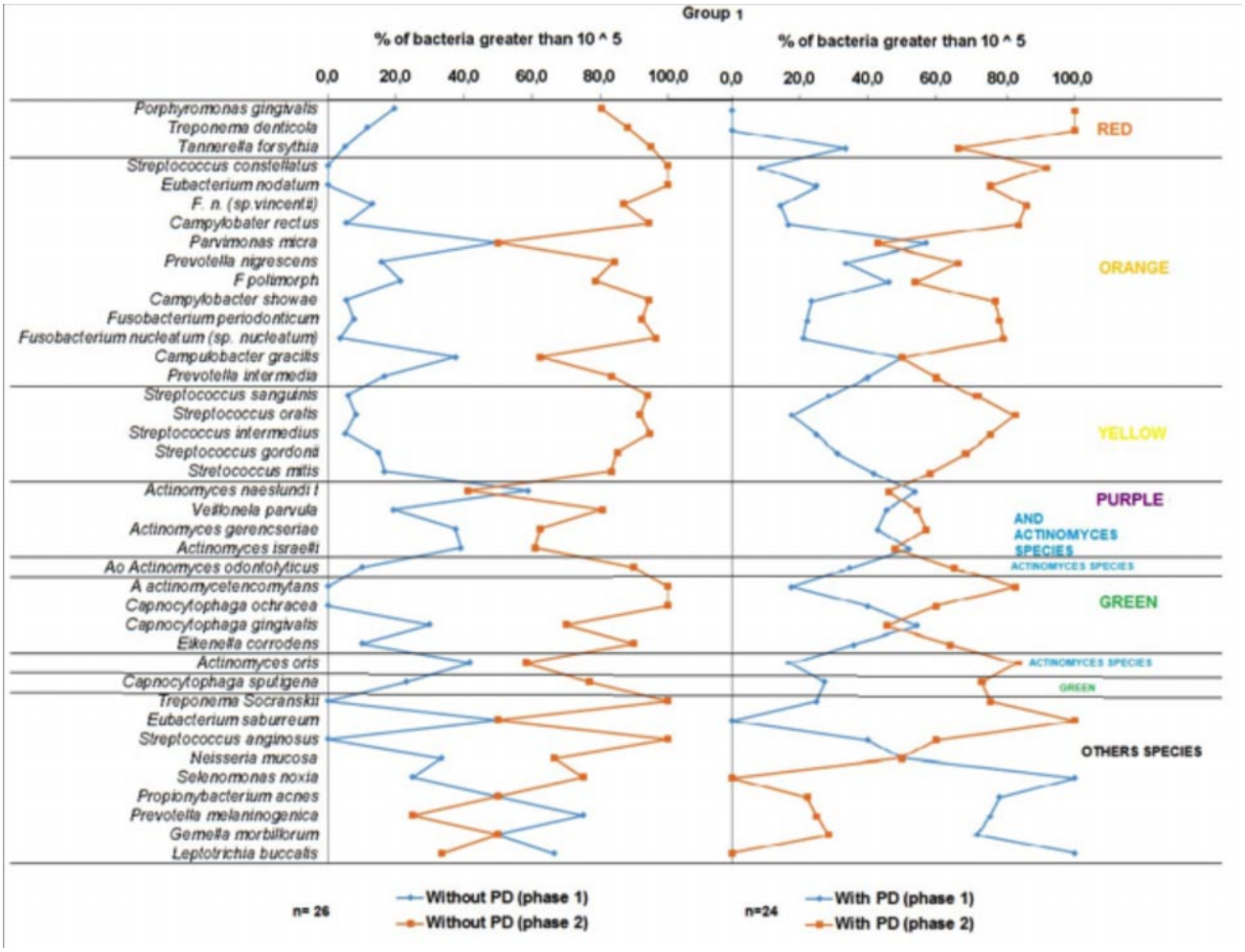

Fig. 1. Description (%) of counts (> 105 bacterial cells) of the 40 test species at Phase 1 and 2 for the Group with cancer in the two treatment groups (1A and 1B). The species were ordered according to the microbial complexes described by Socransky. The counts of individual species were averaged within a subject and then across subjects in each treatment group at each time point. The significance of differences between Phases was assessed using the Wilcoxon test (*p=0.05) compared to the amount of microbiota. Reference value: > 105 bacterial cells adjusted for 40 comparisons (Socransky *et al*., 1991).

In group 1A, phase 2 had higher proportions of bacteria in comparison to phase 1, except bacterial species *Parvimonas micra*, *Actinomyces naeslundi* I, *Actinomyces israelli* and *Capnocytophaga gingivalis* obtained similar proportions between phases. However, *Selenomonas noxia*, *Propionybacterium acnes*, *Prevotella melaninogenica*, *Gemella morbillorum*, and *Leptotrichia buccalis* reached smaller proportions in P2 compared with P1 (Fig. 1).

In Figure 2, the complexes reached higher proportions in almost all evaluations except bacterial species *Tannerella forsythia*, Fn (sp. vicentii), *Campylobacter rectus*, *Parvimonas micra*, *Fusobacterium periodonticum*, *Fusobacterium nucleatum*, *Streptococcus sanguinis*, *Streptococcus oralis*, *Streptococcus gordonii*, *Streptococcus mitis*, *Actinomyces naeslundi* I, *Veillonela parvula*, *Actinomyces israelli*, *Capnocytophaga ochracea* and *Capnocytophaga gingivalis*, *Capnocytophaga sputigena*, *Eubacterium saburreum*, *Streptococcus anginosus*, *Neisseria mucosa* and *Selenomonas noxia* in group 2B. The species *Porphyromonas gingivalis*, *Treponema denticola*, *Tannerela forsythia*, *Streptococcus constellatus*, *Eubacterium nodatum*, *Campylobacter rectus*, *Parvimonas micra*, *Prevotella nigrescens*, *F polimorphum*, *Fusobacterium periodonticum*, *Fusobacterium nucleatum* and *Prevotella intermedia*, *Streptococcus gordonii*, *Actinomyces naeslundi* I, *Veillonela parvula*, *Actinomyces gerencseriae*, *Actinomyces israelli*, *Actinomyces odontolyticus*, *Capnocytophaga ochracea*

and *Capnocytophaga gingivalis*, *Capnocytophaga sputigena*, *Actinomyces oris*, *Eubacterium saburreum*, *Streptococcus anginosus*, *Neisseria mucosa*, *Gemella morbillorum*, and *Leptotrichia buccalis*.

Correlation between subgingival microbiota, clinical data, and age. CAL≥4mm, tooth loss and age, with subgingival microbiota for subjects in the present study are summarized in Tables I and II. In group 2B, CAL ≥4mm was correlated with *Streptococcus anginosus* (r=0,507) to a moderately positive extent. Age correlated with bacterial species *Treponema denticola* (r= 0.514), *F. n. sp. vincentii* (r=0.480), *Campylobacter rectus* (r=0.497), *Eikenella corrodens* (r=0.471), *Treponema socranskii* (r=0.466), and *Propionybacterium acnes* (r=0.521), which were moderately positive. In group 2A, no statistical difference was observed between CAL≥4 mm and bacterial species, but, for age, it was significant with *Selenomonas noxia* (r=0.498) and was considered moderately positive. In Group 1B, CAL ≥ 4mm correlated with *Capnocytophaga ochracea* (r=0,653) and was considered strongly positive, *Eikenella corrodens* (r=0.516) and *Leptotrichia buccalis* (r=0,481) were moderately positive. Age was significantly correlated with *Streptococcus anginosus* (r=0.516) and *Propionybacterium acnes* (r=0.481) and was moderately positive. The tooth loss showed association with *Actinomyces gerencseriae* (r=0.564), *Actinomyces odontolyticus* (r=0.550), *Capnocytophaga gingivalis* (r=0.549) and *Neisseria mucosa* (r=0.515), which were moderately positive and there was strong positive

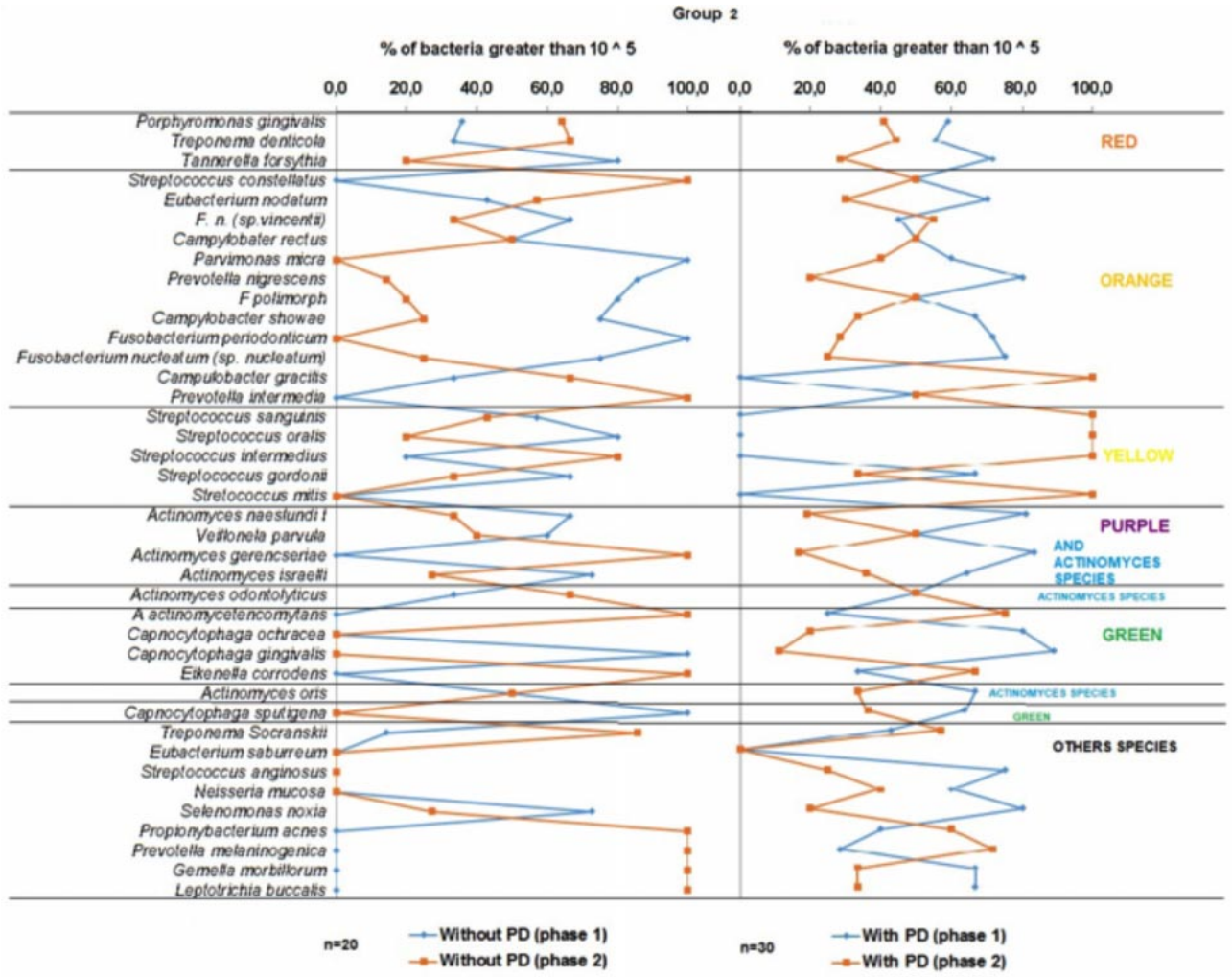

Fig. 2. Description (%) of counts (> 105 bacterial cells) of the 40 test species at Phase 1 and 2 for the Group without cancer in the two treatment groups (2A and 2B). The species were ordered according to the microbial complexes described by Socransky. The counts of individual species were averaged within a subject and then across subjects in each treatment group at each time point. The significance of differences between Phases was assessed using the Wilcoxon test (*p=0.05) compared to the amount of microbiota. Reference value: > 105 bacterial cells adjusted for 40 comparisons (Socransky *et al*., 1991).

correlation with *Treponema socranskii* (r=0.664), *Streptococcus anginosus* (r=0.668) and *Prevotella melaninogenica* (r=0.731). In group 1A positive correlations were revealed between CAL≥4mm and A. actinomycetemcomytans (r=0.468), *Eikenella corrodens* (r=0.437), and *Streptococcus anginosus* (r=0.411) which were moderately positive. Age revealed positive correlations with *Fusobacterium periodonticum* (r=0.539) which was moderately positive. The tooth loss showed a correlation with *Veillonela parvula* (r=0.441), *Eubacterium saburreum* (r=0.496), and *Prevotella melaninogenica* (r=0.526) which were moderately positive (Table I).

When evaluating group 2B, age had a significant correlation with *Treponema denticola* (r=0.451), *Eubacterium nodatum* (r=0.497), *Streptococcus intermedius* (r=0.451), *Actinomyces oris* (r=0.484), *Treponema socranskii* (r=0.519) and *Prevotella melaninogenica* (r=0.533), which was moderately positive. Tooth loss showed association with *Porphyromonas gingivalis* (r=0.537), Campylobacter shower (r=0.448), and *Selenomonas noxia* (r=0.521), which were moderately positive. In group 2A correlations of CAL≥4mm with F. polimorphum (r=0.382), and *Fusobacterium periodonticum* (r=0.570) were revealed, which were moderately positive. Age was correlated with *Capnocytophaga gingivalis* (r= 0.398) and *Gemella morbillorum* (r=0.369) which was moderately positive. Tooth loss was revealed to be associated with *Streptococcus anginosus* (r=0.402), which was moderately positive. In group 1B, age was correlated with *Actinomyces naeslundi* I (r=0.528) and was considered moderately positive. Tooth loss revealed an association with *Streptococcus intermedius* (r=0.432), *Actinomyces israelli* (r=0.419), and *Capnocytophaga ochracea* (r=0.510) which was moderately positive. In group 1A, CAL≥4 mm correlated with *Streptococcus mitis* (r=0.455) and *Actinomyces naeslundi* I (r=0.506), which were moderately positive. Age was associated with *Porphyromonas gingivalis* (r=0.438), *Actinomyces naeslundi* I (r=0.433), *Veillonela parvula* (r=0.439), and *Actinomyces israelli* (r=0.405), which were moderately positive (Table II).

Periodontal Conditions and Tumor Grade. Among participants in Group 1, the most significant association of tooth loss was identified in group 1B with tumor grade II (p=0.003) and tumor grade III (p=0.006) and it was increased in subjects, which occurred less frequently among the participants in Group 1A. Patients with tumor grade III and II had 4.8 and 3.5 times more risk of having tooth loss, respectively.

Subjects with CAL≥4mm and tumor grade II (p=0.005) and III (p=0.001) had a higher level of association in group 1B and a lower number of associations in group 1A. Thus, a patient with tumor grade II and III was at risk of having an increase in CAL of 0.5 and 0.4, respectively, when compared with a patient with tumor grade I (Table III).

DISCUSSION

This study investigated the subgingival microbiota in patients with BC. The bacterial count in groups 1A and 1B revealed the presence of all pathogens of the red, orange, and yellow complexes in P2 compared to P1, but in Groups 2A and 2B was different; all the pathogens of the red, and orange and yellow complexes were practically absent and reached smaller proportions in P2 compared to P1. Although bacteria of the yellow, green, and purple complexes, and Actinomyces species are usually compatible and associated with periodontal health, robust data tend to prove that these bacteria may be associated with PD or systemic diseases (Carrouel *et al*., 2016). The subgingival sulcus is a complex environment that harbors a highly diverse microbiota, so the complexes tend to reflect a succession of microbial events in developing dental biofilms from early to late colonizers (Mestnik *et al*., 2010).

CAL≥4mm increased with species from green complex, *Streptococcus anginosus*, and *Leptotrichia buccalis* in Groups 1A and 1B in Phase 1. *Leptotrichia* spp is associated with both disease and health in periodontitis (Fanas *et al*., 2021). These findings showed the predominance of pathogens considered to be non-aggressive in the groups studied correlating with CAL≥4mm, especially in the groups with cancer either exposed to periodontitis or not. *Streptococcus mitis* increased in groups 1A, and P2. This pathogen comprises one of the early settlers in DP (Mestnik *et al*., 2010) and has been associated with various types of cancer (Chocolatewala *et al*., 2010).

In P1, *Treponema denticola*, F. n. (sp.*vincentii*), *Campylobacter rectus* and other species increased in groups of patients without cancer and species like *Propionybacterium acnes* in group 1B and Fusobacterium periodontium in group 1A increased in correlation with age. More than 60 % of the participants in this study were over 50 years. Many studies confirm the presence of these species in

Table I. Correlation of CAL, Age, and Tooth Loss with Subgingival Microbiota in Phase 1.

PHASE 1	Group 2						Group 1					
	2B			2A			1B			1A		
	CAL≥4	Age	Tooth loss	CAL ≥ 4	Age	Tooth loss	CAL ≥ 4	Age	Tooth loss	CAL ≥ 4	Age	Tooth loss
Porphyromonas gingivalis	-0,047	-0,189	-0,180	0,071	-0,124	0,008	-0,114	0,140	-0,181	-0,075	0,053	0,210
Treponema denticola	-0,082	0,514*	-0,348	0,055	0,080	0,196	0,055	0,105	-0,154	0,150	-0,048	0,356
Tannerella forsythia	0,125	-0,387	-0,209	0,260	0,049	-0,035	-0,255	0,085	-0,271	-0,136	0,347	0,335
Streptococcus constellatus	0,021	-0,392	-0,091	-0,109	0,099	-0,199	-0,238	0,149	-0,349	0,056	0,158	0,214
Eubacterium nodatum	-0,062	-0,277	-0,128	-0,062	0,112	0,092	-0,113	-0,227	-0,213	-0,168	-0,120	0,290
F. n. (sp.vincentii)	-0,065	0,480*	-0,336	0,156	-0,006	-0,185	-0,011	0,386	-0,294	-0,038	0,138	0,303
Campylobacter rectus	-0,090	0,497*	-0,289	0,096	-0,009	-0,190	0,186	0,256	-0,261	-0,107	0,188	0,180
Parvimonas micra	0,055	-0,402	-0,351	0,165	0,118	-0,162	-0,187	0,243	-0,299	-0,074	-0,152	-0,141
Prevotella nigrescens	0,153	-0,381	-0,583**	0,109	0,150	-0,324	-0,302	-0,317	-0,502	-0,244	-0,253	0,305
F polimorphum	0,070	-0,101	0,176	0,271	0,139	-0,270	-0,114	0,227	-0,287	-0,324	0,119	0,150
Campylobacter showae	0,222	-0,406	0,126	0,156	0,173	-0,089	-0,079	0,113	-0,199	0,055	0,077	0,090
Fusobacterium periodonticum	0,214	-0,222	-0,398	0,096	0,126	-0,187	-0,068	0,288	-0,482	-0,075	0,539**	0,208
Fusobacterium nucleatum (sp. nucleatum)	0,141	0,056	0,050	0,113	0,056	-0,262	0,247	0,209	0,031	-0,043	-0,015	0,156
Campylobacter gracilis	0,020	-0,270	0,057	0,111	0,223	-0,152	-0,209	-0,026	0,062	-0,103	-0,142	0,098
Prevotella intermedia	0,150	-0,346	-0,029	0,023	0,023	-0,069	-0,452*	0,052	-0,271	-0,142	-0,007	0,251
Streptococcus sanguinis	0,140	-0,233	-0,118	0,228	0,090	-0,212	-0,441*	-0,164	-0,419	-0,335	-0,224	0,063
Streptococcus oralis	0,265	-0,149	0,081	0,182	0,118	-0,195	-0,222	0,126	-0,207	-0,096	0,045	0,231
Streptococcus intermedius	0,216	-0,199	-0,125	0,054	0,220	-0,302	-0,342	-0,122	-0,201	-0,335	-0,122	0,338
Streptococcus gordonii	0,051	-0,304	0,045	-0,038	0,066	0,011	-0,397*	-0,012	-0,131	-0,100	0,024	0,140
Streptococcus mitis	0,207	-0,323	-0,087	0,139	0,094	-0,280	-0,262	0,376	0,224	-0,207	0,021	0,192
Actinomyces oris	-0,084	-0,131	-0,220	0,091	-0,159	0,003	0,075	0,031	-0,143	0,067	-0,130	-0,110
Actinomyces naeslundi I	-0,016	0,035	0,149		-0,050	-0,341	0,327	-0,020	-0,255	0,244	-0,232	0,266
Veillonela parvula	0,190	0,092	0,169	0,175	0,081	-0,115	0,128	-0,021	0,513	0,235	0,008	0,441*
Actinomyces gerencseriae	0,060	-0,167	0,031	0,007	-0,013	-0,071	0,128	-0,186	0,564*	0,277	-0,200	0,336
Actinomyces israelli	0,325	-0,078	0,041	0,099	0,173	0,195	0,342	-0,150	-0,109	0,353	-0,302	0,310
Actinomyces odontolyticus	0,169	-0,244	-0,039	0,077	0,042	-0,235	-0,041	0,207	0,550*	0,361	-0,003	0,378
A actinomycetencomytans	0,121	-0,105	-0,147	0,140	0,210	-0,051	0,054	0,170	0,462	0,468*	-0,211	0,114
Capnocytophaga ochracea	-0,087	-0,199	0,003	0,257	0,077	-0,287	0,653**	0,192	-0,037	0,054	-0,239	0,163
Capnocytophaga gingivalis	0,187	-0,237	-0,337	0,100	-0,005	0,064	0,127	0,268	0,549*	0,335	-0,153	0,421
Eikenella corrodens	-0,025	0,471*	-0,237	0,116	0,117	-0,241	0,516**	0,322	0,293	0,437*	-0,151	0,321
Capnocytophaga sputigena	0,275	0,266	0,417	-0,117	0,022	-0,022	0,241	-0,123	-0,156	0,272	-0,035	-0,139
Treponema socranskii	-0,483*	0,466*	-0,062	0,118	-0,362*	-0,041	-0,116	0,061	0,664**	0,259	-0,209	0,108
Eubacterium saburreum	-0,039	-0,422	-0,147	0,092	0,144	-0,013	0,337	0,250	0,235	0,218	-0,195	0,496*
Streptococcus anginosus	0,507*	-0,089	-0,050	0,125	-0,105	-0,110	0,088	0,463*	0,668**	0,411*	-0,074	0,083
Neisseria mucosa	0,191	-0,152	0,096	0,136	-0,004	-0,226	0,151	0,295	0,515*	0,315	-0,201	0,316
Selenomonas noxia	0,173	0,048	0,225	0,143	0,498**	0,300	0,260	-0,008	0,161	0,109	-0,210	0,325
Propionybacterium acnes	-0,286	0,521*	-0,011	0,130	-0,399*	-0,032	0,327	0,498**	0,382	0,296	-0,303	0,180
Prevotella melaninogenica	0,455	-0,151	-0,038	0,132	0,043	-0,319	0,002	0,256	0,731**	0,166	0,023	0,526*
Gemella morbillorum	0,404	-0,171	0,051	-0,030	0,068	-0,248	0,191	0,034	0,019	0,143	-0,206	0,214
Leptotrichia buccalis	0,228	-0,277	-0,116	0,051	0,062	-0,239	0,481*	0,298	0,012	0,345	-0,314	0,105

individuals with periodontitis, but in patients without periodontitis, the findings are few. *Treponema denticola* was isolated in individuals without periodontitis (Dashper *et al*., 2011) *F. nucleatum* (sp. *vincentii* was described as a prevalent species in systemically healthy individuals with and without periodontitis (Socransky & Haffajee, 2002) and species such as Campylobacter were associated with healthy and periodontal disease (Macuch & Tanner, 2000).

Table II. Correlation of CAL, Age and Tooth loss with Subgingival Microbiota in Phase 2.

	Group 2						Group 1					
PHASE 2	2B			2A			1B			1A		
	CAL≥ 4mm	Age	Tooth loss	CAL≥ 4mm	Age	Tooth loss	CAL≥ 4mm	Age	Tooth loss	CAL≥ 4mm	Age	Tooth loss
Porphyromonas gingivalis	0,153	0,124	0,537*	0,069	0,223	0,029	0,111	0,208	-0,022	0,142	0,438*	0,307
Treponema denticola	-0,259	0,451*	0,002	0,018	0,127	-0,150	0,064	0,008	-0,019	-0,084	-0,196	-0,019
Tannerella forsythia	-0,283	-0,268	-0,417	0,013	0,010	-0,192	-0,014	-0,151	-0,032	-0,166	-0,103	-0,299
Streptococcus constellatus	-0,024	0,109	0,009	0,367	-0,160	0,027	-0,211	-0,283	0,243	0,234	0,251	-0,294
Eubacterium nodatum	-0,492*	0,497*	0,047	0,019	-0,148	0,242	-0,213	-0,260	-0,128	0,185	0,034	0,127
F. n. (sp.vincentii)	0,040	-0,131	0,094	0,234	-0,095	0,054	-0,430*	-0,010	-0,186	0,192	-0,027	0,044
Campylobacter rectus	-0,011	0,208	-0,297	-0,171	-0,190	0,104	-0,021	0,019	0,246	-0,160	0,026	0,134
Parvimonas micra	0,036	0,180	-0,324	0,019	0,127	-0,237	-0,117	-0,082	-0,008	0,092	0,093	0,137
Prevotella nigrescens	-0,328	0,044	-0,036	0,116	-0,032	0,110	-0,113	-0,233	0,298	0,267	0,214	-0,232
F polimorphum	0,292	-0,064	0,342	0,382*	-0,098	-0,113	-0,077	-0,248	0,277	0,004	0,286	-0,100
Campylobacter showae	-0,125	0,129	0,448*	-0,141	0,116	0,222	-0,092	-0,178	0,199	-0,150	0,110	-0,428*
Fusobacterium periodonticum	-0,380	-0,038	0,085	0,570**	-0,242	0,129	-0,304	0,133	0,375	0,227	0,277	0,162
Fusobacterium nucleatum (sp. nucleatum)	-0,135	0,414	0,081	0,028	-0,014	0,297	0,070	-0,218	0,359	-0,284	-0,258	-0,207
Campylobacter gracilis	-0,210	0,018	0,386	0,229	0,073	-0,153	-0,371	0,056	0,176	0,001	-0,100	0,046
Prevotella intermedia	-0,194	0,314	0,162	-0,009	0,060	0,054	-0,282	0,091	0,114	0,068	-0,079	-0,152
Streptococcus sanguinis	-0,360	0,036	-0,003	0,030	-0,069	-0,084	-0,152	-0,429*	0,128	0,232	0,071	-0,108
Streptococcus oralis	-0,162	-0,434	-0,309	0,148	-0,148	-0,171	-0,027	-0,434*	0,035	0,039	0,339	-0,257
Streptococcus intermedius	0,102	0,451*	0,411	0,294	-0,248	-0,344	-0,094	-0,208	0,432*	0,283	0,299	-0,243
Streptococcus gordonii	-0,405	-0,196	0,038	0,234	0,047	0,011	-0,358	-0,168	0,072	0,018	0,115	-0,457*
Streptococcus mitis	-0,214	0,333	0,219	-0,044	-0,078	-0,134	-0,227	-0,099	-0,150	0,455*	0,265	0,020
Actinomyces oris	-0,031	0,484*	0,385	0,018	0,045	0,283	-0,224	-0,054	0,126	0,003	0,138	-0,201
Actinomyces naeslundi I	-0,169	0,010	0,041	-0,036	-0,237	0,219	0,191	0,528**	0,302	0,506*	0,433*	-0,078
Veillonela parvula	0,064	-0,127	-0,203	0,041	-0,118	0,129	-0,165	0,101	0,183	0,231	0,439*	-0,255
Actinomyces gerencseriae	-0,123	0,396	0,020	0,171	-0,115	0,164	-0,102	-0,208	0,382	0,195	0,287	0,083
Actinomyces israelli	-0,223	0,202	-0,166	0,214	-0,009	0,205	-0,129	-0,182	0,419*	0,186	0,405*	-0,032
A actinomyces odontolyticus	-0,161	0,052	0,089	-0,018	0,167	-0,163	0,043	-0,442*	-0,171	0,186	0,252	0,189
A actinomycetencomytans	-0,054	0,041	-0,385	0,150	0,009	-0,004	-0,322	0,023	-0,156	-0,183	-0,157	-0,238
Capnocytophaga ochracea	0,018	0,049	-0,120	0,170	-0,056	0,234	-0,095	-0,143	0,510**	-0,064	0,073	0,166
Capnocytophaga gingivalis	-0,416	0,164	0,092	0,156	0,398*	0,133	-0,335	-0,083	0,276	-0,126	-0,041	-0,296
Eikenella corrodens	-0,339	-0,043	-0,112	-0,076	0,308	-0,054	-0,560**	0,097	-0,228	0,042	-0,242	0,021
Capnocytophaga sputigena	-0,092	-0,228	0,084	-0,254	0,202	-0,037	-0,231	0,029	0,232	0,168	0,277	0,268
Treponema socranskii	0,069	0,519*	0,096	-0,378*	0,126	0,165	-0,063	-0,052	0,037	0,283	0,287	-0,137
Eubacterium saburreum	-0,205	0,147	0,128	0,244	-0,188	0,229	0,072	-0,278	0,189	0,259	0,081	-0,114
Streptococcus anginosus	-0,219	0,109	-0,011	0,263	-0,057	0,402*	0,020	-0,059	-0,176	0,159	0,035	-0,177
Neisseria mucosa	0,073	0,275	0,051	-0,108	-0,153	0,014	-0,170	-0,248	0,124	-0,071	-0,179	-0,284
Selenomonas noxia	0,364	0,095	0,521*	-0,422*	0,076	0,186	0,058	-0,167	0,025	0,276	0,293	-0,001
Propionybacterium acnes	-0,117	0,242	-0,013	-0,253	0,234	-0,080	-0,049	-0,123	0,158	0,171	-0,047	-0,521*
Prevotella melaninogenica	-0,187	0,533*	-0,081	0,149	-0,051	0,163	-0,146	-0,051	0,279	-0,011	-0,099	-0,363
Gemella morbillorum	0,046	0,133	0,204	0,103	0,369*	0,332	-0,266	0,089	0,243	0,258	0,128	0,135
Leptotrichia buccalis	-0,094	0,115	-0,111	0,186	-0,098	0,172	-0,132	-0,193	0,338	-0,063	0,077	-0,133

Table III. Association of CAL and Tooth Loss with Tumor Grade.

	Tumor	Grade	RR	Robust Standart error	z	p*value	95% confidence interval for RR		Relation
							Inferior limit	Upper limit	
Tooth loss	1B groupe	I	1	-	-	-	-	-	-
		II	3,812	1,734	2,940	**0,003**	1,563	9,298	Increase
		III	4,500	2,461	2,750	**0,006**	1,540	1,314	Increase
	1A groupe	I	1	-	-	-	-	-	-
		III	1,355	0,358	1,150	0,250	0,807	2,275	Stable
CAL	1B groupe	I	1	-	-	-	-	-	-
		II	0,497	0,123	-2,820	**0,005**	0,305	0,808	Increase
		III	0,359	0,104	-3,520	**0,000**	0,203	0,635	Increase
	1A groupe	I	1	-	-	-	-	-	-
		III	0,819	0,159	-1,030	0,302	0,560	1,197	Stable

Elderly individuals are more susceptible to microbial infections than younger ones, which may further magnify the effects of inflammation among those patients. Inflammation and immunosenescence might contribute to the basis of the responses observed in elderly individuals to the periodontal bacterial challenge. Studies have confirmed that older individuals accumulate more biofilm (Feres *et al*., 2016) and can develop severe periodontitis. Tooth loss increased with Actinomyces species, purple and green complexes bacteria species, and other species such as *Eubacterium saburreum* in group 1A and *Treponema socranskii* in group 1B in P1. *Eubacterium saburreum* was found in infection with bacterial species of red, orange, and yellow complexes and these were associated with an increased likelihood of having oral bone loss as measured by alveolar crestal height (Brennan *et al*., 2007). *Actinomyces gerencseriae* can induce the destruction of soft and hard periodontal tissues (Vielkind *et al*., 2015) such as tooth loss. *T. socranskii* was frequently detected together with *T. denticola* or *P. gingivalis* and they are associated with the severity of periodontitis, however, few studies have examined the distribution and role of *T. socranskii* in periodontitis and cancer (Takeuchi *et al*., 2001). In P2, *Porphyromonas gingivalis*, Campylobacter showae increased in group 2B and *Streptococcus intermedius*, *Actinomyces israelli*, *Capnocytophaga ochracea* in group 1B. The species *P. gingivalis*, at low levels of colonization, remodeled a symbiotic community into a dysbiotic state, triggering inflammatory bone loss. However, it caused periodontitis in the absence of commensal bacterial species (Hajishengallis & Lambris, 2012).

Actinomyces israelli and Actinomyces species are not virulent, they break in the integrity of the mucous membranes and the presence of devitalized tissue to invade deeper body structures and cause human illness (Karpinski, 2019). Periodontal inflammation may contribute to several microbial successions during chemotherapy and pathogens such as Capnocytophaga may pose a systemic risk in individuals with cancer (Meurman, 2010). In P1, there was an increase in the correlation of age with *Streptococcus anginosus*, *Propionybacterium acnes* in group 1B, and *Fusobacterium periodonticum* in group 1A. In P2, there was an increase in the correlation of age with *Porphyromonas gingivalis*, *Treponema denticola* and some species in group 1A and *Treponema denticola* in group 2B. Key pathogens in periodontitis progression such as *Treponema denticola*, *Porphyromonas gingivalis*, and species of orange complex were found in groups 1A and 2B. The effects of human aging on periodontal tissues are based on biomolecular changes in periodontal ligament cells that exacerbate bone loss in elderly patients with periodontitis (Huttner *et al*., 2009). Studies have suggested that supragingival biofilm levels modify the effects of subgingival bacteria on CAL in older adult women (Tezal *et al*., 2006).

Studies have demonstrated that the Actinomyces species were in significantly higher proportions in the subgingival biofilm of subjects over 60 years of age. On the other hand, the prevalence of subjects colonized by *P. gingivalis* increased with aging. In addition, the decline in function of the immune system is brought on by natural age advancement, generally referred to as “immunosenescence” (Karpinski, 2019). The bacterial profiles of periodontally healthy elders might be more diverse than those of young and middle-aged adults (Feres *et al*., 2016). V. parvula is present in the mouth flora and is an important pathogen of periodontitis (Matera *et al*., 2009). Chocolatewala *et al*. (2010) concluded in a review that studies showed diversities of species such as V. parvula in tumor tissues. Yang *et al*. (2018), in investigations, found species of V. parvula and Actinomyces in abundance in oral squamous cell carcinoma. Concerning the Actinomyces species, this species co-aggregates with *E. corrodens* from the green

complex through the GalNAc-specific lectin, which stimulates the mitogenic activity of B lymphocytes (Carrouel *et al*., 2016) such as *F. nucleatum* in breast tumor samples correlated with high levels of GalNAc-specific lectin (Parhi *et al*., 2020). The persistent accumulation of species *Propionybacterium acnes* can lead to resistance to phagocytosis in several organs and tissues, culminating in a chronic inflammatory response and the ability to produce enzymes that facilitate tissue destruction in periodontitis (Eady & Eileen, 1994).

Streptococcus anginosus revealed correlations with tooth loss and increased with age (Ammann *et al*., 2013) as showed in groups 1B and CAL≥4mm in group 1A. *Selenomonas noxia* and *Streptococcus anginosus* have been identified in patients with severe or aggressive periodontitis (Dhillon *et al*., 2019). Concerning the green complex, our study showed that in healthy periodontal subjects, *C. ochracea* and *C. sputigena* were expressed at lower levels than those found in a previous study (Henne *et al*., 2014). A. gerencseriae is able to induce soft and hard tissue destruction (Vielkind *et al*., 2015) such as tooth loss in group 1B in P1. Our study highlighted *E. corrodens* and *C. ochracea* which are correlated with several pathogens and they are the first bacteria to constitute the supragingival and subgingival biofilms and are evidence of periodontally healthy patients (Teles *et al*., 2013). In a cross-sectional study of women with BC, the five bacterial species were found at the highest levels such *Actinomyces gerencseriae* and *Neisseria mucosa* (Lopes *et al*., 2019). Three bacterial species (*Capnocytophaga gingivalis*, *Prevotella melaninogenica*, and *Streptococcus mitis*) were suggested as diagnostic markers and were found to predict 80 % of cancer cases (Karpinski *et al*., 2019).

The analysis of tumor grade revealed the prevalence of tooth loss and CAL in Group 1B in comparison to Group 1A. Some differences were observed with regard to tooth loss which was shown to be higher after clinical follow-up and periodontal therapy. Longitudinal monitoring of these subjects will be important in order to determine whether the therapy would produce sustained beneficial changes in the subgingival microbial profile and periodontal clinical parameters over time. The correlation between cancer and periodontitis depends on the presence of specific pathogens present in subgingival biofilm along with CAL and tooth loss analyses that characterized the advancement of periodontitis and the short- term changes in the microbiota profile may to define the periodontal clinical stability.

Final considerations

It can be concluded from this study that: We showed a remarkable correlation of periodontal clinical conditions with the development of periodontitis, and surprisingly, its relationship with the level of aggressiveness of the tumor. Periodontal disease has progressed even with basic periodontal therapy, which suggests that prevention or treatment of periodontitis in cancer patients should also consider the effects of antineoplastics on periodontal tissues. This study, despite limitations, sought to establish causality between periodontal disease and breast cancer, since the temporal relationship between exposure and the result is difficult to determine and contributes to greater dissemination of the subgingival microbiota in populations with cancer, even for patients without established risk factors. An understanding of the mechanisms involved in the onset and progression of periodontal diseases could greatly help to establish effective ways to prevent and treat these diseases and decrease the risk factors for relevant systemic disorders.

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RESUMEN: El objetivo de este estudio fue comparar la microbiota subgingival de sujetos con y sin cáncer de mama (CM). Pacientes con CM (Grupo 1; n= 50) y sin CM (Grupo 2; n=50) con periodontitis (A) y sin periodontitis (B). El estudio se realizó en das fases (P1 y P2). Se recogió una muestra de biopelícula de cada sujeto y se analizó mediante hibridación ADN-ADN (tablero de ajedrez ADN-ADN). La abundancia relativa de la microbiota subgingival difirió entre

los grupos de Caso y Control. Sin embargo, algunas especies fueron más altas en los pacientes del Caso que en los sujetos del Control y difirieron entre los grupos en ambas fases. La composición de la comunidad microbiana subgingival según el complejo de Socransky se relacionó con la enfermedad periodontal, seguida por el nivel de inserción clínica (CAL≥4mm), la edad y la pérdida de dientes, que se mostró abundante en los casos en comparación con los controles. Los pacientes con Tumor Grado II y III tuvieron mayor prevalencia de pérdida dental y CAL≥4mm. Se concluyó que en individuos con CM la microbiota subgingival presentó cambios atípicos, pero sin embargo, desarrollaron enfermedad periodontal.

PALABRAS CLAVE: microbiota subgingival, enfermedad periodontal, tumores de mama, hibridación ADN-ADN, investigación dental.

REFERENCES

Ammann, T. W.; Belibasakis, G. N. & Thurnheer, T. Impact of early colonizers on in vitro subgingival biofilm formation. *PLoS One, 8(12)*:e83090, 2013.

Arora, M.; Weuve, J.; Fall, K.; Pedersen, N. L. & Mucci, L. A. An exploration of shared genetic risk factors between periodontal disease and cancers: a prospective co-twin study. *Am. J. Epidemiol., 171(2)*:253-9, 2010.

Brennan, R. M.; Genco, R. J.; Wilding, G. E.; Hovey, K. M.; Trevisan, M. & Wactawski-Wende, J. Bacterial species in subgingival plaque and oral bone loss in postmenopausal women. *J. Periodontol., 78(6)*:1051-61, 2007.

Carrouel, F.; Viennot, S.; Santamaria, J.; Veber, P. & Bourgeois, D. Quantitative molecular detection of 19 major pathogens in the interdental biofilm of periodontally healthy young adults. F*ront. Microbiol., 7*:840, 2016.

Chocolatewala, N.; Chaturvedi, P. & Desale, R. The role of bacteria in oral cancer. *Indian J. Med. Paediatr. Oncol., 3(4)*:126-31, 2010.

Darveau, R. P. Periodontitis: a polymicrobial disruption of host homeostasis. *Nat. Rev. Microbiol., 8(7)*:481-90, 2010.

Dashper, S. G.; Seers, C. A.; Tan, K. H. & Reynolds, E. C. Virulence factors of the oral spirochete *Treponema denticola*. *J. Dent. Res., 90(6)*:691-703, 2011.

Dhillon, N.; Kingsley, K. & Howard, K.M. Prevalence of *Selenomonas noxia* among pediatric and adult orthodontic patients. *Int. J. Res. Rep. Dent., 2(1)*:1-7, 2019.

Dowsett, M. *et al*. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. *Journal of the National Cancer Institute, 103(22)*:1656-1664, 2011.

Eady, E. A. & Eileen, I. Propionibacterium acnes - friend or foe? *Rev. Med. Microbiol., 5(3)*:163-73, 1994.

Elston, C. W. & Ellis, O. Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. *Histopathology, 19(5)*:403-10, 1991.

Fanas, S. A.; Brigi, C.; Varma, S. R.; Desai, V.; Senok, A. & D'Souza, J. The prevalence of novel periodontal pathogens and bacterial complexes in Stage II generalized periodontitis based on 16S rRNA next-generation sequencing. *J. Appl. Oral Sci., 29*:e20200787, 2021.

Feres, M.; Teles, F.; Teles, R.; Figueiredo, L. C. & Faveri, M. The subgingival periodontal microbiota of the aging mouth. *Periodontol. 2000, 72(1)*:30-53, 2016.

Hajishengallis, G. & Lambris, J. D. Complement and dysbiosis in periodontal disease. *Immunobiology, 217(11)*:1111-6, 2012.

Henne, K.; Fuchs, F.; Kruth, S.; Horz, H. P. & Conrads, G. Shifts in Campylobacter species abundance may reflect general microbial community shifts in periodontitis progression. *J. Oral Microbiol., 6*:25874, 2014.

Huttner, E. A.; Machado, D. C.; de Oliveira, R. B.; Antunes, A. G. & Hebling, E. Effects of human aging on periodontal tissues. *Spec. Care Dentist., 29(4)*:149-55, 2009.

Karpin´ski, T. M. Role of oral microbiota in cancer development. *Microorganisms, 7(1)*:20, 2019.

Kinane, D. F.; Stathopoulou, P. G. & Papapanou, P. N. Periodontal diseases. *Nat. Rev. Dis. Primers, 3*:17038, 2017.

Lopes, G. R. S.; Feitosa, A. C. R.; Suaid, F. F.; Matos, J. D. M.; Vasconcelos, J. E. L.; Vaz, S. L. A.; Andrade, V. C.; Nishioka, R. S. & Guerra, S. M. G. Evaluation of peri-implant condition in periodontally compromised patients. *J. Indian Prosthodont. Soc., 19(4)*:283-9, 2019.

Macuch, P. J. & Tanner, A. C. Campylobacter species in health, gingivitis and periodontitis. *J. Dent. Res., 79(2)*:785-92, 2000.

Mantovani, A.; Allavena, P.; Sica, A. & Balkwill, F. Cancer-related inflammation. *Nature, 454(7203)*:436-44, 2008.

Matera, G.; Muto, V.; Vinci, M.; Zicca, E.; Abdollahi-Roodsaz, S.; van de Veerdonk, F. L.; Kullberg, B. J.; Liberto, M. C.; van der Meer, J. W.; Focà, A.; *et al*. Receptor recognition of and immune intracellular pathways for Veillonella parvula lipopolysaccharide. *Clin. Vaccine Immunol., 16(12)*:1804-9, 2009.

Mestnik, M. J.; Feres, M.; Figueiredo, L. C.; Duarte, P. M.; Lira, E. A. G. & Faveri, M. Short-term benefits of the adjunctive use of metronidazole plus amoxicillin in the microbial profile and in the clinical parameters of subjects with generalized aggressive periodontitis. *J. Clin. Periodontol, 37(4)*:353-65, 2010.

Meurman, J. H. *Oral microbiota and cancer*. J. Oral Microbiol., 2, 2010. DOI: https://www.doi.org/10.3402/jom.v2i0.5195

Meyer, M. S.; Joshipura, K.; Giovanucci, E. & Michaud, D. S. A review of the relationship between tooth loss, periodontal disease, and cancer. *Cancer Causes Control, 19(9)*:895-907, 2008.

Michaud, D. S.; Joshipura, K.; Giovannucci, E. & Fuchs, C. S. A prospective study of periodontal disease and pancreatic cancer in US male health professionals. *J. Natl. Cancer Inst., 99(2)*:171-5, 2007.

Michaud, D. S.; Liu, Y.; Meyer, M.; Giovannucci, E. & Joshipur, K. Periodontal disease, tooth loss and cancer risk in male health professionals: a prospective cohort study. *Lancet Oncol., 9(6)*:550-8, 2008.

Parhi, L.; Alon-Maimom, T.; Sol, A.; Nejman, D.; Shhadeh, A.; Fainsod-Levi, T.; Yajuk, O.; Isaacson, B.; Abed, J.; Maalouf, N.; *et al*. Breast cancer colonization by *Fusobacterium nucleatum* accelerates tumor growth and metastatic progression. *Nat. Comun., 11(1)*:3259, 2020.

Socransky, S. S. & Haffajee, A. D. Dental biofilms: difficult therapeutic targets. *Periodontol. 2000, 28*:12-55, 2002.

Socransky, S. S.; Haffajee, A. D.; Smith, C. & Dibart, S. Relation of counts of microbial species to clinical status at the sampled site. *J. Clin. Periodontol., 18(10)*:766-75, 1991.

Socransky, S. S.; Smith, C.; Martin, L.; Paster, B. J.; Dewhirst, F. E. & Levin, A. E. "Checkerboard" DNA-DNA hybridization. *Biotechniques, 17(4)*:788-92, 1994.

Söder, B.; Yakob, M.; Meurman, J. H.; Andersson, L. C.; Björn, K. & Söder, P. Ö. Periodontal disease may associate with breast cancer. *Breast Cancer Res. Treat., 127(2)*:497-502, 2011.

Takeuchi, Y.; Umeda, M.; Sakamoto, M.; Benno, Y.; Huang, Y. & Ishikawa, I. *Treponema socranskii*, *Treponema denticola*, and *Porphyromonas gingivalis* are associated with severity of periodontal tissue destruction. *J. Periodontol., 72(10)*:1354-63, 2001.

Teles, R.; Teles, F.; Frias-Lopez, J.; Paster, B. & Haffajee, A. Lessons learned and unlearned in periodontal microbiology. *Periodontol. 2000, 62(1)*:95-162, 2013.

Tezal, M.; Scannapieco, F. A.; Wactawski-Wende, J.; Grossi, S. G. & Genco, R. J. Supragingival plaque may modify the effects of subgingival bacteria on attachment loss. *J. Periodontol., 77(5)*:808-13, 2006.

Viale, G. The current state of breast cancer classification. *Ann. Oncol., 23 Suppl. 10*:x207-10, 2012.

Vielkind, P.; Jentsch, H.; Eschrich, K.; Rodloff, A. C. & Stingu, C. S. Prevalence of Actinomyces spp. in patients with chronic periodontitis. *Int. J. Med. Microbiol., 305(7)*:682-8, 2015.

Von Elm, E.; Altman, D. G.; Egger, M.; Pocock, S. J. & Gøtzsche, P. C.; Vandenbroucke, J. P. & STROBE Initiative. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Ann. Intern. Med., 147(8)*:573-7, 2007.

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