Microbiological characterization of a population affected by periodontitis with different levels of bone health

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ABSTRACT

Introduction: Both osteoporosis and periodontitis are diseases characterized by deficits at the level of bone tissue, where bone resorption exceeds neo-formation, resulting in bone loss.

Materials and methods: In the context of a monocentric cross-sectional observational study carried out through the collaboration between the University of Florence and the private dentistry institute Excellence Dental Network, 110 subjects affected by periodontitis were recruited. Of these, 71 were also affected by osteoporosis or osteopenia and 39 were not osteoporotic or osteopenic. Data were collected on oral microbiota, oral health and bone health.

Results: The collected data reveal a population of frail subjects in terms of fracture risk. The most common forms of periodontitis were essentially chronic in nature, and mainly of a moderate or even severe type, with high gingival recession and periodontal pocket depth values. The microbial species associated with Socransky's red and orange complexes showed concentration values nearly always above 10^3 copies/mL.

Conclusions: The relationship between osteoporosis and periodontitis still needs to be explored in depth. Data from our analyses are certainly interesting and provide a basis for designing further studies in larger populations.

KEYWORDS

Periodontal disease, bone disease, oral microbiota.

Introduction

Osteoporosis is a systemic skeletal disease characterized by reduced bone mass and qualitative changes in the material properties of the macro- and micro-architecture of the bones, leading to an increased risk of fracture even in the case of minor traumas [1]. According to the World Health Organization (WHO), densitometric diagnosis of osteoporosis is based on bone mineral density (BMD) evaluated using the dual-energy X-ray absorptiometry (DXA) technique and expressed as the value of the standard deviation with respect to the mean BMD value of healthy adult subjects (T-score) [1]. It has been observed that the risk of fracture begins to increase exponentially with densitometric T-score values <-2.5 SD, a value which therefore represents the threshold for diagnosing the presence of osteoporosis. Based on the diagnostic criteria defined by the WHO, around 6% of men and 22% of women aged 50-84 years have osteoporosis, which affects a total of 27.6 million men and women in the EU [2].

Periodontitis is a multifactorial inflammatory disease in which bacterial infection of periodontal tissues is both necessary and sufficient for the onset and progression of the oral pathology. Yet, numerous other factors, like smoking, hormonal changes, endocrine or systemic comorbidities, and poor oral hygiene, negatively affect the course of the disease. Periodontitis is a chronic disease of polymicrobial and site-specific etiology affecting the supporting tissues of the teeth. According to the Global Burden of Disease 2010 study, the global prevalence of severe periodontitis, standardized by age, in the twenty-year period 1990-2010 was 11.2%, making it the sixth most common disease in the world [3]. Both osteoporosis and periodontitis are diseases characterized by an imbalance in bone tissue, where bone resorption exceeds neo-formation, resulting in bone loss. Furthermore, recent studies have raised the possibility that the two pathologies are connected to each other, one acting as a risk factor for the onset of the other [4,5].

Characteristic microbial populations, termed microbiota, which typically coexist in harmony with the host, colonize different areas of the human body. Over the years, more than 700 different bacterial species have been identified in the oral cavity, making up the oral microbiota [6]. Currently, the main method for characterizing the microbial species inhabiting the oral cavity involves analysis of the small subunit (16S) of the ribosomal RNA (rRNA) gene, which contains highly conserved regions between other more variable ones that are characteristic of the various individual species. The alignment and com-

Article history
Received 8 Mar 2023 – Accepted 19 May 2023

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parison of 16S rRNA sequences from different microorganisms therefore allows us to build phylogenetic trees that represent their evolutionary relationships [7].

Different types of microorganisms, including obligate aerobes and facultative and obligate anaerobes, are present within the oral cavity. The oral bacterial community is dominated by species belonging to the phyla *Firmicutes*, *Bacteroides*, *Proteobacteria*, *Actinobacteria*, *Spirochaete* and *Fusobacteria*, which account for 96% of the identified species [8].

Since the mouth is an open system and frequently exposed to colonization by exogenous bacteria found in food, water and air, defining the precise composition of the oral microbiota is very complex. In addition, in humans, the effect of social contacts must also be considered.

To date we are still not able to fully define which microbial species are involved in the pathogenesis of periodontitis.

Several pathogens have been shown to be involved in destructive periodontal disease, and their concentrations in the study population will be shown later. The objective of this study was to analyze the association between the presence of osteoporosis and periodontal disease and to explore qualitative-quantitative differences in the composition of the oral microbiota between osteoporotic/osteopenic versus non-osteoporotic/non-osteopenic individuals with periodontitis.

Materials and method

This single-center observational cross-sectional study, approved by the local ethics committee, was carried out from November 2019 to December 2021, through the collaboration between the University of Florence (UNIFI) and the Excellence Dental Network (EDN), a private dental institute in Florence. A total of 110 adult patients, men and women, affected by periodontitis, 71 with osteoporosis/osteopenia and 39 with a normal BMD value, were included in the analysis.

Inclusion criteria were: signature of the informed consent form, age > 18 years, and a diagnosis of periodontitis. We excluded patients presenting at least one of the following exclusion criteria: A) medical therapy with antibiotic and/or steroids in the three months prior to enrollment in the study; B) presence of a parathyroid disease or a bone and mineral metabolism disease other than osteoporosis; C) development of a malignancy within the 5 years prior to inclusion in the study; D) eating disorders; E) pregnancy.

The participants were patients from EDN clinics who met the inclusion criteria for the study. The project was explained to the them and they were given the necessary documents (Patient information, Informed consent to participation, and Consent to the processing of personal data). All data collected were made anonymous by assigning each participant a specific alphanumeric code. The data were collected in an electronic database and stored for the purposes of this scientific research. All data collected at EDN and at an external laboratory, Biomolecular Diagnostic Srl (BD), were transcribed and archived in a database prepared ad hoc for this study.

The following parameters were evaluated:

- bone mass: patients who were advised by the dental staff to evaluate their BMD underwent an ultrasound scan (QUS) during their dental appointment. In subjects referred for mineralometry (MOC), T-scores, both lumbar and femoral, were recorded. T-scores were collected in subjects > 50 years of age and in post-menopausal women, while in individuals younger than 50 years bone status was evaluated on the basis of Z-score values.
- blood markers of bone and mineral metabolism: serum levels of 25(OH)D, alkaline phosphatase, bone alkaline phosphatase, parathormone (PTH), calcium, and phosphate.
- periodontal disease grading: the dental staff collected periodontal pocket depth (PPD), gingival recession (REC), and plaque index values. Periodontitis was classified as aggressive or chronic (Amitage CG, 1994).
- periodontal microbial profile: subgingival plaque samples collected from each of the subjects were evaluated to identify the main periodontal pathogens:
  - *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythensis*, *Porphyromonas gingivalis*, *Treponema denticola*, *Peptostreptococcus micros*, *Filifactor alocis, Synergistetes*, *Porphyromonas endodontalis* (red complex, associated with severe or advanced periodontitis);
  - *Fusobacterium nucleatum spp*, *Campylobacter rectus* and *Prevotella intermedia*, *Leptotrichia hofstadii*, *Rothia dentocariosa* (orange complex, associated with moderate or early periodontitis);*Eikenella corrodens*, *Cardiobacterium hominis* (green complex, associated with a healthy oral cavity).

To characterize the periodontal microbial profiles, the genetic material was extracted from the samples and analyzed through the real time qPCR procedure with the collaboration of BD.

Results

During the two years of the project, a total of 181 patients were invited to participate in the study. Of these only 110, 36 males and 74 females, agreed to take part.

Average age and anthropometry

The patients had a mean age of 55.2 + 13.2 years at the time of study inclusion. Anthropometric characteristics of the analyzed population, expressed as mean values + standard deviation (SD), are summarized in tables I-III, referring, respectively, to all the patients, the males and the females.

Bone turnover markers

Serum values of markers of bone and mineral metabolism in the analyzed population, expressed as mean values + SD, are summarized in table IV. Interestingly, our population had an average 25(OH)D level corresponding to vitamin D insufficiency.

Bone mineralogy

The available mineralometric data, obtained by MOC or QUS, and expressed as mean values + SD, are summarized in table V.

According to the DXA or QUS T-score values (or Z-score value for individuals < 50 years), patients were classified as osteoporotic (16.4%), osteopenic (48.2%), or normal BMD (35.5%), as reported in table VI.

Table I Age average and anthropometry.

	N. STATISTICS	MINIMUM STATISTICS	MAXIMUM STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS	ASYMMETRY STATISTICS
Age	110	27	101	55.22	13.208	.478
Weight (kg)	107	41.0	120.0	67.250	15.0090	.835
Height (m)	107	1.53	1.89	1.6832	.08513	.271
BMI	107	15.6226	41.9143	23.652578	4.6736212	1.449

Table II Age average ad anthropometry in males.

MALE	N. STATISTICS	MINIMUM STATISTICS	MAXIMUM STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS	ASYMMETRY STATISTICS
Age	36	27	81	56.83	12.707	-.442
Weight (kg)	36	55.0	120.0	78.236	12.8755	.998
Height (m)	36	1.55	1.89	1.7650	.06670	-.837
BMI	36	19.7210	41.6233	25.136001	4.2250091	1.953

Table III Age average and anthropometry in females.

FEMALES	N. STATISTICS	MINIMUM STATISTICS	MAXIMUM STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS	ASYMMETRY STATISTICS
Age	74	29	101	54.43	13.459	.875
Weight (kg)	71	41.0	110.0	61.679	12.8328	1.317
Height (m)	71	1.53	1.78	1.6417	.05967	.259
BMI	71	15.6226	41.9143	22.900419	4.7376427	1.547

Table IV Mean levels of bone turnover markers analyzed.

BONE TURNOVER MARKERS	N. STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS
25OHD3 (ng/mL)	81	27.3035	8.97985
PTH (pg/mL)	80	37.3475	19.28684
Calcemia (mg/dL)	34	9.1532	2.52885
Phosphatemia (mg/dL)	30	3.5627	.71254
Alkaline Phosphatase (U/L)	16	68.31	14.202
Bone alkaline phosphatase (ug/L)	10	13.000	5.2993

Table V Mineralometric data.

MINERALOMETRIC DATA	N. STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS
T-score lumbar	15	-2.093	1.5917
T-score femur tot	13	-1.823	.9671
T-score femur neck	14	-2.157	.9788
T-score right foot	99	-.961	1.2538
T-score left foot	99	-.948	1.2966

Table VI Mineralometric examination result.

EXAM RESULT	FREQUENCY	VALID PERCENTAGE
Normal	39	35.5
Osteopenia	53	48.2
Osteoporosis	18	16.4
Total	110	100.0

Periodontal disease classification, periodontal pocket depth, gingival recession, plaque index. Periodontal disease severity was rated using the classification proposed by Amitage CG (1999). The analysis revealed that the patients always presented generalized periodontitis, with chronic forms prevailing over juvenile/aggressive ones; in particular, 16.4% had a mild chronic form, 41.8% a moderate chronic form, and 32.7% a severe chronic form (Table VII). The average PPD detected on probing was 5.54 mm, therefore beyond the values considered physiological, while the average gingival recession (REC) was 0.59 mm. The average plaque index was found to be 31.16% (Table VIII).

Analysis of species with reference to the Socransky classification

For detection of microorganisms, we applied the minimum threshold value for the real-time PCR detection method, which is 10^2 copies/uL.

Table VII Types of periodontitis found in the population.

PERIODONTITIS (AMITAGE CG 1999)	FREQUENCY	VALID PERCENTAGE
Chronic. mild. generalized	18	16.4
Chronic. moderate. generalized	46	41.8
Chronic. severe. generalized	36	32.7
Aggressive. mild. generalized	1	.9
Aggressive. moderate. generalized	1	.9
Aggressive. severe. generalized	8	7.3
Total	110	100.0

The concentrations of microbial species belonging to Socransky's red, orange and green complexes were measured and tended to be above the physiological values (i.e., above 10^3 copies/uL) for all types of microorganism, where detected (Figure 1, Tables IX,X).

Listed below are the microorganisms present at non-physiological values (more than 10^3 copies/uL) together with the percentages of subjects in which they were found:

- *P. gingivalis* (red complex), 68.2%;
- *P. endodontalis* (red complex), 78%;
- *T. forsythensys* (red complex), 74.5%;
- *P. micros* (red complex), 62.7%;
- *F. alocis* (red complex), 59.6%;
- *Synergistetes* (red complex), 83.5%;
- *F. nucleatum* (orange complex), 81.8%;
- *C. rectus* (orange complex), 56.4%;
- *E. corrodens* (green complex), 53.6%;
- *C. hominis* (green complex), 72.5%.

Table VIII Average PPD, REC and plaque index in the population.

	N. STATISTICS	MINIMUM STATISTICS	MAXIMUM STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS
PPD average (mm)	110	2.20	15.00	5.5414	1.42438
REC average (mm)	110	.00	2.40	.5877	.59648
Plaque Index (%)	97	0	90.48	31.1609	25.10672

Figure 1 Average microbial concentrations.

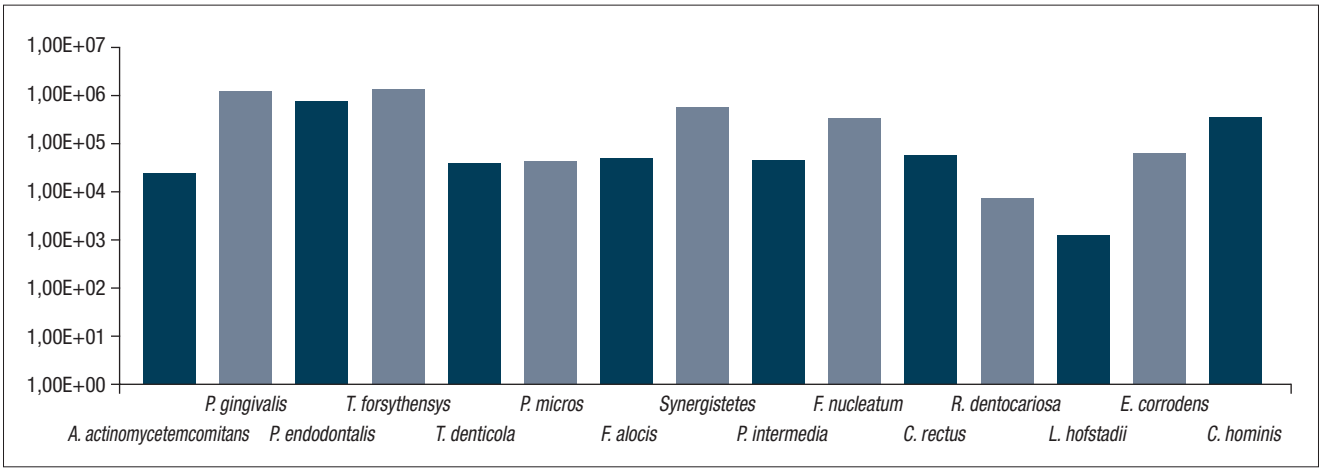

Table IX Mean microbial concentrations in the population.

	N. STATISTICS	MINIMUM STATISTICS	MAXIMUM STATISTICS	AVERAGE STATISTICS	STANDARD DEVIATION STATISTICS
Total bacterial copies	110	80000	200000000	11568978.18	25992242.871
(copies/µl) *A. actinomycetemcomitans*	110	0	2300000	22842.77	219295.046
(copies/µl) *P. gingivalis*	110	0	55500000	1094530.42	5576959.645
(copies/µl) *P. endodontalis*	109	0	23400000	699523.29	2686757.504
(copies/µl) *T. forsythensys*	110	0	35200000.0	1221587.318	4767461.6500
(copies/µl) *T. denticola*	110	0	759000	36960.53	118372.970
(copies/µl) *P. micros*	110	0	1160000	44375.78	168354.163
(copies/µl) *F. alocis*	109	0	1140000	46624.16	169666.354
(copies/µl) *Synergistetes*	109	0	5280000	546933.16	1017021.850
(copies/µl) *P. interaverage*	110	0	1140000	40878.37	160754.154
(copies/µl) *F. nucleatum*	110	0	25800000	356869.09	2473601.143
(copies/µl) *C. rectus*	109	0	2550000	52376.53	273987.392
(copies/µl) *R. dentocariosa*	109	0	201000.0	6914.349	26050.8026
(copies/µl) *L. hofstadii*	109	0	72000	1242.63	7018.584
(copies/µl) *E. corrodens*	110	0	3840000.0	62757.527	380029.8547
(copies/µl) *C. hominis*	109	0	30000000.0	345441.239	2874905.8636

Table X Percentages of subjects with pathological or physiological concentrations for each microrganism analyzed.

MICROORGANISM	N.	% OF SUBJECTS WITH PHYSIOLOGICAL CONCENTRATIONS	% OF SUBJECTS WITH PATHOLOGICAL CONCENTRATIONS
A. actinomycetemcomitans	110	93.6	6.4
P. gingivalis	110	31.8	68.2
P. endodontalis	109	22	78
T. forsythensys	110	25.5	74.5
T. denticola	110	58.2	41.8
P. micros	110	37.3	62.7
F. alocis	109	40.4	59.6
Synergistetes	109	16.5	83.5
P. interaverage	110	62.7	37.3
F. nucleatum	110	18.2	81.8
C. rectus	110	43.6	56.4
R. dentocariosa	110	57.8	42.2
L. hofstadii	110	85.3	14.7
E. corrodens	110	46.4	53.6
C. hominis	109	27.5	72.5

Discussion

The data collected show that the present study population tended to be over 50 years old, with BMI values within the recommended range, in both the male and female subpopulations. The bone turnover and mineralometric data describe a population with serum values of PTH, calcium and phosphate levels in line with the desirable values, but with below-optimal 25(OH) D values. Furthermore, the T-score values from both the QUS and MOC, when available, identify a mainly fragile population from the point of view of the risk of fracture and osteoporosis, with a smaller number of subjects showing values tending to normal. To be recruited, subjects had to be affected by periodontal disease. In this regard, the forms most frequently found had a chronic rather than an aggressive nature, and were mainly moderate or severe, with high gingival recession values and PPDs in the tested sites. As regards the analysis of the microbiota, it was logical to expect that the most common microbial species linked to periodontal disease would be the ones usually associated with severe and moderate pathology in the population in question; and infact the species associated with Socransky's red and/or orange complex were practically ubiquitous in the tested subjects, with concentrations almost always above the "threshold of pathogenicity", defined as 10^3 copies/mL.

Conclusions

The relationship between osteoporosis and periodontitis remains to be extensively explored. However, our results consolidate the idea that there is a relationship between these two diseases. The analyses presented here may be of great interest for the development of future studies aiming to expand the sample size and to reduce the confounding factors present at the level of the studied population.

References

1. Adami G, Fassio A, Rossini M, et al. Osteoporosis in rheumatic diseases. Int J Mol Sci. 2019;20(23):5867.
2. Hernlund E, Svedbom A, Ivergård M, et al. Osteoporosis in the European Union: medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). Arch Osteoporos. 2013;8(1):136.
3. Kassebaum NJ, Bernabé E, Dahiya M, Bhandari B, Murray CJ, Marcenes W. Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression. J Dent Res. 2014;93(11): 1045-53.
4. Tezal M, Wactawski-Wende J, Grossi SG, Dmochowski J, Genco RJ. Periodontal disease and the incidence of tooth loss in postmenopausal women. J Periodontol. 2005;76(7):1123-8.
5. Straka M, Straka-Trapezanlidis M, Deglovic J, Varga I. Periodontitis and osteoporosis. Neuro Endocrinol Lett. 2015;36(5):401-6.
6. Krzysciac W, Jurczak A, Piątkowski J. The role of human oral microbiome in dental biofilm formation. In Dhanasekaran D, Thajuddin N, eds. Microbial biofilms: Importance and applications. Intech Open. 2016. Doi: 10.5772/63492.
7. Giovannoni SJ, Britschgi TB, Moyer CL, Field KG. Genetic diversity in Sargasso Sea bacterioplakton. Nature 1990;345(6270):60-3.
8. Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. J Bacteriol. 2010;192(19):5002-17.