

Oral Microbiota, Periodontal Status, and Osteoporosis in Postmenopausal Females

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Background: Alterations in hormone levels during menopause decrease bone density and may worsen oral health, favoring the growth of periodontal pathogens, whose detection could improve the diagnosis of periodontitis. The aim of this study is to detect and quantify the main periodontal pathogens in the oral microbiota of postmenopausal females and to explore the relationship between clinical and periodontal parameters.

Methods: This was an observational cross-sectional study of 76 postmenopausal females. Dental examinations and sampling for microbiologic evaluation were performed, and a history of osteoporosis/osteopenia was collected. Real-time polymerase chain reaction was used for detecting and quantifying *Fusobacterium nucleatum* (*Fn*), *Aggregatibacter actinomycetemcomitans* (*Aa*), *Porphyromonas gingivalis* (*Pg*), *Campylobacter rectus* (*Cr*), and *Tannerella forsythia* (*Tf*). The results obtained were subjected to statistical analyses. Statistical significance was defined as $P < 0.05$.

Results: Periodontitis was detected in 77.1% of females with osteoporosis/osteopenia ($P > 0.05$). A significant correlation was found between osteoporosis and missing teeth. *T. forsythia* and *C. rectus* were detected in 100% of the samples, *Fn* and *Pg* in 98.7%, and *Aa* in 73.7%.

Conclusions: Osteoporosis did not influence the prevalence of periodontitis among postmenopausal females. The presence of periodontopathogenic bacteria was not sufficient to confirm disease. A preventive maintenance program for postmenopausal females, particularly osteoporotic females, who are at greater risk of tooth loss, could minimize the potential effects of bone loss on periodontal tissues. *J Periodontol* 2016;87:124-133.

KEY WORDS

Bacteria, anaerobic; oral health; osteoporosis Periodontal diseases; postmenopause; real-time polymerase chain reaction.

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Menopause is the permanent cessation of menstrual periods, whether naturally or induced by surgery, chemotherapy, or radiation. It is accompanied by hormonal changes, most prominently decreases in estrogen and androgen levels, which produce a variety of signs and symptoms: 1) vasomotor symptoms; 2) decreased bone density; 3) hot flashes; 4) profuse perspiration; 5) atrophic vaginitis; and 6) oral cavity alterations.¹

The oral symptoms during menopause include dry mouth (xerostomia), burning mouth, and changes in taste perception and are a common complaint of postmenopausal females.² If xerostomia is left untreated, it may change the oral pH and increase the risk of developing oral diseases. These symptoms have been attributed to reduced salivary flow, and oral pathologies seem to be affected by changes in salivary and gingival crevicular fluid (GCF) composition.³ Saliva contributes to maintenance of the oral pH by neutralizing acids from food and beverages, as well as from bacterial activity, thereby reducing the risk of periodontal disease.³ After menopause, reduced salivary flow rate and low pH may make females more prone to oral health problems.⁴ Decreased estrogen levels, in addition to worsening periodontal conditions, increase the risk of osteoporosis in postmenopausal females.³ Osteoporosis is a systemic skeletal disease characterized by a generalized and progressive loss of bone mineral density (BMD) and an

increased propensity to fracture.^{5,6} Its prevalence increases with age, and it affects all bones of the body, including the jaw bone, resulting in cortical thinning and a reduction of cancellous bone.⁶

For females, in addition to age, the menopausal transition itself causes a period of rapid bone loss (12% to 20% in the first 5 years after menopause) such that the risk of fracture in postmenopausal females is higher than in males of the same age.⁷

The changes in bone composition also have implications for oral health,⁸ including the risk of enhanced progression of periodontitis.^{5,6} Thus, bone loss is a feature shared by periodontal disease and osteoporosis. The relationship between the skeletal loss of BMD and the increased periodontal bone loss has been ascribed to several factors.⁵ Greater periodontal bone loss may occur simply because the bone surrounding the teeth is less dense and therefore less resistant to resorption.⁵ In addition, the loss of alveolar BMD may leave bone more susceptible to periodontal bacteria, increasing the risk for periodontitis and tooth loss.⁹

Osteoporotic females show greater clinical attachment loss and gingival recession than females with a normal BMD, which suggests a greater severity of periodontitis.¹⁰ The early diagnosis of reduced BMD, before the establishment of a significant negative influence on the periodontal tissues, has been recommended.⁹

The pathogenic microbial population involved in periodontitis is highly complex. However, the literature contains reports of associations among periodontal disease and certain microorganisms, such as *Aggregatibacter actinomycetemcomitans* (Aa), *Prevotella intermedia* (Pi), *Porphyromonas gingivalis* (Pg), *Campylobacter rectus* (Cr), *Treponema denticola*, *Fusobacterium nucleatum* (Fn), and *Tannerella forsythia* (Tf).^{11,12} A quantitative analysis of these periodontal pathogens could improve both the diagnosis and evaluation of periodontitis and the understanding of its relationship to osteoporosis.

The aim of this study is to detect and quantify the main periodontal pathogens in the oral microbiota of postmenopausal females to explore the relation between the microbiota and clinical and periodontal parameters. The measurement of pH was done as a general parameter because it is related strongly with consumption of sugar and acidic beverages, xerostomia, and oral microbiota.³

MATERIALS AND METHODS

Study Population

This is an observational cross-sectional study of 76 postmenopausal females who were treated at the Dental Hospital of the University of Barcelona, Barcelona, Spain. Dental examinations and sampling for microbiologic

evaluation were performed between May 2009 and September 2012.

The study population was selected from 694 females who participated in a questionnaire related to fracture risk factors in the Rheumatology Service of the University Hospital of Bellvitge, Hospitalet, Barcelona, Spain, in 1999. Only 137 of the 427 females who could be contacted agreed to participate. Subgingival samples were collected from 76 of these females, who were finally included in the study (aged 40 to 71 years; median age: 63 years). None of them were under specific estrogen replacement treatment.

The study was approved by the Ethical Committee of the Faculty of Dentistry, University of Barcelona. The study was conducted in accordance with the Declaration of Helsinki of 1975 (revised in 2013). All of the patients were informed about the objectives and methodology and provided written informed consent to participate in the study.

Oral Examination

All of the participants received a complete oral examination. The following variables were assessed: 1) number of missing teeth, not including third molars (28 maximum); 2) probing depth (PD), assessed with a World Health Organization (WHO) probe in all teeth; 3) oral hygiene status, determined according to the criteria of the index of Greene and Vermillion;¹³ 4) the carious, absent, and obturated teeth (CAOD) index, which was obtained by summing carious, missing, and obturated teeth and dividing by the total number of teeth; 5) the presence of mucosal injury; 6) periodontal treatment needs, examined by using the Community Periodontal Index of Treatment Needs recommended by the WHO; 7) oral pH, measured by using pH indicator strips[¶] that were placed on the floor of the mouth; and 8) xerostomia, determined by using a specific questionnaire and performing the test of the mirror (sticking of a dental mirror to the buccal mucosa was taken as a positive result for xerostomia).

Diagnosis of Osteoporosis/Osteopenia

Osteopenia was assessed from densitometry reports. The methods and criteria used in the diagnosis of osteoporosis were those established by the WHO.¹⁴ According to these criteria, osteoporosis is defined as BMD ≥ 2.5 standard deviations (SDs) below the average value for young healthy females (T score ≤ 2.5 SDs), and osteopenia is defined as 1 to 2.5 SDs (T score between -1.0 and -2.5 SDs). Association between osteopenia and oral bone loss and between osteopenia and oral state were accomplished as recommended in the literature.^{15,16}

¶ Merck, Darmstadt, Germany

Diagnosis of Periodontitis

Females were considered to have periodontitis if they met the criteria established by the American Academy of Periodontology, which state that the patient must have at least one site with PD and clinical attachment level ≥ 4 mm.¹⁷

Specimen Collection

Subgingival samples were taken after supragingival plaque removal. Two paper points (size 30)[#] were inserted in the deepest periodontal pocket for 20 seconds and placed in labeled sterile test tubes containing 500 μ L bidistilled water. The vials were immediately frozen at -80°C . Despite the limitation derived from the sampling of only one pocket, the samples were harvested from the deepest one, because deeper pockets offer a better environment for anaerobes and are useful as a general parameter.

Reference Bacterial Strains

Standard reference strains of *Fn* DMSZ 20482, *Aa* DSMZ 8,324, *Pg* ATCC 33277, *Cr* ATCC 33238, and *Tf* ATCC 43037 were used.

DNA Extraction

The tubes containing the paper points were thawed, vortexed for 2 to 3 minutes, and then centrifuged at 14,000 rpm for 10 minutes. The pellet was processed with a commercial DNA extraction kit** following the protocol of the manufacturer for Gram-negative bacteria. DNA purity and concentrations were determined spectrophotometrically using a spectrophotometer.^{††} DNA from the controls (reference bacterial strains) was obtained using the same method. Purified genomic DNA of *Pg*, *Aa*, *Tf*, *Cr*, and *Fn* was obtained from 1 mL viable bacteria containing 10^8 , 10^9 , 10^8 , 10^6 , and 10^6 colony forming units (CFU)/mL, respectively. Ten-fold serial dilutions of each DNA were prepared in sterile water.^{‡‡} All DNA samples were frozen at -20°C until used.

Quantitative Real-Time Polymerase Chain Reaction

Primers and probes. The primers^{§§} and probes^{|||} sequences used to target the 16S ribosomal ribonucleic acid (rRNA) gene are listed in Table 1.^{18,19} The oligonucleotide probes were labeled at the 5' end with fluorescent 6-carboxyfluorescein and at the 3' end with 6-carboxytetramethylrhodamine.

Quantitative real-time polymerase chain reaction conditions. Polymerase chain reaction (PCR) amplification was performed in a total reaction mixture volume of 10 μ L, containing 8 μ L of 2 \times master mixture^{¶¶} and 2 μ L DNA from the samples. Each DNA sample was analyzed in duplicate. The no template control (NTC) consisted of 8 μ L master mixture without DNA. The samples were placed in white 384-well plates^{##} and subjected to an initial amplification cycle

of 95°C for 10 minutes, followed by 40 cycles at 95°C for 15 seconds and 60°C for 1 minute in a thermocycler.^{***} To avoid carryover DNA, the laboratory was separated into pre-PCR and post-PCR areas, and strict barrier measures were used. However, none of these methods prevented the amplification of unspecific bacteria in some of the NTCs.

Data analyses. Quantification cycle (Cq) values were determined using a software package.^{†††}

Quantitation of the DNA samples was based on accurate standard curves for each bacterium in each run, using appropriate external standards of known concentration. Standard curves were constructed by plotting the Cq values generated from the quantitative PCR (qPCR) against *Pg*, *Tf*, *Cr*, *Fn*, and *Aa* (see supplementary Figures 1 through 5 in online *Journal of Periodontology*) and total cell concentrations (log CFU/mL). The correlation between Cq values and CFU/mL was automatically generated.

All assays were developed with a linear quantitative detection range established by a slope of 3.2 to 3.7 cycles/log decade, $r^2 > 0.994$ and an efficiency range of 1.8 to 2.0. The limit of detection was calculated using the Cq value from the last point on the standard curve that differed by 3 units from the lowest Cq value of the NTC. Values below this detection range may be detectable, but they are not quantifiable. Samples were considered positive when the Cq values were above the detection limit.

Statistical Analyses

A descriptive statistical analysis was performed based on the mean or median according to normality criteria (Kolmogorov–Smirnov test) and the SD or interquartile range (IQR), respectively. A bivariate analysis was performed using the χ^2 test for the comparison of qualitative variables. Student *t* test, analysis of variance, the Mann–Whitney *U* test, or the Kruskal–Wallis test for quantitative variables was used according to normality criteria and number of categories studied. Pearson or Spearman correlation tests were used to compare quantitative variables when appropriate. A multivariate analysis using binary logistic regression was conducted to determine the association between osteoporosis and periodontitis. The statistical significance of the two-way interactions between BMD and other variables in the main effects model was assessed using forward stepwise regression

DENTSPLY Maillefer, Ballaigues, Switzerland.

** DNeasy blood and tissue kit, Qiagen, Hilden, Germany.

†† NanoDrop 1000 spectrophotometer, Thermo Fisher Scientific, Waltham, MA.

‡‡ PCR grade water, Roche Diagnostics, Mannheim, Germany.

§§ Thermo Fisher Scientific.

||| Thermo Fisher Scientific.

¶¶ LightCycler 480 Probes Master, Roche Diagnostics.

LightCycler 480 Multiwell Plate 384, Roche Diagnostics.

*** LightCycler 480 II, Roche Diagnostics.

††† LightCycler 480 Software v.1.5, Roche Diagnostics.

Table 1.
Primers and Probes Used for Quantification of Genomic DNA From the Target Bacteria

Sequence (5'-3')	Concentration (nM)	Target gene	Reference
<i>Aa</i> Forward, GAA CCT TAC CTA CTC TTG ACA TCC GAA Reverse, TGC AGC ACC TGT CTC AAA GC Probe, AGA ACT CAG AGA TGG GTT TGT GCC TTA GGG	Primers, 300 Probe, 100	16S rRNA (80 bp)	Boutaga et al. ¹⁸
<i>Pg</i> Forward, GCG CTC AAC GTT CAG CC Reverse, CAC GAA TTC CGC CTG C Probe, CAC TGA ACT CAA GCC CGG CAG TTT CAA	Primers, 300 Probe, 300	16S rRNA (67 bp)	Boutaga et al. ¹⁸
<i>Cr</i> Forward, TTT CGG AGC GTA AAC TCC TTT TC Reverse, CGC TTG CAC CCT CCG TAT Probe, TCC GTG CCA GCA GCC GC	Primers, 900 Probe, 100	16S rRNA	Figuro et al. ¹⁹
<i>Tf</i> Forward, GGG TGA GTA ACG CGT ATG TAA CCT Reverse, ACC CAT CCG CAA CCA ATA AA Probe, CCC GCA ACA GAG GGA TAA CCC GG	Primers, 300 Probe, 200	16S rRNA	Boutaga et al. ¹⁸
<i>Fn</i> Forward, GGA TTT ATT GGG CGT AAA GC Reverse, GGC ATT CCT ACA AAT ATC TAC GAA Probe, CTC TAC ACT TGT AGT TCC G	Primers, 600 Probe, 300	16S rRNA (162 bp)	Boutaga et al. ¹⁸

Primers and probes targeted the 16S rRNA gene.

to test whether other variables modified the effect of BMD on periodontitis. To select the most accurate model, the area under the curve of each model was analyzed according to the receiving operating characteristics curves. A statistical package^{†††} was used for all statistical analyses. Statistical significance was defined as $P < 0.05$.

RESULTS

The general characteristics and relevant variables regarding the oral health status of the 76 females who participated in the study are listed in Table 2. For some females, the data were incomplete because they could not attend all sessions of data collection. All participants were kept in the analysis because the percentage of missing data was <10% of the total for all variables.

The participants had a median age of 63.0 years (IQR = 2). Periodontitis was detected in 47.9% of the patients, and 41.1% required periodontal treatment. Most (78.6%) had been diagnosed previously with osteopenia or osteoporosis.

Table 3 provides a cross-tabulation of periodontal disease versus osteoporosis, age, number of missing teeth, oral pH, CAOD index, need for treatment, oral hygiene, xerostomia, and mucosal injury. Periodontitis was detected in 22.9% of the females with no previous diagnosis of osteopenia/osteoporosis, in 57.1% of the females with osteopenia, and in 20% of those with osteoporosis, although there was no association between osteoporosis/osteopenia and periodontitis ($P = 0.52$). There was a significant correlation between periodontitis and oral hygiene ($P = 0.003$) and between periodontitis and the need for treatment ($P = 0.001$). The associations between periodontitis and missing teeth and between periodontitis and xerostomia were not significant ($P = 0.12$ and $P = 0.10$, respectively).

An analysis of the correlation among osteoporosis and the same clinical variables (data not shown) showed that, among the two groups (normal/osteopenia and osteoporosis), only the relation to the number of missing teeth was significant ($P < 0.05$).

††† SPSS v.17.0 for Windows, IBM, Chicago, IL.

Table 2.
Characteristics of the Patients (n = 76)

Variable	Value
Age (years), median (IQR)	63.00 (2.00)
Missing teeth, median (IQR)	5.00 (8.75)
CAOD index, mean (SD)	17.21 (8.00)
Treatment need, n (%)*	
No	5 (6.8)
Profound hygiene	38 (52.1)
Periodontal treatment	30 (41.1)
Periodontitis, n (%)*	
Negative	38 (52.1)
Positive	35 (47.9)
Mucosal injury, n (%)*	
No	45 (61.6)
Yes	28 (38.4)
Xerostomia, n (%)*	
No	67 (91.8)
Yes	6 (8.2)
pH, median (IQR)	7.0 (1.0)
pH, n (%)*	
Basic (>7)	14 (20.3)
Neutral (6.5 to 7)	27 (39.1)
Acid (<6.5)	28 (40.6)
Oral hygiene, n (%)*	
Good	24 (32.9)
Regular	32 (43.8)
Bad	17 (23.3)
Osteoporosis, n (%)*	
No	16 (21.3)
Osteopenia	40 (53.3)
Osteoporosis	19 (25.3)

* Information missing for some patients.

The most prevalent bacteria were *Tf* and *Cr*, which were detected in 100% of the samples, followed by *Fn* and *Pg* (98.7% in both cases) and *Aa* (73.7%). It should be highlighted that, in all cases but *Pg*, it was feasible to quantify bacteria when present. *Pg* was quantifiable in only 84.2% of the samples, because in 14.5% the value was below the minimum needed to quantify. Quantification of periodontal bacteria according to the presence or absence of periodontitis is reported in Table 4.

The box plot in Figure 1A shows the distribution of the five periodontal bacteria according to the presence or absence of periodontitis; the differences were statistically non-significant ($P > 0.05$). The box plot in Figure 1B shows the distribution of bacteria in osteo-

porotic, osteopenic, and normal patients. Again, the differences between the groups were not significant ($P > 0.05$).

Scatter plots were used to analyze the relationship between the periodontal pathogens *Fn*, *Tf*, *Cr*, *Pg*, and *Aa*, and non-parametric Spearman correlation coefficients were calculated (Table 5). Statistically significant positive relationships were found between log *Cr* and log *Fn* ($r = 0.815$, $P < 0.001$) and log *Tf* and log *Fn* ($r = 0.510$, $P < 0.001$). Other significant relationships among bacteria included a moderately positive relationship between log *Pg* and log *Tf* ($r = 0.410$, $P < 0.001$) and slightly positive relationship between log *Tf* and log *Cr* ($r = 0.381$, $P = 0.001$), log *Pg* and log *Cr* ($r = 0.328$, $P = 0.004$), log *Pg* and log *Fn* ($r = 0.268$, $P = 0.02$), and log *Aa* and log *Tf* ($r = 0.273$, $P = 0.04$).

Spearman correlation was used to compare periodontal pathogens and oral parameters. A significant highly positive relationship was found between missing teeth and CAOD index ($r = 0.770$, $P < 0.001$) and a slightly positive relation between log *Pg* and pH ($r = 0.285$, $P = 0.02$).

Analysis of the associations between clinical variables and pH values (data not shown) failed to demonstrate a significant correlation between oral hygiene and pH ($P = 0.50$). Only the differences among basic, neutral, and acidic pH groups and the log CFU/mL of *Aa* were significant ($P = 0.01$). For *Pg*, the P value was 0.053.

DISCUSSION

According to the present findings, the presence of osteoporosis/osteopenia does not influence the prevalence of periodontitis among postmenopausal females. The criteria for the diagnosis of periodontitis were clinical examination parameters. In a systematic review by Martínez-Maestre et al.,²⁰ five of nine studies based on the same criteria concluded that the correlations between osteoporosis and periodontitis were negative. In contrast, in most of the studies based on maxillary radiologic criteria, a positive association was determined. Martínez-Maestre et al.²⁰ highlighted the need for well-controlled studies to better determine the complexity of this relationship.

The results of two recent studies performed on samples from postmenopausal females also reached conflicting conclusions, thus adding to the current controversy and emphasizing the need for greater uniformity in the methodologies to study the relationship between osteoporosis and periodontitis.^{21,22} Methodologic limitations include small sample sizes, non-comparable study populations, and the diverse criteria used to define osteoporosis and periodontitis.

Although there was no correlation between both diseases, when the patients were evaluated in groups

Table 3.
Correlation Among Periodontitis and Clinical and Oral Variables

Variable	Periodontitis		Total	P
	Negative	Positive		
Osteoporosis, n (%)				0.52
No	8 (21.1)	8 (22.9)	16 (21.9)	
Osteopenia	18 (47.4)	20 (57.1)	38 (52.1)	
Osteoporosis	12 (31.6)	7 (20.0)	19 (26.0)	
Age (years), median (IQR)	62.5 (3.0)	63.0 (2.0)	63.0 (2.0)	0.62
Age (years), n (%)				0.42
≤60	2 (5.3)	4 (11.4)	6 (8.2)	
>60	36 (94.7)	31 (88.6)	67 (91.8)	
Missing teeth, median (IQR)	4.0 (6.0)	7.0 (13.0)	5.0 (8.75)	0.12
pH, n (%)				0.55
Basic	7 (19.4)	7 (21.9)	14 (20.6)	
Neutral	12 (33.3)	14 (43.8)	26 (38.2)	
Acid	17 (47.2)	11 (34.4)	28 (41.2)	
CAOD index, mean (SD)	16.53 (7.47)	17.94 (8.60)	17.21 (8.00)	0.45
pH, median (IQR)	7.0 (1.0)	7.0 (1.0)	7.2 (1.0)	0.32
Treatment need, n (%)				0.001
No	4 (10.5)	1 (2.9)	5 (6.8)	
Profound hygiene	26 (68.4)	12 (34.3)	38 (52.1)	
Periodontal treatment	8 (21.1)	22 (62.9)	30 (41.1)	
Oral hygiene, n (%)				0.003
Good	19 (50.0)	5 (14.3)	24 (32.9)	
Regular	14 (36.8)	18 (51.4)	32 (43.8)	
Bad	5 (13.2)	12 (34.3)	17 (23.3)	
Xerostomia, n (%)				0.10
No	37 (97.4)	30 (85.7)	67 (91.8)	
Yes	1 (2.6)	5 (14.3)	6 (8.2)	
Mucosal injury, n (%)				0.22
No	26 (68.4)	19 (54.3)	45 (61.6)	
Yes	12 (31.6)	16 (45.7)	28 (38.4)	

Numbers in bold represent statistical significance ($P < 0.05$).

of normal/osteopenia and osteoporosis, significant differences were found with respect to the number of missing teeth. The associations between dental status and skeletal bone density in healthy postmenopausal females have been investigated previously. Some findings support the hypothesis that systemic bone loss contributes to tooth loss.²³

Tooth loss is considered the ultimate outcome of periodontal disease.²⁴ The chronic inflammation triggered by the bacteria that cause periodontal disease underlies both breakdown of the jaw bone and tooth loss. Tezal et al.²⁵ reported that periodontal disease is a strong and independent predictor of tooth loss in postmenopausal females. However, in the patients in the present study, a relationship between periodontitis

and missing teeth could not be established. One explanation for the different findings is that patients with chronic periodontitis (CP) undergoing therapy may have lower rates of tooth loss.²⁶⁻²⁸ For example, the systemic statins frequently used in lipid-lowering therapies have an anti-inflammatory effect on periodontal disease and protect against tooth loss.²⁹ Higher rates of tooth loss have also been associated with low socioeconomic status, a low level of education, and low income and with age and smoking habits.³⁰ However, this information was not collected from the participants in the present study. The results showed that, in the present patients, periodontitis was correlated with the level of oral hygiene. Therefore, the potential effects of osteoporotic factors on the periodontal tissues could

Table 4.
Quantification of Subgingival *Pg*, *Tf*, *Cr*, *Fn*, and *Aa* in the Total Study Sample (N = 76)

Variable	Periodontitis, median (IQR)		Total, median (IQR)	P
	Negative (n = 38)	Positive (n = 35)		
Log <i>Pg</i>	3.03 (2.30)	3.40 (2.49)	3.00 (2.56)	0.53
Log <i>Tf</i>	4.86 (1.86)	5.25 (1.67)	5.09 (1.75)	0.08
Log <i>Cr</i>	5.25 (1.04)	5.42 (1.29)	5.40 (1.19)	0.59
Log <i>Fn</i>	6.23 (0.84)	6.31 (0.81)	6.26 (0.84)	0.46
Log <i>Aa</i>	2.35 (1.88)	2.72 (3.21)	2.50 (1.94)	0.09

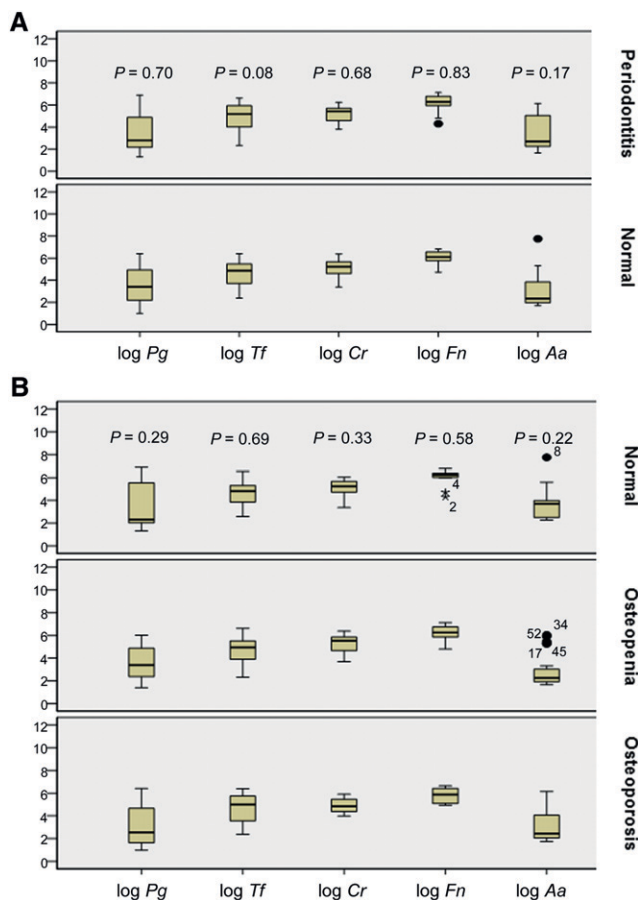


Figure 1.
A) Box plot of normal patients and the periodontitis group and quantification of periodontal bacteria. **B)** Box plot of normal, osteopenic, or osteoporotic patients and quantification of periodontal bacteria.

be minimized by combining good oral hygiene with regular check-ups.

The association between periodontitis and xerostomia was not significant, but a positive trend was evident because five of the six females with dry-mouth symptoms were diagnosed with periodontitis. More

patients with xerostomia are required to better analyze this relationship. Salivary flow rates are lower in postmenopausal females, although hormone therapy has been shown to improve saliva production.³¹⁻³²

In this study, qPCR assays are used successfully to determine the abundance of periodontal pathogens known to be associated with the progression and severity of periodontal disease in paper-point samples collected from postmenopausal females. The samples were harvested only from the deepest pocket, because deeper pockets offer a better environment for anaerobes and, consequently, their more extensive colonization and are useful as a general parameter. The prevalences of four of the five periodontal bacteria analyzed (*Pg*, *Tf*, *Cr*, and *Fn*) were very high (98.7% to 100%), and *Aa* was detected in 73.7% of the patients. In a similar study, Brennan et al.³³ determined the prevalence of infection with periodontal bacterial species in a sample of postmenopausal females. They found *Tf* in 37.9% of the samples and *Cr*, *Pg*, and *Fn* in 14% to 17%. Disparities between study methods may account for the differences in their sensitivities with respect to the detection and quantification of periodontal pathogens. Brennan et al.³³ used indirect immunofluorescence microscopy, which has important drawbacks compared with quantitative PCR, including a limited capability for accurate quantification and higher limits of detection.³⁴

Macuch and Tanner³⁵ identified *Cr* in 90% of their patients with newly diagnosed and established periodontitis, in 20% of those with gingivitis, and in 10% of those considered healthy. However, the samples in that study originated from a different source population than in the present study and relied on bacterial culturing methods, which have a lower sensitivity of detection.³⁵

The sensitivity and specificity of qPCR are higher because this method overcomes most of the limitations of the other techniques.^{34,36,37} Therefore, the use of qPCR is one of the strengths of the present work.

Table 5.
Spearman Correlation Coefficients (r) and P Values for all Pairs of Variables Shown.

Variables	Log <i>Pg</i>	Log <i>Tf</i>	Log <i>Cr</i>	Log <i>Fn</i>	Log <i>Aa</i>	Age	Missing Teeth	CAOD Index	pH
Log <i>Pg</i>									
<i>r</i>	1.000	0.410 [†]	0.328 [†]	0.268*	0.082	-0.117	-0.031	-0.070	0.285*
<i>P</i>	—	<0.001	0.004	0.02	0.55	0.32	0.79	0.56	0.02
Log <i>Tf</i>									
<i>r</i>	0.410 [†]	1.000	0.381 [†]	0.510 [†]	0.273*	-0.162	0.022	-0.031	-0.056
<i>P</i>	<0.001	—	0.001	<0.001	0.04	0.16	0.85	0.80	0.65
Log <i>Cr</i>									
<i>r</i>	0.328 [†]	0.381 [†]	1.000	0.815 [†]	0.009	-0.021	0.062	0.065	0.024
<i>P</i>	0.004	0.001	—	<0.001	0.95	0.86	0.60	0.59	0.84
Log <i>Fn</i>									
<i>r</i>	0.268*	0.510 [†]	0.815 [†]	1.000	0.116	-0.052	0.027	-0.002	-0.083
<i>P</i>	0.02	<0.001	<0.001	—	0.40	0.66	0.82	0.98	0.50
Log <i>Aa</i>									
<i>r</i>	0.082	0.273*	0.009	0.116	1.000	-0.195	0.008	0.192	-0.183
<i>P</i>	0.55	0.04	0.95	0.40	—	0.15	0.96	0.16	0.20
Age									
<i>r</i>	-0.117	-0.162	-0.021	-0.052	-0.195	1.000	0.100	0.064	-0.045
<i>P</i>	0.32	0.16	0.86	0.66	0.15	—	0.39	0.59	0.71
Missing teeth									
<i>r</i>	-0.031	0.022	0.062	0.027	0.008	0.100	1.000	0.770 [†]	-0.041
<i>P</i>	0.79	0.85	0.60	0.82	0.96	0.39	—	<0.001	0.74
CAOD index									
<i>r</i>	-0.070	-0.031	0.065	-0.002	0.192	0.064	0.770 [†]	1.000	-0.059
<i>P</i>	0.56	0.79	0.59	0.98	0.16	0.59	<0.001	—	0.63
pH									
<i>r</i>	0.285*	-0.056	0.024	-0.083	-0.183	-0.045	-0.041	-0.059	1.000
<i>P</i>	0.02	0.65	0.84	0.50	0.20	0.71	0.74	0.63	—

P values in boldface are statistically significant.

* *P* < 0.05.

† *P* < 0.01.

Riep et al.³⁶ examined the prevalence of 10 periodontal bacterial species in three groups of patients, those with CP or generalized aggressive periodontitis and those in a periodontitis-resistant control group. PCR of 16S rRNA gene fragments and subsequent dot-blot hybridization were used as the detection method. The prevalence of *Tf*, *Pg*, and *Pi* was high in all groups (60% to 95% of positive patients), without significant differences among them, although these species are considered to be highly associated with advanced periodontitis.³¹ The prevalence of some of the target species in that study are similar to those detected in this study.

In examining the potential correlation between bacterial species, significant associations were found between *Fn* and *Cr*; this will fit with the orange complex of Socransky et al.,³⁷ *Fn* and *Tf*, and *Pg* and *Tf* (red

complex), among others. The simultaneous presence of these bacteria suggests their interdependence, which could further promote the progression of periodontal disease.

The lower presence of *Aa* detected in this study is correlated with an acidic oral environment. These results are in agreement with the observed optimal pH range for growth of this bacterium.³⁸ The findings also suggest a greater abundance of *Pg* under acidic conditions (pH below 7), although optimum pH for growth is close to neutrality.³⁹ Maybe the association could be attributable to low salivary flow more than to pH. This information could help clinicians choose the best approach for treating periodontal infections caused by these microorganisms.

The bacterial species investigated in this study do not show significant quantitative differences among

healthy, osteopenic, and osteoporotic patients. The correlations between females with periodontitis and healthy patients were also negative. Therefore, the presence of subgingival periodontopathogenic bacteria in almost all of the present patients demonstrates that the appearance of these species is not sufficient to confirm the presence of periodontal disease. In fact, the mere presence of oral pathogens is recognized as being necessary but not sufficient for disease occurrence. Factors that influence disease development include the expression of virulence by the pathogen, the presence of interacting bacterial species, and the susceptibility of the individual host.^{28,40-42} Still, disease will not necessarily develop as long as the levels of the pathogen do not exceed the threshold for the host.³⁸ In the present study, some of the healthy patients may have had early-stage, albeit, by the criteria of this study, non-detectable disease, which may have blunted any significant differences between patients with periodontitis and those who were apparently healthy.

CONCLUSIONS

In the present study, osteoporosis did not influence the prevalence of periodontitis among postmenopausal females. The presence of periodontopathogenic bacteria was not sufficient to confirm disease, although the influence of PD on the prevalence of the pathogens analyzed was not examined. Probably, the detection of these bacterial species may be more closely related to the depth of the sampled pocket than to the diagnosis of periodontitis, because deeper pockets offer a better environment for anaerobes and, consequently, their more extensive colonization.^{36,43} A preventive maintenance program for postmenopausal females, particularly osteoporotic females, who are at greater risk of tooth loss, could minimize the potential effects of bone loss on periodontal tissues. Additional studies are needed to examine the influence of confounding factors for periodontitis and osteoporosis in postmenopausal females.

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