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Prevalence of undiagnosed diabetes and prediabetes related to periodontitis and its risk factors in elderly individuals

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ABSTRACT

Objective: The prevalence of undiagnosed diabetes was estimated to increase with age and can reach 3.5%. The purpose of the study was to evaluate the prevalence of undiagnosed diabetes and prediabetes in the elderly patients who attended a dental clinic and to find common risk factors.
Methods: Male patients, older than 50 years, attended their first dental visit to the School of Dentistry for a period of two years, and it was proposed to evaluate undiagnosed type 2 diabetes mellitus. Periodontal, biochemical, microbiological examinations, nutritional profile, and physical activity were performed.
Results: A total of 106 patients were examined, 6 (5.6%) had diabetes, and 37 (34.9%) had prediabetes without prior diagnosis. The severity of periodontitis was greater in patients with diabetes. Most of the patients were overweight and had increased systolic blood pressure. Patients with prediabetes and periodontitis had a low adherence to the Mediterranean diet. *Tannerella forsythia* was present in more patients with periodontitis, and the prevalence of *Aggregatibacter actinomycetemcomitans* is practically absent in groups with periodontitis, except for the group with diabetes.
Conclusions: In the population studied, the prevalence of patients without a diagnosis of diabetes and prediabetes was very high and underestimated. The increased severity of periodontitis in patients with diabetes and in conjunction with the high level of cortisol seen in patients with periodontitis, especially those with diabetes, emphasize the dysregulation of the immunoinflammatory system.
Clinical significance: It is essential to add all this data to our dental practice to cover patient health with a broader landscape.

1. Introduction

Non-communicable diseases (NCDs) can induce a systemic pro-inflammatory response and are responsible for more than 70% of all deaths worldwide, along with a significant economic impact. These NCDs often share negative behaviors such as smoking and alcoholism, unhealthy diet, lack of physical activity [1,2] and are based on three pillars: dysbiosis, low-grade inflammation, and immune dysfunction [3]. Several chronic diseases are part of that group, such as type 2 diabetes mellitus (DM2), obesity, metabolic syndrome, cardiovascular diseases, and periodontitis, which is now considered one of the six most important NCDs [4,5].

Diabetes mellitus is one of the most serious chronic diseases and one of the fastest growing in the 21st century, where it is estimated that in 20 years there will be more than 643 million people with diabetes in the world [6]. DM2 is the most common form of diabetes and one of the leading causes of premature death in the world, considered a global epidemic [7,8]. It is characterized by a hyperglycemic condition due to insulin resistance (IR) or partial/total deficiency in insulin production, resulting in an altered immunoinflammatory response that can result in

Abbreviations: Periodontitis, Per; Type 2 diabetes mellitus, DM2; Western Diet, WD.

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micro and macrovascular complications [6].

Impaired glucose tolerance (IGT) and impaired fasting glucose (IFG) are also called "prediabetes" or borderline diabetes by some authors. Prediabetes is not a clinical entity, but a risk marker for the appearance of DM2, cardiovascular disease, and / or adverse outcomes. [6,9-11] The increasing prevalence of prediabetes represents a public health problem, and its early detection is an important measure for the implementation of interventions to prevent diabetes and other possible complications. [6,12]

Periodontitis is a multifactorial chronic inflammatory disease, considered a major public health problem present in developed and developing countries that has increased in incidence since 1990. Its global prevalence is around 45–50%, and 11% of the world's population suffers from its severe form, making it the sixth most common disease in the world [13]. Periodontitis is recognized as the sixth most classic complication of diabetes, and its presence is associated with a greater presence of complications related to diabetes [14,15].

Periodontitis is associated with dysbiotic dental biofilm that causes progressive host-mediated connective tissue damage and loss of alveolar bone loss [16]. Dental biofilm represents only 20% of the direct risk of developing periodontitis and the remaining 80% are represented by modifiable risk factors (such as diabetes and smoking) and non-modifiable risk factors (such as age, sex, genetic predisposition genetics) [17]. Periodontitis negatively affects chewing ability, leading to poor nutrition and poor quality of life [16,18].

DM2 and periodontitis [4] have a "bidirectional" relationship and this association is considered a source of comorbidity [19,20]. Chronic hyperglycemia is one of the main risk factors for periodontitis and increases its prevalence, severity and progression, just as periodontitis increases insulin resistance and worsens glycemic control [21]. These diseases share several pathological mechanisms that involve impaired activation of the immune system and increased oxidative stress, primarily in people with uncontrolled diabetes [22–25]. Periodontal health and oral health in general should be considered part of an individual's overall health, and preventive strategies that are appropriate for most chronic diseases can also be appropriate to prevent or reduce the progression rate of periodontitis progression [26].

The global population over 60 years of age is expanding, estimated at 1 in 11 people in the world, and its prevalence is expected to double in the coming years until 2050 [27]. Elderly people are also an important factor to consider in this scenario because they cover a group of patients who are more susceptible to chronic diseases, polymedication therapies, and fewer people with missing teeth, making oral health even more important, necessary, and complex [28]. In fact, understanding the interaction between accelerated aging and these inflammatory diseases is essential for the follow-up and treatment of patients.

The prevalence of periodontitis and diabetes mellitus varies according to sociodemographic characteristics, suggesting that lifestyle can play a role in the development of the disease [13,29,30]. Today we face an aging population with chronic diseases and exposure to risk factors that are extremely difficult to treat and define a health parameter for these people [31]. The combination of lifestyle changes (dietary counseling, regular physical exercise, quitting smoking) and dental care in the same medical care program showed an improvement in glycemic and periodontal status in patients with NCD, especially those with elderly [18,32–34]. Considering the great impact of oral complications on quality of life, it is crucial that both the doctor and dentist deepen their knowledge of the pathological relationship of these diseases to carry out adequate treatment and recognize the patient as the center of a dynamic environment and suffering from complex diseases [18,35].

Diabetes is a silent disease and is often undiagnosed because it initially has few or even no symptoms. Around 50% of people with diabetes are estimated to have a correct diagnosis [6]. In view of this and all the above, the main objectives of this study are to find all diabetes and prediabetes conditions in a group of elderly male patients and associated risk factors, attended in a dental clinic.

2. Materials and methods

2.1. Study population

This study was designed as a descriptive analysis of patients who attended the first visit to the School of Dentistry. Inclusion criteria: male individuals, more than 50 years old. Exclusion criteria: previous treated for periodontal disease and intake of antibiotics in previous 6 months. All patients signed an informed consent. The protocol was approved by the Ethics Committee (1588-N-20, 12–21–2016). The minimum clinical difference to be considered in the HbA1c (glycated hemoglobin) variable was estimated to be 0.15, the statistical power greater than 80% for a 5% margin of error for a minimum of seven patients.

2.2. Clinical data

General data were recorded: age, history of other diseases that may be related to the purpose of the study and the drugs used, anthropometric parameters (height, weight, body mass index [BMI], waist circumference), cardiovascular parameters (systolic pressure, diastolic pressure, and heart rate), and smoking. We considered a smoker to be someone who smokes at least one cigarette per day, and nonsmokers as those who currently do not smoke.

2.3. Dietary evaluation

Diet assessment was carried out using the Mediterranean Diet Adherence Screener (MDA) [36], consisting of 12 questions on the frequency of food consumption and two questions about food intake habits considered characteristic of the Spanish Mediterranean diet. Each question was scored 0 or 1 and classified as low (0- 7) and high (8–14).

2.4. Physical activity

The average hours of weekly exercise were obtained based on Minnesota Leisure Time Physical Activity [37]. The patients were asked about the hours they spent walking, sports, home activity and other physical activity per week, the average weekly exercise hours were obtained.

2.5. Periodontal examination

A calibrated clinical investigator recorded the probing depth (PD) (distance between the free gingival margin and the pocket depth), the clinical attachment level (CAL) (distance between the cement-enamel junction and the pocket depth) and bleeding on probing (BoP) after 30 s at six sites per tooth [38]. The diagnosis of periodontitis was made according to the World Workshop on the Classification of Periodontal and Peri-implant Diseases and Conditions 2017 [16]. The percentage of teeth with PD greater than or equal to 4 mm, at 2 or more interproximal sites was obtained as an index of severity of PD [39]. Also, the mean PD, CAL, and BoP were obtained. Additionally, the number of teeth present was recorded.

2.6. Biochemical parameters

Venous blood samples were obtained after a fasting period. The following biochemical parameters were obtained: fasting plasma glucose (FPG), cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), urea, creatinine, uric acid, GPT, GGT, albumin, total bilirubin, phosphorus, calcium, thyroid-stimulating hormone (TSH), cortisol for all patients. Participants were evaluated for the presence of prediabetes or diabetes based mainly on HbA1c levels where the normal group has HbA1c less than 5.7%, the prediabetes group 5.7% to 6.4% and the groups with type 2 diabetes mellitus greater than 6.4% according to the

recommendations of the American Diabetes Association [11].

2.7. Microbiological parameters

For the analysis of the microbiological profile, the three deepest periodontal pockets of each patient were selected. Before harvesting, a supragingival scaling was performed. Paper points were subgingivally inserted for 30 s and then the three samples of each patient were pooled and stored in an Eppendorf tube and processed immediately. DNA was extracted in 24 h using the High Purity PCR Template Preparation Kit (Roche, Mannheim, Germany) and aliquoted for the microIDent PCR test (Hain Lifescience, Nehren, Germany). DNA-STRIP technology was used to record the species *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi) and Aggregatibacter actinomycetemcomitans (Aa)*, as previously described [40]. To determine analytical sensitivity, a series of plasmids containing target sequences detectable with micro-IDent®plus11. The following detection limits were determined: Species *Aa*: 50–100 genome equivalents/PCR or 1000- 5000 genome equivalents/paper point; all other species: 500–1000 genome equivalents/PCR or 10,000- 50,000 genome equivalents/paper point. The results were classified into 5 groups according to the bioburden: not detectable ($<10^4$, *Aa* $<10^3$), borderline (10^4, *Aa* 10^3), increased ($<10^5$, *Aa* $<10^4$), high (<106, *Aa* $< 10^5$), very high (10^6, *Aa* $\geq 10^5$).

2.8. Statistical analysis

Statistical analysis was performed using SPSS version 21.0 software (SPSS Inc., Chicago, IL, USA). Data were presented as means and standard deviations for continuous variables and as percentages for qualitative variables. Pairwise comparisons of qualitative variables were made using the Chi square test (Chi^2), to determine the groups that made the difference, corrected standardized Haberman residuals were used, and a normality test was previously applied. Regarding numerical variables, those that do not follow a normal distribution have been used using the Mann-Whitney *U* test (dichotomous variables) or the Kruskal-Wallis test (for variables with more than two categories). For those with normal distributions, analysis of variance (ANOVA) has been applied. To determine the groups that make the difference (Post-hoc) in the case of variables with more than two categories, the Bonferroni test has been applied.

3. Results

3.1. General data

From 2018 and 2020 a total of 106 individuals agreed to participate in the study. Among them, 45 patients without periodontitis and 61 with periodontitis. Of these 106 patients, only 15 patients had a previous diagnosis of DM2, and analyzing the HbA1c values in the entire sample, we found 37 (34.9%) patients with prediabetes and 6 (5.6%) patients with DM2, without a previous diagnosis. Table 1 shows the proportion of periodontitis in each group, prediabetes and diabetes patients had a higher number of periodontitis cases, but with nonsignificant statistical differences.

A total of six groups were established: 1) no diabetes without periodontitis (H), prediabetes without periodontitis (PreDM), diabetes without periodontitis (DM), no diabetes with periodontitis (P), prediabetes with periodontitis (PreDMP), and diabetes with periodontitis (DMP). Systolic blood pressure was significantly higher in patients with DMP than in the other groups (H, PreDM, P, and PreDMP), except for those with DM. No significant differences were found for age, weight, BMI, waist circumference, diastolic blood pressure, heart rate, and smoking between the groups considered, except for height and systolic blood pressure. (Tables 2 and 3)

Table 1
Periodontitis according to HbA1c levels ($n = 106$).

	Periodontitis	
	No	Yes
Normoglycemic ($n = 48$, 45.3%)	24 (50%)	24 (50%)
Prediabetes ($n = 37$, 34.9%)	14 (37.8%)	23 (62.2%)
Diabetes ($n = 21$, 19.8%)	7 (33.3%)	14 (66.4%)

Regarding the analysis of adherence to the Mediterranean diet (MDA), it was possible to observe a significant difference between the 6 groups. Patients with diabetes had a good adaptation to the Mediterranean diet, especially patients with DMP (85.7%). Another interesting aspect is the difference in the adequacy of patients with prediabetes, where almost half of the patients with PreDMP were in the low category, while all patients with PreDM had a high adequacy to MDA. The PreDMP and DMP groups were those with the lowest average weekly exercise per year. However, no statistically significant differences were found between the groups in these parameters. Smoking habit did not show significant differences among the 6 groups.(Table 4)

3.2. Periodontal evaluation

Table 5 shows the periodontal evaluation with statistical differences between all variables. The number of teeth present did not show differences between the groups. The PD in H was different between P, PreDMP, and DMP; in PreDM it was different from DM; DMP was different from H and PreDM. CAL measurements showed a marked difference between the non-periodontitis and periodontitis groups. BoP shows differences between H and P, PreDMP and DMP with the highest value in DMP. The severity index shows differences between H and P, PreDMP and DMP; DM was different from PreDMP.

Among the 106 patients, 24 of the patients were smokers and 82 were nonsmokers. When analyzing the percentage of BoP and smoking (Table 6), it is observed that in the group of non-smoking patients, the DMP group had significantly higher bleeding compared to the other subgroups, H shows statistical differences with P and DMP. No statistically significant differences were observed between smoking patients, although a visibly higher percentage of bleeding was observed in the periodontitis groups, except for the DMP group.

3.3. Analysis of the microbiological profile

The microbiological results are shown in Table 7. *A. actinomycetemcomitans* was found to be present in all groups except the P and PreDMP groups, which were completely absent. The bacterial load of *T. forsythia* differs between the groups with and without periodontitis, with the highest and most significant values in the P and DMP groups. *T. forsythia* and *A. actinomycetemcomitans* were the only ones who showed a marked statistical difference in their bacterial load, while the others did not have statistically significant differences.

3.4. Biochemical analysis

Most biochemical parameters did not show statistical differences between groups, except HbA1c and FPG. HbA1c shows differences between all groups; FPG shows differences in H with DM and DMP, DM was different between H, P and DMP. Furthermore, cortisol levels also highlighted a statistically significant difference between the groups, especially between the H group compared to patients with periodontitis (DMP, PreDMP and P groups). (Table 8)

4. Discussion

From a clinical point of view, the most relevant finding of this study was that 40% of patients did not know about their diagnosis of prediabetes (34.9%) and diabetes (5,6%). Furthermore, in our sample we

Table 2
General data.

	H[a] (n = 24; 22,60%)	PreDM[b] (n = 14; 13,20%)	DM[c] (n = 7; 6,60%)	P[d] (n = 24; 22,60%)	PreDMP[e] (n = 23; 21,70%)	DMP[f] (n = 14; 13,20%)
Age (years)	60.63 ± 8.85	63.00 ± 9.00	62.57 ± 9.54	60.79 ± . 48	60.48 ± 7.77	65.79 ± 8.75
Weight (kg)	85.50 ± 12.24	80.00 ± 9.92	62.57 ± 8.86	76.33 ± 9.83	81.07 ± 12.14	83.64 ± 15.66
Height (cm)	175.42 ± 6.71[c,f]	171.79 ± 7.46	167.00 ± 6.03[a]	171.42 ± 6.19	171.87 ± 5.38	170.21 ± 7.71[a]
BMI (kg/m2)	27.79 ± 3.71	27.08 ± 2.55	30.13 ± 3.84	26.00 ± 3.33	27.48 ± 28.93	28.93 ± 5.44
WC (cm)	107.08 ± 8.92	103.57 ± 11.41	111.86 ± 9.06	101.24 ± 8.75	108.17 ± 11.07	109.57 ± 12.79

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; BMI: body mass index; WC: waist circumference. Mean ± standard deviation. Statistically significant differences between groups are indicated by the letters a,b,c,d,e,f which indicate statistical differences.

Table 3
Cardiovascular parameters.

	H[a]	PreDM[b]	DM[c]	P[d]	PreDMP[e]	DMP[f]
SP (mmHg)	145.46 ± 20.49[f]	133.50 ± 22.68[f]	156.57 ± 27.71	144.04 ± 21.80[f]	145.30 ± 26.48[f]	165.21 ± 21.32[a,b,d,e]
DP (mmHg)	83.96 ± 10.84	81.36 ± 10.09	91.57 ± 10.85	80.08 ± 17.62	83.04 ± 12.48	88.64 ± 13.57
Heart rate (b/min)	67.71 ± 10.12	69.79 ± 12.83	75.57 ± 11.12	68.24 ± 13.79	71.61 ± 11.46	73.71 ± 10.48

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; SP: systolic pressure; DP: diastolic pressure. Mean ± standard deviation. Statistically significant differences between groups are indicated by the letters a,b,c,d,e,f which indicate statistical differences.

Table 4
General information regarding on habits such as smoking, diet, and physical exercise.

	H[a]	PreDM[b]	DM[c]	P[d]	PreDMP[e]	DMP[f]
Smokers	12.5%	21.4%	14.3%	29.2%	34.8%	14.3%
MDA						
Low	33.3	0.0*	42.9	16.7	47.8*	14.3
High	66.7	100.0*	57.1	83.3	52.2*	85.7
Weekly exercise (h)/year	9.48 ± 7.68	11.64 ± 8.39	8.71 ± 6.90	9.75 ± 8.39	5.27 ± 4.82	6.07 ± 3.98

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; MDA: Mediterranean diet adherence. Mean ± standard deviation. + statistical differences chi^2.

Table 5
Periodontal evaluation.

	H[a]	PreDM[b]	DM[c]	P[d]	PreDMP[e]	DMP[f]
Remaining teeth	23.4 ± 4,8[b,c,d]	20.7 ± 4.3 [a]	23.1 ± 3.4[a]	18.1 ± 7.1 [a]	20.3 ± 6,0	20.8 ± 5.4
PD	2.4 ± 0.2 [d,e,f]	2.58 ±0.1 [c]	2.56 ±0.2 [b]	2.88 ±0.4 [a]	2.8 ± 0.4 [a]	2.95 ±0.4 [a,b]
CAL	2.61 ±0.3 [d,e,f]	2.8 ± 0.2 [d,e,f]	2.72 ±0.2 [d,e,f]	4.31 ±1.2 [a,b,c]	4.03±1.0 [a,b,c]	4.11 ±1.3 [a,b,c]
BoP (%)	2.78 ±3.5 [d,e,f]	6.29 ±8.1	5.80 ±4.5 [a]	15.34 ±17.3 [a]	9.86 ±10.7 [a]	21.93 ±24.3 [a]
Severity	3.36 ±6.8 [d,e,f]	10.18 ±13.6	13.00 ±16.9[e]	39.56 ±31.9 [a]	38.74 ±35.06 [a,c]	48.13 ±33.5 [a]

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; PD: probing depth; CAL: clinical attachment level; BoP: bleeding on probing. Mean ± standard deviation. Statistically significant differences between groups are indicated by the letters a,b,c,d,e,f.

observed a high number of patients with periodontitis (61; 57,54%) and the highest proportion (37; 60,65%) was present in the prediabetes and diabetes groups. Considering that we are dealing with a population restricted to men and older than 50 years of age, we can say that these results demonstrate a high prevalence and often underestimated, for these two diseases, especially when we consider previous data (44,7% of adults between 20 and 79 years of age with undiagnosed diabetes [6], 22% of people in developed countries with undiagnosed diabetes) [41].

The prevalence of adult patients with diabetes and prediabetes without previous diagnosis was analyzed in the United States and their results emphasize how the prevalence increases with age, where the percentage of patients without a previous diagnosis of diabetes was 4.8% in adults over 65 years, 4.4% in the group of 45 to 64 years, and 1.8% in patients 20 to 44 years. The same pattern was observed in prediabetic cases, with a prevalence of 54.6%, 42.2%, and 25.1% for those older than 65, 45–64 and 20–44 years, respectively [42].

Many studies have already demonstrated the importance of dentists in detecting patients without a previous diagnosis of diabetes or prediabetes in dental settings [43–48]. Include the fact that there is a significant number of patients who do not usually visit their doctors in the same proportion as they attend dental offices [49]. Bearing in mind that our sample was restricted to care at the Faculty of Dentistry, this rate is quite considerable and shows the importance of the dentist in the comprehensive evaluation of the patient to reduce the enormous rate of undiagnosed patients in the world.

Possible reasons for this high prevalence of undiagnosed patients may be related to the fact that diabetes does not have symptoms in most cases and there are currently five definitions of prediabetes in clinical use [50] which is a challenge to establish optimal approaches to its detection, diagnosis and management [51]. This controversy exists mainly due to the use of HbA1c as the only parameter for the detection of this condition and the possible variation of its levels may occur according to some characteristics of individuals such as ethnicity and age [52]. But it is already clear that high levels of HbA1c are strongly associated with adverse outcomes and highly specific for long-term hyperglycemia, in addition to being a non-fasting test with less pre-analytical variability (fewer factors that can influence test results) and with low intra-individual variability compared to fasting plasma glucose, in addition to being a guide for decision- making about treatment [50].

A small number of patients reported the use of medications to control hypertension, and an even smaller number confirmed a previous

Table 6
Bleeding on probing and smoking.

	BoP (%)					
	H[a]	PreDM[b]	DM[c]	P[d]	PreDMP[e]	DMP[f]
Smokers	2.49 ± 0.98	3.97 ± 3.46	5.3 ± 0.00	24.13 ± 28.00	9.58 ± 8.28	4.75 ± 0.16
Non-smokers	2.82 ± 3.83[d,f]	6.92 ± 9.00	5.89 ± 4.97	12.24 ± 11.41[a]	10.01 ± 12.23	24.79 ± 25.29[a]

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis. Mean ± standard deviation. Statistically significant differences between groups are indicated by the letters a,b,c,d,e,f.

Table 7
Microbiological analysis.

	Categories	H	PreDM	DM	P	PreDMP	DMP
Aa	Not detectable	91.7	85.7	71.4*	100.0	100.0	85.7
	Borderline	8.3	7.1	0.0	0.0	0.0	0.0
	Increased	0.0	0.0	0.0	0.0	0.0	7.1*
	High	0.0	0.0	14.3*	0.0	0.0	0.0
	Very High	0.0	7.1	14.3	0.0	0.0	7.1
Pg	Not detectable	45.8	50.0	57.1	25.0	43.5	28.6
	Borderline	16.7	7.1	14.3	4.2	0.0	7.1
	Increased	12.5	21.4	0.0	4.2	4.3	0.0
	High	4.2	0.0	0.0	0.0	8.7	7.1
	Very High	20.8	21.4	28.6	66.7	43.5	57.1
Tf	Not detectable	50.0*	21.4	14.3	8.3	17.4	0.0*
	Borderline	8.3	7.1	14.3	0.0	0.0	7.1
	Increased	16.7	21.4	28.6	16.7	4.3	0.0
	High	0.0*	14.3	28.6	8.3	26.1	21.4
	Very High	25.0*	35.7	14.3	66.7*	52.2	71.4*
Td	Not detectable	45.8	21.4	28.6	12.5	17.4	28.6
	Borderline	12.5	0.0	14.3	4.2	0.0	0.0
	Increased	16.7	21.4	0.0	16.7	4.3	7.1
	High	8.3	28.6	42.9	45.8	43.5	42.9
	Very High	16.7	28.6	14.3	20.8	34.8	21.4
Pi	Not detectable	62.5	50.0	71.4	54.2	56.5	57.1
	Borderline	12.5	21.4	14.3	8.3	4.3	0.0
	Increased	16.7	21.4	14.3	8.3	17.4	21.4
	High	4.2	7.1	0.0	20.8	13.0	14.3
	Very High	4.2	0.0	0.0	8.3	8.7	7.1

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; *Porphyromonas gingivalis (Pg), Tannerella forsythia (Tf), Treponema denticola (Td), Prevotella intermedia (Pi) and Aggregatibacter actinomycetemcomitans (Aa)*. Statistically significant differences between the groups are indicated by *.

diagnosis of hypertension. However, in this study, we observed that most patients have a systolic blood pressure greater than 140 mmHg and a diastolic blood pressure below 90 mmHg. According to the literature, isolated systolic hypertension occurs when we have this blood pressure profile [53]. Systolic blood pressure was significantly higher in the DMP group and this is consistent with some studies showing a negative change in blood pressure in patients with diabetes and becoming even more severe when these patients have periodontitis [5,41,54].

Therefore, as we find patients without a diagnosis of diabetes or prediabetes, it is possible that, by evaluating more carefully, we can find patients with some type of undiagnosed hypertension, especially in patients with active periodontitis and poor glycemic control. Furthermore, although we did not find statistical differences between the six groups, we observed that all patients were overweight and the DM group was already at the obesity threshold, since they all also had a waist circumference greater than 94 cm according to the parameters used for the evaluation of obesity in the literature [55]. A recent study reports that more than 80% of people with prediabetes are overweight or obese [50].

Taking in account the lipid profile, it is also possible to observe a tendency to dyslipidemia in these patients. Several studies highlight the importance of these parameters, such as BMI, waist circumference, dyslipidemia, and blood pressure, in addition to glycemic analysis, in evaluating DM2 or even in its prevention [55]. Therefore, we must emphasize the importance of evaluating these clinical and biochemical parameters in the first contact with the patient so that attention is not diverted to a single aspect.

Screening to investigate the presence of diabetes or prediabetes in dental offices should also be implemented considering the presence of periodontitis as one of the risk factors associated with diabetes. According to our study, of the three groups of patients with periodontitis, the diabetes group (DMP) had the highest degree of severity of

Table 8
Biochemical parameters.

	H[a]	PreDM[b]	DM[c]	P[d]	PreDMP[e]	DMP[f]
HbA1c (%)	5.37 ± 0.26[b,c,e,f]	5.91 ± 0.19[a,c,d,f]	7.40 ± 1.28[a,b,d,e]	5.31 ± 0.22[b,c,e,f]	5.93 ± 0.22[a,c,d,f]	7.34 ± 1.55[a,b,d,e]
FPG (mg/dL)	91.57 ± 12.66[c,f]	106.79 ± 33.4	155.43 ± 68.20[a,d,e]	90.63 ± 14.72[c,f]	99.43 ± 15.96[c,f]	135.64 ± 43.20[a,d,e]
Cholesterol (mg/dL)	190.04 ± 39.95	193.93 ± 49.86	188.43 ± 37.95	196.63 ± 42.53	191.30 ± 34.91	193.71 ± 49.12
Triglycerides (mg/dL)	108.75 ± 51.46	137.43 ± 64.79	167.29 ± 80.34	120.21 ± 59.44	143.00 ± 99.83	180.50 ± 187.50
HDL(mg/dL)	52.75 ± 15.26	46.71 ± 13.48	40.14 ± 9.65	55.42 ± 14.76	51.57 ± 19.11	50.79 ± 18.09
LDL(mg/dL)	115.38 ± 32.70	119.50 ± 43.51	118.43 ± 35.82	117.21 ± 34.82	112.78 ± 29.35	99.50 ± 50.52
VLDL(mg/dL)	21.75 ± 32.70	27.50 ± 43.51	29.00 ± 35.82	23.92 ± 34.82	28.61 ± 29.35	45.64 ± 50.52
Urea (mg/dL)	35.96 ± 8.19	32.86 ± 6.86	31.14 ± 3.08	31.04 ± 7.54	31.83 ± 8.28	35.46 ± 5.36
Creatinine (mg/dL)	0.93 ± 0.20	0.86 ± 0.16	0.88 ± 0.13	0.82 ± 0.15	0.82 ± 0.17	0.83 ± 0.16
Uric acid (mg/dL)	5.57 ± 1.21	5.79 ± 1.08	5.77 ± 0.95	5.69 ± 1.57	5.95 ± 1.38	5.43 ± 1.64
GPT	25.00 ± 13.99	21.93 ± 10.22	26.14 ± 7.65	27.83 ± 21.96	27.52 ± 17.96	19.79 ± 8.80
GGT	51.54 ± 62.47	39.64 ± 33.86	28.71 ± 18.80	56.75 ± 82.68	47.55 ± 35.26	44.57 ± 34.88
Albumin (g/dL)	4.32 ± 0.28	4.36 ± 0.43	4.23 ± 0.43	4.32 ± 0.52	4.38 ± 0.31	4.55 ± 0.21
Total bilirubin (mg/dL)	0.48 ± 0.19	0.6 ± 0.21	0.44 ± 0.21	0.6 ± 0.24	0.58 ± 0.28	0.47 ± 0.14
Phosphorus (mg/dL)	3.23 ± 0.91	2.86 ± 0.53	3.19 ± 0.60	2.98 ± 0.46	3.04 ± 0.42	3.08 ± 0.42
Calcium (mg/dL)	9.23 ± 0.42	9.28 ± 0.48	9.29 ± 0.41	9.35 ± 0.40	9.21 ± 0.45	9.49 ± 0.44
TSH (mUI/L)	2.55 ± 1.66	1.74 ± 0.74	2.3 ± 0.99	2.4 ± 1.48	2.16 ± 1.48	1.97 ± 0.96
Cortisol (nmol/L)	85.5 ± 57.76[d,e,f]	111.2 ± 62.24	119.07 ± 65.07	133.56 ± 59.53[a]	139.26 ± 53.42[a]	140.11 ± 54.35[a]

Abbreviations: H: no diabetes without periodontitis; PreDM: prediabetes without periodontitis; DM: diabetes without periodontitis; P: no diabetes with periodontitis; PreDMP: prediabetes with periodontitis; DMP: diabetes with periodontitis; FPG: fasting plasma glucose; Continuous variables are presented as mean ± SD. Statistically significant differences between groups are indicated by the letters a,b,c,d,e,f.

periodontitis, a strong positive correlation between HbA1c and PD levels greater than or equal to 6, CAL greater than or equal to 5 and the highest percentage of BoP zones. This is in line with the literature where BoP in periodontitis patients is indicative of the severity of the disease and periodontal instability, and people with higher BoP appear to have more risk factors [56].

However, in smokers, the manifestation of BoP can generally be altered, due to the fact that smoking has the ability to cause a decrease in the inflammatory response and therefore reduce this sign of inflammation in patients with periodontitis. [56,57] In this study, we observed that the differences between the groups have been significant in non-smokers and not in smokers. We can suggest that the BoP may be an excellent parameter to assess the state of the current disease only in non-smoking patients.

Regarding the microbiological profiles studied, *A. actinomycetemcomitans* was not detectable in most patients with periodontitis, these data agree with the low detection in the Spanish population [58], The exception is in DM2, which is observed differently in other studies [59]. This result represent *A. actinomycetemcomitans* serotypes with greater virulence for the manifestation of periodontitis [60]. However, when we compared their concentration in patients with DM2 with and without periodontitis, we had similar results to some studies evaluated in a systematic review [61]. Thus, these findings in patients with diabetes may also be associated with the fact that *A. actinomycetemcomitans* has been reported as an early colonizer and that changes in the immune system may influence its survival [62].

The bacteria most prevalent among periodontitis patients was *T. forsythia*, which belongs to the red group that is most associated with the manifestation of this disease, while the other bacteria in the red complex did not present statistical differences. This is in line with recent studies, mainly in patients with DM2 [61,63], while in another study no differences were observed in this microbial profile [59]. Regardless of statistical significance, we can assume that a dysbiosis profile is established that compares the groups with and without periodontitis.

Many studies have shown how the oral or intestinal microbiota can trigger altered metabolic responses and lead to a disease state. [64–69]. Furthermore, they also show the connection of this dysbiotic microbiota and its influence on the central nervous system (CNS) through the hypothalamic-pituitary-adrenal (HPA) axis, focused on the cortisol release [69]. It is known that cortisol is directly related to the hosts inflammatory response, suppressing its immune response, and its high levels also negatively affect blood pressure, glucose levels, and IR [70–72]. This feedback induces a dysregulation of this axis that chronically contributes to the maintenance of hyperglycemia, oxidative stress, mitochondrial dysfunction, and bone metabolism deterioration [73]. According to some authors, dysregulation of the HPA axis may be involved in the pathogenesis of diabetes and periodontitis [74].

An interesting finding in this study was that patients with periodontitis, especially those with diabetes, had significantly higher serum cortisol levels. Furthermore, cortisol was directly associated with HbA1c levels in the P group. However, many studies linking the microbiota with the CNS are preclinical studies, and caution should be exercised when translating the data from these studies into clinical data for humans using existing microbiome studies. But we can confirm that the recent research trend has focused on the fourth role of the microbiome: guiding the maturation and functionality of the host immune system [75]. For this reason, it is important to understand how to reverse this dysbiosis in these patients, which can positively impact several axes of patient metabolism, especially in older and systemically compromised patients.

In addition to the local and systemic factors linked to these diseases, we know that stressors of modern life accompanied by an unhealthy diet and inadequate physical activity may also implicate abnormal chronic activation of the HPA axis along with increased obesity, in addition to perpetuating a chronic systemic pro-inflammatory response. This could fuel the development of diabetes, other metabolic diseases, and periodontitis [63,76].

Patients with diabetes in this study show a good adaptation to the Mediterranean diet, especially patients with DMP, and this may be related to the fact that most of these patients already had a diagnosis of DM2 and therefore were already aware of the need for dietary changes, since this is the first intervention proposed by endocrinologists. Improving the diet of patients with diabetes is undoubtedly a fundamental approach in their treatment, as it is known that the consumption of high refined carbohydrates, saturated fats, and fructose is the main responsibility for maintaining the low degree of systemic inflammation involved in metabolic disorders [77,78].

On the other hand, in patients with prediabetes, those who are periodontally healthy have a very good adaptation to the Mediterranean diet, while many patients with periodontitis adhere poorly to a healthier diet. From the data of prediabetes patients, we can infer that the unhealthy diet may have helped develop periodontitis in these patients. This would be in line with a recent study that stated that a diet was associated with a lower extension of CAL [79] and that nutrition is a key component of the prevention of periodontitis [78].

In addition to diet, physical exercise is also a very important point to consider in these patients. In this study, although there are no statistically significant differences, it is possible to see that most of the patients are overweight or obese, and if we look at the average hours of weekly exercise, patients with diabetes have the lowest values, especially if we consider those with diabetes, prediabetes, and periodontitis.

Physical exercise, when practiced with the appropriate intensity and frequency, has the ability to improve various physiological functions of the body, such as improving glycemic control by improving insulin sensitivity, the immune-inflammatory response, and can even induce a rebalancing of the microbiota [80–83], also it can reduce the chances of periodontitis [84]. Based on our data, we can suggest that lack of physical exercise may also have had a negative impact on the development of periodontitis and poor glycemic control in these patients, mainly those of the pre-DMP and DMP groups.

Older age is one of the strongest risk factors for the development of diabetes/prediabetes and other chronic diseases [27,28]. To our knowledge, our study is the only one to include patients older than 50 years. Older people were mentioned to be often underrepresented in clinical trials for the treatment or prevention of diabetes and many questions remain about the detection, management, and prevention of prediabetes in this group of patients. The authors emphasize two approaches that may not be applicable in the group of older patients and patients with prediabetes: weight loss due to an increased possibility of bone demineralization and risk of fracture, and the use of hypoglycemic drugs, which would be questionable given that these patients are already elderly and cannot be exposed for a long time to the hyperglycemic condition in a way that leads to complications [51].

Taking these data into account, we can say that the detection and counseling of diabetes risk should always be in the pragmatic context of patient comorbidities, and this should begin at age 35, and patients with negative results should be retested every 3 years [11]. Additionally, asymptomatic adults with DM2 and / or prediabetes should be tested for DM2 and / or prediabetes when they are overweight or obese along with other risk factors such as hypertension ($\geq$140 / 90 mmHg or in treatment for hypertension), HDL ($<$35 mg/dL) and triglycerides ($>$250 mg/dL), and physical inactivity [11]. These parameters were evaluated in our study, the vast majority of our patients had a BMI $\geq$26 and blood pressure values very close to what they considered limit, although we did not find mean values close to the stipulated lipid parameters, perhaps due to the high adequacy to the Mediterranean diet in all groups.

According to a recent study, some degree of regression can be expected in populations receiving lifestyle interventions to mitigate the risk of prediabetes, with a 28% to 58% reduction in the risk of developing diabetes through lifestyle modifications, diet, and exercise practice that resulted in weight loss [50]. *In addition, the patient must change* his behavior as a passive recipient of care towards care focused on their collaboration for a

change in his lifestyle and commitment to treatment [85].

Data show that only one-third of people with prediabetes have received diet or exercise advice from their health care providers [86]. Possible explanations for this behavior, in addition to the lack of defined consensus, can include lack of knowledge of the possible effect of interventions to reduce the risk of diabetes, lack of access to nutritional and exercise counseling providers, and inertia therapy [87]. Therefore, it is very important to emphasize the need for a detailed evaluation to determine the patient profile we are dealing with, as the global obesity epidemic and the global increase in the global prevalence of prediabetes are of great concern, and this does not bode well for future prospects for diabetes and its complications worldwide [51].

Furthermore, it is possible to observe that most of the scientific evidence within the medical class still does not recognize the importance of multidisciplinary care for this patient profile together with dentists. It is important to emphasize more and more the association of these comorbidities with periodontitis and how it is not possible to approach these patients unilaterally.

This study is limited to the analyze of male patients older than 50 years restricted to a university dental clinic, and therefore these results should not be generalized to the entire community. We chose to use a sample with only male patients, because we believe that it is the best way to study risk factors, to eliminate any bias associated with possible different metabolic responses associated with gender. In recent decades, some studies have shown that endogenous sex hormones can differentially modulate glycemic status and risk in men and women [88,89].

Low testosterone levels in men are associated with an increased risk of developing DM2, while higher levels are needed in women to predict an increased risk of diabetes [88,90]. Furthermore, testosterone in men is directly associated with high HDL levels and is inversely associated with BMI and waist circumference [88]. We still do not know what the real effect of these hormones is on glycemic control and the development of DM2, but in a recent study, serum estradiol was positively and independently correlated with HbA1c levels in men, while testosterone showed no correlation [89].

In another study, the authors suggest that the incidence of hypertension is higher in women compared to men among participants with better glycemic control, but higher in men compared to women among those with worse glycemic control [90]. In addition to structural differences, environmental and developmental stressors can elicit a distinctive physiological response by sex [90]. The biological mechanisms responsible for the sex-related differences observed in cardiovascular aging are unraveling in recent decades [27] and there is evidence of how diabetes can influence the propensity of patients to develop cardiovascular complications, with women being the group at highest risk [91].

Evaluation of the limited microbiological profile of a few pathogens could be considered a limitation in our study. However, it is currently known that there is no specific microbiota in the biofilm, and its composition varies depending not only on the patient's health status but also on their exposure to various secondary factors related to the environment in which they live, habits, and psychosocial conditions [92]. Perhaps for this reason, our analysis did not show a characteristic profile for each group, although it agrees with the literature showing a higher number of pathogens in the red group and a reduced amount of *A. actinomycetemcomitans* in patients with periodontitis [61,93,94].

Furthermore, some parameters such as CRP levels, vitamin D, and inflammatory markers are not addressed in this study, and we suggest that they should be included in future studies in search of more detail of the patient's immunological profile. In general, it is possible to observe that there are still many questions about microbiological and metabolic parameters and the influence of secondary factors such as negative habits and the environment in which the individual lives. However, the importance of performing a complete evaluation of the patient in the dental office is indisputable [43], mainly in elderly patients, not only to investigate possible diseases in their initial stage or those that already appear silently, such as diabetes, but also to better target the treatment for each patient in a personalized way.

5. Conclusions

In the population studied, the prevalence of patients without a diagnosis of diabetes and prediabetes is very high and underestimated, especially considering the condition of prediabetes in elderly patients. The increased severity of periodontitis in patients with diabetes, along with the high level of cortisol seen in patients with periodontitis, mainly those with diabetes, emphasize the dysregulation of the immunoinflammatory system in these patients. All red complex bacteria were more prevalent in patients with periodontitis; however, only *T. forsythia* had a marked difference in relation to normoglycemic patients. However, the prevalence of *A. actinomycetemcomitans* was the most striking, since it was practically absent in periodontitis groups, except for the group with diabetes, which may be related to its altered immune system. Perhaps the diet profile and lack of physical exercise had an influence on the predisposition of some patients to develop periodontitis when we evaluated the patient's lifestyle in this study.

CRediT authorship contribution statement

Juliana Portes: Conceptualization, Methodology, Investigation, Supervision, Data curation, Writing – review & editing, Visualization. **Beatriz Bullón:** Conceptualization, Investigation, Resources. **Isabel Gallardo:** Data curation, Investigation, Resources. **Patricia Fernandez-Riejos:** Data curation, Investigation, Resources. **Jose Luis Quiles:** Conceptualization, Writing – review & editing. **Francesca Giampieri:** Conceptualization, Writing – review & editing. **Pedro Bullón:** Conceptualization, Methodology, Investigation, Supervision, Data curation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] GBD 2015 Risk Factors Collaborators, Global, regional, and national comparative risk assessment of 79 behavioural, environmental and occupational, and metabolic risks or clusters of risks, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015, Lancet 388 (2016) 1659–1724, https://doi.org/10.1016/S0140-6736(16)31679-8.

[2] N. Agarwal, F. Bull, R. Caixeta, M. Cho, A. Commar, C.C. Cortez, Noncommunicable Diseases Progress Monitor 2020, World Health Organization, Geneva, 2020.

[3] T. Haahtela, L. Von Hertzen, J.M. Anto, C. Bai, A. Baigenzhin, E.D. Bateman, et al., Helsinki by nature: the nature step to respiratory health, Clin. Transl. Allergy 9 (2019) 57, https://doi.org/10.1186/s13601-019-0295-2.

[4] I. Chapple, N. Wilson, Chronic non-communicable diseases, Br. Dent. J. 216 (2014) 487, https://doi.org/10.1038/sj.bdj.2014.357.

[5] M. Sanz, A. Marco Del Castillo, S. Jepsen, J.R. Gonzalez-Juanatey, F. D'Aiuto, P. Bouchard, et al., Periodontitis and cardiovascular diseases: consensus report, J. Clin. Periodontol. 47 (2020) 268–288, https://doi.org/10.1111/jcpe.13189.

[6] International Diabetes Federation, IDF Diabetes Atlas, 10th ed. Brussels, Belgium, 2021. Available from, www.diabetesatlas.org.

[7] C.D. Mathers, D. Loncar, Projections of global mortality and burden of disease from 2002 to 2030, PLoS Med 3 (2006) e442, https://doi.org/10.1371/journal.pmed.0030442.

[8] M. Sanz, A. Ceriello, M. Buysschaert, I. Chapple, R.T. Demmer, R.T.F. Graziani, et al., Scientific evidence on the links between periodontitiss and diabetes: consensus report and guidelines of the joint workshop on periodontitiss and diabetes by the International Diabetes Federation and the European Federation of Periodontology, J. Clin. Periodontol. 45 (2018) 138–149, https://doi.org/10.1111/jcpe.12808.

[9] B. Richter, B. Hemmingsen, M.I. Metzendorf, Y. Takwoingi, Development of type 2 diabetes mellitus in people with intermediate hyperglycaemia, Cochrane Database Syst. Rev. 10 (2018), CD012661, https://doi.org/10.1002/14651858.CD012661.pub2.
[10] S. Colagiuri, Definition and classification of diabetes and prediabetes and emerging data on phenotypes, Endocrino.l Metab. Clin. North Am. 50 (2021) 319–336, https://doi.org/10.1016/j.ecl.2021.06.004.
[11] American Diabetes Association Professional Practice Committee, 2. Classification and diagnosis of diabetes: standards of medical care in diabetes-2022, Diabetes Care 45 (2022) S17–S38, https://doi.org/10.2337/dc22-S002.
[12] Diabetes Prevention Program Research Group, 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study, Lancet 374 (2009) 1677–1686, https://doi.org/10.1016/S0140-6736(09)61457-4.
[13] N.J. Kassebaum, E. Bernabé, M. Dahiya, B. Bhandari, C.J. Murray, W. Marcenes, Global burden of severe periodontitis in 1990-2010: a systematic review and meta-regression, J. Dent. Res. 93 (2014) 1045–1053, https://doi.org/10.1177/0022034514552491.
[14] F. Graziani, S. Gennai, A. Solini, M. Petrini, A systematic review and meta-analysis of epidemiologic observational evidence on the effect of periodontitis on diabetes an update of the EFP-AAP review, J. Clin. Periodontol. 45 (2018) 167–187, https://doi.org/10.1111/jcpe.12837.
[15] H. Loe, Periodontal Disease: the sixth complication of diabetes mellitus, Diabetes Care 16 (1993) 329–334.
[16] P.N. Papapanou, M. Sanz, N. Buduneli, T. Dietrich, M. Feres, D.H. Fine, et al., Periodontitis: consensus report of workgroup 2 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, J. Periodontol. 89 (2018) S173–S182, https://doi.org/10.1002/JPER.17-0721.
[17] N.P. Lang, P.M. Bartold, Periodontal health, J. Periodontol. 89 (2018) S9–S16, https://doi.org/10.1002/JPER.16- 0517.
[18] M.J.L. Verhulst, B.G. Loos, V.E.A. Gerdes, W.J. Teeuw, Evaluating all potential oral complications of diabetes mellitus, Front. Endocrinol. 10 (2019) 56, https://doi.org/10.3389/fendo.2019.00056.
[19] M.A. González-Moles, P. Ramos-García, State of evidence on oral health problems in diabetic patients: a critical review of the literature, J. Clin. Med. 10 (2021) 5383, https://doi.org/10.3390/jcm10225383.
[20] D. Polak, T. Sanui, F. Nishimura, L. Shapira, Diabetes as a risk factor for periodontal disease- plausible mechanisms, Periodontol 83 (2020) 46–58, https://doi.org/10.1111/prd.12298.
[21] S. Jepsen, J.G. Caton, J.M. Albandar, N.F. Bissada, P. P.Bouchard, P. Cortellini, et al., Periodontal manifestations of systemic diseases and developmental and acquired conditions: consensus report of workgroup 3 of the 2017 World Workshop on the Classification of Periodontal and Peri-Implant Diseases and Conditions, J. Periodontol. 89 (2018) S237–S248, https://doi.org/10.1002/JPER.17-0733.
[22] P. Bullon, M.D. Cordero, J.L. Quiles, J.M. Morillo, M. Ramirez-Tortosa, M. Battino, Mitochondrial dysfunction promoted by Porphyromonas gingivalis lipopolysaccharide as a possible link between cardiovascular disease and periodontitis, Free Radic. Biol. Med. 50 (2011) 1336–1343, https://doi.org/10.1016/j.freeradbiomed.2011.02.018.
[23] P. Bullon, H.N. Newman, M. Battino, Obesity, diabetes mellitus, atherosclerosis and chronic periodontitis: a shared pathology via oxidative stress and mitochondrial dysfunction? Periodontol 64 (2014) 139–153, https://doi.org/10.1111/j.1600-0757.2012.00455.x.
[24] J. Portes, B. Bullón, J.L. Quiles, M. Battino, P. Bullón, Diabetes mellitus and periodontitis share intracellular disorders as the main meeting point, Cells 10 (2021) 2411, https://doi.org/10.3390/cells10092411.
[25] A. Varela-López, F. Giampieri, P. Bullón, P. Battino, M.J.L. Quiles, Role of lipids in the onset, progression and treatment of periodontal disease. A systematic review of studies in humans, Int. J. Mol. Sci. 17 (2016) 1202, https://doi.org/10.3390/ijms17081202.
[26] M.S. Al-Zahrani, E.A. Borawski, N.F. Bissada, Periodontitis and three health-enhancing behaviors: maintaining normal weight, engaging in recommended level of exercise, and consuming a high-quality diet, J. Periodontol. 76 (2005) 1362–1366, https://doi.org/10.1902/jop.2005.76.8.1362.
[27] V. Dela Justina, J.S.G. Miguez, F. Priviero, J.C. Sullivan, F.R. Giachini, R.C. Webb, Sex differences in molecular mechanisms of cardiovascular aging, Front Aging 2 (2021), 725884, https://doi.org/10.3389/fragi.2021.725884.
[28] I.B. Lamster, Geriatric periodontology: how the need to care for the aging population can influence the future of the dental profession, Periodontol 72 (2016) 7–12, https://doi.org/10.1111/prd.12157.
[29] M. Blüher, Obesity: global epidemiology and pathogenesis, Nat. Rev. Endocrinol. 15 (2019) 288–298, https://doi.org/10.1038/s41574-019-0176-8.
[30] M.A. Peres, L. Macpherson, R.J. Weyant, B. Daly, B.R. Venturelli, M.R. Mathur, et al., Oral diseases: a global public health challenge, Lancet 394 (2019) 249–260, https://doi.org/10.1016/S0140-6736(19)31146-8.
[31] M. Huber, J.A. Knottnerus, L. Green, H. van der Horst, A.R. Jadad, D. Kromhout, et al., How should we define health? B.M.J. 343 (2011) d4163, https://doi.org/10.1136/bmj.d4163.
[32] S. Saengtipbovorn, S. Taneepanichskul, Effectiveness of lifestyle change plus dental care (LCDC) program on improving glycemic and periodontal status in the elderly with type 2 diabetes, B.M.C. Oral Health 14 (2014) 1–10, https://doi.org/10.1186/1472-6831-14-72.
[33] B.G. Loos, T.E. Van Dyke, The role of inflammation and genetics in periodontal disease, Periodontol 83 (2020) 26–39, https://doi.org/10.1111/prd.12297.
[34] T. Simpson, I. Needleman, S. Wild, E. Mills, Treatment of periodontal disease for glycaemic control in people with diabetes (Review), Cochrane Collect 5 (2010) 1–52, https://doi.org/10.1002/14651858.CD004714.pub2.
[35] P.M. Bartold, Lifestyle and periodontitis: the emergence of personalized periodontics, Periodontol 78 (2018) 7–11, https://doi.org/10.1111/prd.12237.
[36] H. Schröder, M. Fitó, R. Estruch, M.A. Martínez-González, D. Corella, J. Salas-Salvadó, et al., A short screener is valid for assessing Mediterranean diet adherence among older Spanish men and women, J Nutr 141 (2011) 1140–1145, https://doi.org/10.3945/jn.110.135566.
[37] H.L. Taylor, D.R. Jacobs Jr, B. Schucker, J. Knudsen, A.S. Leon, G. Debacker, A questionnaire for the assessment of leisure time physical activities, J. Chronic. Dis. 31 (1978) 741–755, https://doi.org/10.1016/0021-9681(78)90058-9.
[38] A. Badersten, R. Nilvéus, J. Egelberg, Effect of nonsurgical periodontal therapy. VII. Bleeding, suppuration and probing depth in sites with probing attachment loss, J Clin Periodontol 12 (1985) 432–440, https://doi.org/10.1111/j.1600-051x.1985.tb01379.x.
[39] P.I. Eke, B.A. Dye, L. Wei, G.O. Thornton-Evans, R.J. Genco, CDC Periodontal Disease Surveillance workgroup: James Beck (University of North Carolina, Chapel Hill, USA), Gordon Douglass (Past President, American Academy of Periodontology), Roy Page (University of Washin. Prevalence of periodontitis in adults in the United States: 2009 and 2010, J Dent Res 91 (2012) 914–920, https://doi.org/10.1177/0022034512457373.
[40] E. Lanza, A. Magan-Fernandez, A. Bermejo, J. de Rojas, R. Marfil-Alvarez, F. Mesa, Complementary clinical effects of red complex bacteria on generalized periodontitis in a caucasian population, Oral Dis 22 (2016) 430–437, https://doi.org/10.1111/odi.12471.
[41] Z. Negahban, M. Rezaei, M.M. Daei, M. Mirzadeh, Evaluation of the systolic and diastolic right ventricular function: a comparison between diabetes, prediabetes and normal patients without coronary artery disease, Curr, Probl. Cardiol. 46 (2021), 100817, https://doi.org/10.1016/j.cpcardiol.2021.100817.
[42] A. Menke, S. Casagrande, L. Geiss, C.C. Cowie, Prevalence of and trends in diabetes among adults in the United States, 1988-2012, J.A.M.A. 314 (2015) 1021–1029, https://doi.org/10.1001/jama.2015.10029.
[43] S. Masiero, A. Alberti, S. Corbella, L. Francetti, Chairside screening for undiagnosed diabetes and prediabetes in patients with periodontitis, Int. J. Dent. 2022 (2022), 9120115, https://doi.org/10.1155/2022/912011.
[44] E. Montero, P. Matesanz, A. Nobili, J. Herrera-Pombo, M. Sanz, A. Guerrero, et al., Screening of undiagnosed hyperglycaemia in the dental setting: the DiabetRisk study. A field trial, J. Clin. Periodontol. 48 (2021) 378–388, https://doi.org/10.1111/jcpe.13408.
[45] N.C.R. Holm, D. Belstrøm, J.A. Østergaard, S. Schou, P. Holmstrup, M.B. Grauballe, Identification of individuals with undiagnosed diabetes and pre-diabetes in a danish cohort attending dental treatment, J. Periodontol. 87 (2016) 395–402, https://doi.org/10.1902/jop.2016.150266.
[46] M. Bossart, K. Calley, J.R. Gurenlian, B. Mason, R. Ferguson, T. Peterson, A pilot study of an HbA1c chairside screening protocol for diabetes in patients with chronic periodontitis: the dental hygienist's role, Int. J. Dent, Hyg. 14 (2016) 98–107, https://doi.org/10.1111/idh.12140.
[47] R.J. Genco, R.E. Schifferle, R.G. Dunford, K.L. Falkner, W.C.J. Hsu, Balukjian, Screening for diabetes mellitus in dental practices: a field trial, J. Am. Dent. Assoc. 145 (2014) 57–64, https://doi.org/10.14219/jada.2013.7.
[48] E. Lalla, C. Kunzel, S. Burkett, B. Cheng, I.B. Lamster, Identification of unrecognized diabetes and pre-diabetes in a dental setting, J, Dent. Res. 90 (2011) 855–860, https://doi.org/10.1177/0022034511407069.
[49] S.M. Strauss, M.C. Alfano, D. Shelley, T. Fulmer, Identifying unaddressed systemic health conditions at dental visits: patients who visited dental practices but not general health care providers in 2008, Am. J. Public Health. 102 (2012) 253–255, https://doi.org/10.2105/AJPH.2011.300420.
[50] J.B. Echouffo-Tcheugui, E. Selvin, Prediabetes and what it means: the epidemiological evidence, Annu. Rev. Public Health. 42 (2021) 59–77, https://doi.org/10.1146/annurev-publhealth-090419-102644.
[51] D. Tsilingiris, N.G. Vallianou, M. Dalamaga, Prediabetes screening: questionable benefits in the golden years, Metabol. Open. 10 (2021), 100091, https://doi.org/10.1016/j.metop.2021.100091.
[52] K.A.M. White, S. Daneshvari, J. Lilyquist, L. Luo, L.E. Steffen, A. Bivin, et al., Prediabetes: the variation between HbA1c and fasting plasma glucose, Int. J. Diabetol. Vasc. Dis. Res. (Suppl 2) (2015) 001, https://doi.org/10.19070/2328-353X-SI02001.
[53] F.L.J. Visseren, F. Mach, Y.M. Smulders, D. Carballo, K.C. Koskinas, et al., ESC National Cardiac Societies; ESC Scientific Document Group. 2021 ESC Guidelines on cardiovascular disease prevention in clinical practice, Eur. Heart J. 42 (2021) 3227–3337, https://doi.org/10.1093/eurheartj/ehab484.
[54] J.D. Beck, P.N. Papapanou, K.H. Philips, S. Offenbacher, Periodontal medicine: 100 years of progress, J. Dent. Res. 98 (2019) 1053–1062, https://doi.org/10.1177/0022034519846113. PMID: 31429666.
[55] D. Durrer Schutz, L. Busetto, D. Dicker, N. Farpour-Lambert, R. Pryke, H. Toplak, et al., European practical and patient-centred guidelines for adult obesity management in primary care, Obes. Facts. 12 (2019) 40–66, https://doi.org/10.1159/000496183.
[56] C.A. Ramseier, D. Mirra, S. Schütz, A. Sculean, N.P. Lang, C. Walter, et al., Bleeding on probing as it relates to smoking status in patients enrolled in supportive periodontal therapy for at least 5 years, J. Clin. Periodontol. 42 (2015) 150–159, https://doi.org/10.1111/jcpe.12344.
[57] T.S. Miranda, M.L. Almeida, L.M. Marins, H.D.P. da Silva, M. Feres, et al., Might smoking assuage the pro-inflammatory effect of diabetes in periodontal sites? Oral Dis 26 (2020) 200–212, https://doi.org/10.1111/odi.13206.
[58] M. Sanz, A.J. van Winkelhoff, D. Herrera, N. Dellemijn-Kippuw, R. Simón, E. Winkel, Differences in the composition of the subgingival microbiota of two periodontitis populations of different geographical origin. A comparison between

Spain and The Netherlands, Eur J Oral Sci 108 (2000) 383–392, https://doi.org/10.1034/j.1600-0722.2000.108005383.x.
[59] M. Akherati, E. Shafaei, H. Salehiniya, H. Abbaszadeh, Comparison of the frequency of periodontal pathogenic species of diabetics and non-diabetics and its relation to periodontitis severity, glycemic control and body mass index, Clin. Exp. Dent. Res. 7 (2021) 1080–1088, https://doi.org/10.1002/cre2.453.
[60] M. Mínguez, X. Pousa, D. Herrera, A. Blasi, M.C. Sánchez, R. León, M. Sanz, Characterization and serotype distribution of Aggregatibacter actinomycetemcomitans isolated from a population of periodontitis patients in Spain, Arch Oral Biol 12 (2014) 1359–1367, https://doi.org/10.1016/j.archoralbio.2014.07.021.
[61] L.S. Liu, N. Gkranias, B. Farias, D. Spratt, N. Donos, Differences in the subgingival microbial population of chronic periodontitis in subjects with and without type 2 diabetes mellitus-a systematic review, Clin. Oral Investig. 22 (2018) 2743–2762, https://doi.org/10.1007/s00784-018-2660-2.
[62] D.H. Fine, A.G. Patil, S.K. Velusamy, Aggregatibacter actinomycetemcomitans (Aa) under the radar: myths and misunderstandings of Aa and its role in aggressive periodontitis, Front. Immunol. 10 (2019) 728, https://doi.org/10.3389/fimmu.2019.00728.
[63] M. Montevecchi, L. Valeriani, M.R. Gatto, G. D'Alessandro, G. Piana, Subgingival pathogens in chronic periodontitis patients affected by type 2 diabetes mellitus: a retrospective case-control study, J. Periodontal Implant Sci. 51 (2021) 409–421, https://doi.org/10.5051/jpis.2100180009.
[64] M. Branchereau, F. Reichardt, P. Loubieres, P. Marck, A. Waget, V. Azalbert, et al., Periodontal dysbiosis linked to periodontitis is associated with cardiometabolic adaptation to high-fat diet in mice, Am. J. Physiol. Gastrointest. Liver Physiol. 310 (2016) G1091–G1101, https://doi.org/10.1152/ajpgi.00424.2015.
[65] F.Le Sage, O. Meilhac, M.P. Gonthier, Porphyromonas gingivalis lipopolysaccharide induces pro- inflammatory adipokine secretion and oxidative stress by regulating Toll-like receptor-mediated signaling pathways and redox enzymes in adipocytes, Mol. Cell. Endocrinol. 446 (2017) 102–110, https://doi.org/10.1016/j.mce.2017.02.022.
[66] J. Li, H. Lu, H. Wu, S. Huang, L. Chen, Q. Gui, et al., Periodontitis in elderly patients with type 2 diabetes mellitus: impact on gut microbiota and systemic inflammation, Aging 12 (2020) 25959–25980, https://doi.org/10.18632/aging.202174.
[67] G. Simionescu, O.D. Ilie, A. Ciobica, B. Doroftei, R. Maftei, D. Grab, et al., Mini-review on the possible interconnections between the gut-brain axis and the infertility-related neuropsychiatric comorbidities, Brain Sci 10 (2020) 1–15, https://doi.org/10.3390/brainsci10060384.
[68] L. Xue, X. Zou, X.Q. Yang, F. Peng, D.K. Yu, J.R. Du, Chronic periodontitis induces microbiota-gut- brain axis disorders and cognitive impairment in mice, Exp. Neurol. 326 (2020) 1–12, https://doi.org/10.3390/brainsci10060384.
[69] X. Jia, R. Yang, J. Li, L. Zhao, X. Zhou, X. Xu, Gut-bone axis: a non-negligible contributor to periodontitis, Front. Cell Infect. Microbiol 11 (2021) 1–16, https://doi.org/10.3390/brainsci10060384.
[70] A. Farzi, E.E. Fröhlich, P. Holzer, Gut microbiota and the neuroendocrine system, Neurotherapeutics 15 (2018) 5–22, https://doi.org/10.3390/brainsci10060384.
[71] L.G. Bermúdez-Humarán, E. Salinas, G.C. Ortiz, L.J. Ramirez-Jirano, J.A. Morales, O.K. Bitzer-Quintero, From probiotics to psychobiotics: live beneficial bacteria which act on the brain-gut axis, Nutr. 11 (2019) 890, https://doi.org/10.3390/brainsci10060384.
[72] B. Misiak, I. Łoniewski, W. Marlicz, D. Frydecka, A. Szulc, L. Rudzki, et al., The HPA axis dysregulation in severe mental illness: can we shift the blame to gut microbiota? Prog. Neuropsychopharmacol. Biol. Psychiatry. 102 (2020) 1–9, https://doi.org/10.1016/j.pnpbp.2020.109951.
[73] A.P. Shoubridge, C. Fourrier, J.M. Choo, C.G. Proud, T.J. Sargeant, G.B. Rogers, Gut microbiome regulation of autophagic flux and neurodegenerative disease risks, Front. Microbiol. 12 (2021) 1–10, https://doi.org/10.3390/brainsci10060384.
[74] K. Azuma, Q. Zhou, M. Niwa, K. Kubo, Association between mastication, the hippocampus, and the HPA Axis: a comprehensive review, Int. J. Mol. Sci. 18 (2017) 1–14, https://doi.org/10.3390/ijms18081687.
[75] Y. Wang, L.H. Kasper, The role of microbiome in central nervous system disorders, Brain Behav. Immun. 38 (2014) 1–12, https://doi.org/10.1016/j.bbi.2013.12.015.
[76] K. Watanabe, B.J. Petro, A.E. Shlimon, T.G. Unterman, Effect of periodontitis on insulin resistance and the onset of type 2 diabetes mellitus in Zucker diabetic fatty rats, J. Periodontol. 79 (2008) 1208–1216, https://doi.org/10.1902/jop.2008.070605.
[77] N. Jaiswal, S. Agrawal, A. Agrawal, High fructose-induced metabolic changes enhance inflammation in human dendritic cells, Clin. Exp. Immunol. 197 (2019) 237–249, https://doi.org/10.1111/cei.13299.
[78] P. Martinon, L. Fraticelli, A. Giboreau, C. Dussart, D. Bourgeois, F. Carrouel, Nutrition as a key modifiable factor for periodontitis and main chronic diseases, J, Clin. Med. 10 (2021) 197, https://doi.org/10.3390/jcm10020197.
[79] D.M. Wright, G. McKenna, A. Nugent, L. Winning, G.J. Linden, J.V. Woodside, Association between diet and periodontitis: a cross-sectional study of 10,000 NHANES participants, Am. J. Clin. Nutr. 112 (2020) 1485–1491, https://doi.org/10.1093/ajcn/nqaa266.
[80] A.M. Petersen, B.K. Pedersen, The anti-inflammatory effect of exercise, J. Appl. Physiol. 98 (2005) 1154–1162, https://doi.org/10.1152/japplphysiol.00164.2004.
[81] A.P. Anderson, Y.M. Park, D. Shrestha, J. Zhang, J. Liu, A.T. Merchant, Cross-sectional association of physical activity and periodontal antibodies, J. Periodontol. 89 (2018) 1400–1406, https://doi.org/10.1002/JPER.17-0709.
[82] B. Cerdá, M. Pérez, J.D. Pérez-Santiago, J.F. Tornero-Aguilera, R. González-Soltero, M. Larrosa, Gut microbiota modification: another piece in the puzzle of the benefits of physical exercise in health? Front. Physiol. 7 (2016) 51, https://doi.org/10.3389/fphys.2016.00051.
[83] S.J. Han, K.H. Bae, H.J. Lee, S.J. Kim, H.J. Cho, Association between regular walking and periodontitis according to socioeconomic status: a cross-sectional study, Sci. Rep. 9 (2019) 12969, https://doi.org/10.1038/s41598-019-49505-2.
[84] M. Almohamad, E. Krall Kaye, D. Mofleh, N.L. Spartano, The association of sedentary behaviour and physical activity with periodontal disease in NHANES 2011-2012, J. Clin. Periodontol. 49 (2022) 758–767, https://doi.org/10.1111/jcpe.13669.
[85] A. Karve, R.A. Hayward, Prevalence, diagnosis, and treatment of impaired fasting glucose and impaired glucose tolerance in nondiabetic U.S. adults, Diabetes Care 33 (2010) 2355–2359, https://doi.org/10.2337/dc09-1957.
[86] A.G. Mainous 3rd, R.J. Tanner, R. Baker, Prediabetes diagnosis and treatment in primary care, J. Am, Board, Fam. Med. 29 (2016) 283–285, https://doi.org/10.3122/jabfm.2016.02.150252.
[87] Y. Xu, W. Cao, Y. Shen, J. Tang, Y. Wang, X. Ma, et al., The relationship between sex hormones and glycated hemoglobin in a non-diabetic middle-aged and elderly population, B.M.C. Endocr. Disord. 22 (2022) 91, https://doi.org/10.1186/s12902-022-01002-w.
[88] M. Karakas, S. Schäfer, S. Appelbaum, F. Ojeda, K. Kuulasmaa, B. Brückmann, et al., Testosterone levels and type 2 diabetes-no correlation with age, differential predictive value in men and women, Biomolecules 8 (2018) 76, https://doi.org/10.3390/biom8030076.
[89] E.L. Ding, Y. Song, V.S. Malik, S. Liu, Sex differences of endogenous sex hormones and risk of type 2 diabetes: a systematic review and meta-analysis, J.A.M.A 295 (2006) 1288–1299, https://doi.org/10.1001/jama.295.11.1288.
[90] T. Costacou, T.J. Orchard, Differential effect of glycemia on the incidence of hypertension by sex: the epidemiology of diabetes complications study, Diabetes Care 36 (2013) 77–83, https://doi.org/10.2337/dc12-0708.
[91] R.J. Phang, R.H. Ritchie, D.J. Hausenloy, J.G. Lees, S.Y. Lim, Cellular interplay between cardiomyocytes and non-myocytes in diabetic cardiomyopathy, Cardiovasc. Res. (2022) cvac049, https://doi.org/10.1093/cvr/cvac049.
[92] R. Pianeta, M. Iniesta, D.M. Castillo, G.I. Lafaurie, M. Sanz, D. Herrera, Characterization of the subgingival cultivable microbiota in patients with different stages of periodontitis in Spain and Colombia. A cross-sectional study, Microorganisms 9 (2021) 1940, https://doi.org/10.3390/microorganisms9091940.
[93] C.A. Field, M.D. Gidley, P.M. Preshaw, N. Jakubovics, Investigation and quantification of key periodontal pathogens in patients with type 2 diabetes, J. Periodontal Res. 47 (2012) 470–478, https://doi.org/10.1111/j.1600-0765.2011.01455.x.
[94] M. Feres, B. Retamal-Valdes, C. Gonçalves, L. Cristina Figueiredo, F. Teles, Did Omics change periodontal therapy? Periodontol 85 (2021) 182–209, https://doi.org/10.1111/prd.12358.