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Journal of Dental Health, Oral Disorders & Therapy

Subgingival Microbiota of an Indigenous Mexican Population with Chronic Periodontitis and T2DM

Abstract

Background: Otomi-Indians (OI) exhibit a high prevalence of type 2 diabetes (T2DM) and periodontal disease; however, to date there is only scarce information published regarding the subgingival microbiota associated with this and other indigenous Mexican populations. The aim of this study was to describe and compare the subgingival microbiota of Mestizo Mexicans and OI with and without T2DM, exhibiting periodontal health (PH) and chronic periodontitis (CP).

Methods: 115 Mestizos and 63 OI (N=178) were distributed in 4 groups: non-diabetic (non-T2DM) and T2DM individuals each with PH and CP. Subgingival plaque samples were processed to determine the levels, prevalence, and proportion of 40 microorganisms using DNA-probes. Blood-chemistry data (BCD) and obesity parameters were also determined in T2DM subjects.

Results: In non-T2DM, OI harbored higher total bacterial counts than Mestizos (p<0.001), as well as a greater proportion of red-complex species (p<0.001) and a lower proportion of *Actinomyces* sp. (p<0.01); however, the comparisons were only significant for PH subjects. In CP, T2DM subjects, both OI and Mestizos, exhibited a higher prevalence (Prev) and proportion (Prop) than non-T2DM individuals of the following species: *Actinomyces georgiae*, *Streptococcus anginosus*, *Streptococcus intermedius* (Prev & Prop: p<0.001), *Veillonella parvula* (Prev: p<0.01 & Prop: p<0.05), *Capnocytophaga ochracea*, *Streptococcus constellatus* (Prev & Prop: p<0.001), *Prevotella nigrescens* and *Parvimonas micra* (Prev: p<0.001 & Prop: p<0.01). No significant microbial differences were associated with BCD parameters. T2DM subjects with obesity presented increases in *S. intermedius* (Levels: p<0.01 & Prop: p<0.05), *Treponema denticola* and *Neisseria mucosa* (Levels: p<0.05) than individuals with normal bodyweight.

Conclusions: Non-T2DM OI individuals with PH harbored a microbial profile suggestive of a higher predisposition for periodontal diseases. A common subgingival profile associated with T2DM in CP subjects from both ethnic groups was identified, consisting of elevated proportions of putative periodontal pathogens and *Streptococcus* sp., as well as by lower proportion of red-complex species.

Keywords: Type 2 diabetes; Chronic periodontitis; Subgingival plaque; Microbiology; DNA-DNA hybridization; Mexican ethnic groups, Indigenous populations.

Research Article

Volume 7 Issue 2 - 2017

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Received: April 16, 2017 | **Published:** May 08, 2017

Abbreviations: T2DM: Type 2 Diabetes Mellitus; Non-T2DM: Non Diabetic; PH: Periodontal Health; CP: Chronic Periodontitis; OI: Otomi Indians; AL: Attachment Level; PD: Pocket Depth; PLA: Plaque Accumulation; GE: Gingival Erythema; BOP: Bleeding on Probing; SUP: Suppuration; BCD: Blood Chemistry Data; BMI: Body Mass Index; HbA_{1c}: Glycated Hemoglobin; TL: Total Lipids; TG: Triglycerides; TC: Total Cholesterol; HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; UG: Ungrouped; ATCC: American Type Culture Collection; SEM: Standard Error of the Mean; NS: Not Significant; UNAM: National Autonomous University of Mexico; UAEH: Autonomous University of the State of Hidalgo

Introduction

The subgingival microbiota has been extensively studied in populations from different geographical locations. Such studies that aim to improve the outcomes of clinical periodontal treatments, have reported a number of differences in the prevalence and proportion of periodontal pathogens in specific populations. Several studies have shown evidence that major differences in the proportion or prevalence of species such as *Tannerella forsythia*, *Porphyromonas gingivalis*, *Treponema denticola* and *Aggregatibacter actinomycetemcomitans*, may occur in populations with periodontitis from Chile, Sweden, USA, Brazil, Spain and the Netherlands [1,2]. In previous studies, our research group has described that the subgingival microbiota of Mexican Mestizo populations with aggressive and chronic periodontitis (CP) was characterized by elevated proportions of red-complex species and a decrease in the proportion of *Actinomyces* sp. [3,4]. However, to date, there are no published studies describing the subgingival microbiota of the indigenous populations of Mexico. Natives from a Brazilian study were described as having an infrequent occurrence of *T. denticola* [5]. Similarly, *T. denticola* and

Eubacterium nodatum were found to be uncommon in a population of indigenous individuals from Guatemala, such pathogens were detected in less than 10% of the subjects evaluated [6]. These studies concluded that there may be important differences in subgingival microbial profiles between urban communities and the often marginalized indigenous populations in developing countries in which oral healthcare regimens and other basic medical services can be scarce [5]. Thus, the study of indigenous populations can provide an opportunity to research the "natural" microbiota of untreated periodontitis. In 2005, Mexican natives comprised 9.8% of the country's population. These individuals consisted of 62 different ethnic groups [7], one of them being the Otomi-Indians (OI). Recent population statistics have reported 646,872 OI living within the Mexican territory, and a prevalence of 4.4% of type 2 diabetes mellitus (T2DM) in this ethnic group [8]. One of the most relevant reports of high prevalence of T2DM is the population of Pima Indians from the Gila River in Arizona. They have reported the highest predisposition to T2DM and a threefold increased risk of periodontitis compared to individuals without T2DM (non-T2DM) [9]. Studies in Mexican populations from urban areas have reported a high prevalence of Metabolic Syndrome components including obesity, insulin resistance, dyslipidaemia, hypertension [10] and a high prevalence of T2DM [11,12]. Indigenous and urban Mexican populations are often settled in different geographical locations and have distinct cultural, nutritional and healthcare regimens. There is a need to study each population individually in order to better understand the factors that influence and predispose the initiation and progression of both periodontitis and T2DM in each one, and thus, to be able to provide specific and effective preventive and therapeutic strategies [13].

To date, published studies have presented contradictory information regarding the subgingival microbial composition in T2DM subjects. While some studies have reported a higher prevalence, levels or proportion of periodontal pathogens in CP T2DM individuals than in non-T2DM [14-16], others have suggested a lower frequency of detection of "red complex" species in T2DM [17,18]. Furthermore, few studies have evaluated the subgingival microbiota of PH subjects with T2DM, thus, making it difficult to understand if a microbial profile exists that is associated specifically with T2DM, irrespective of the periodontal status [19-21]. The purpose of the present study was to describe and compare the subgingival microbiota of Mexican Mestizo individuals and Otomi-Indians (OI) with T2DM exhibiting both periodontal health (PH) and CP.

Materials and Methods

Subject population

The present study was approved by the Ethics Committee of the School of Dentistry of the National University of Mexico (UNAM). Subjects were asked to sign informed-consent forms acknowledging their willingness to participate in the study. Subjects were randomly selected from either the Clinic for the Prevention, Detection and Comprehensive Care of Diabetic Patients (Santiago de Anaya, Mexico) or one of the dental care clinics at the School of Dentistry of UNAM. The subject population is summarized in Tables 1A & 1B. The population consisted of 178 currently non-smoking subjects: 115 Mestizos [PH non-T2DM (*n*=41), CP non-T2DM (*n*=59), PH T2DM (*n*=5), CP T2DM (*n*=10)] and 63 OI [PH non-T2DM (*n*=18), CP non-T2DM (*n*=8), PH T2DM (*n*=9), CP T2DM (*n*=28)]. Subjects had not received periodontal therapy in the past, presented at least 20 teeth (excluding third molars) and were born in Mexico. OI individuals had a direct indigenous lineage, having been born themselves, both their parents and all of their grandparents in the Valle del Mezquital region, in the state of Hidalgo. Similarly, Mestizo subjects were selected as such by having been born themselves, both their parents and all of their grandparents in Mexico City. Diabetic subjects were diagnosed with T2DM at least one year prior to their inclusion in the study by medical evaluation and levels of glycated hemoglobin (HbA_{1c}) ≥6.5%. PH subjects had ≤4 sites with attachment level (AL) of 4 mm, no sites with AL ≥5 mm and were at least 18 years of age. CP subjects had ≥8 sites with AL ≥5 mm and were ≥35 years of age. Exclusion criteria included current smokers, pregnancy, lactation, antibiotic-therapy in the 3 months prior to sampling, and any systemic condition (except T2DM in the corresponding groups) such as HIV/AIDS or autoimmune diseases. Subjects receiving insulin or presenting type 1 diabetes were also excluded from the study.

Clinical evaluation and sample collection

Clinical data and samples were collected in a single visit. General information obtained included medical history, age, gender, smoking status, weight, height, blood pressure and recent systemic antibiotic therapy. Patients were defined as overweight if they exhibited a body mass index (BMI: kg weight/m^2 height) of 25.0-29.9 and as having obesity class I with a BMI of 30.0-34.9, class II 35.0-39.9 and class III ≥ 40.0. Periodontal parameters included previous periodontal treatment, number of missing teeth and clinical measurements taken at 6 sites per tooth from all teeth, excluding third molars (168 sites per subject), as previously described [22]. Measurements assessed were pocket depth (PD, mm), AL (mm), plaque accumulation (PLA, 0/1: undetected/detected), gingival erythema (GE, 0/1), bleeding on probing (BOP, 0/1) and suppuration (SUP, 0/1). PD and AL were recorded twice by the same examiner using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). The average of the pair of measurements was used in the analyses. After drying and isolating with cotton rolls, supragingival plaque was removed with curettes and subgingival plaque samples were obtained with individual sterile Gracey curettes (Hu-Friedy) from the mesiobuccal site of up to 28 teeth (excluding third molars). Samples were placed in individual tubes containing 150 µl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.6), dispersed and mixed with 100 µl of 0.5 M NaOH. A 5 ml sample of peripheral blood was collected from all T2DM subjects using a standard venipuncture technique and placed in vacutainer tubes containing sodium heparin (Becton, Dickinson and Co., Franklin Lakes, NJ, USA). Tubes were inverted several times and processed to determine blood-chemistry data (BCD) including serum levels of HbA_{1c}, total lipids (TL), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL).

Microbial assessment

Digoxigenin labelled whole genomic DNA-probes were

prepared using a random primer technique [23]. Samples were processed individually for the detection and enumeration of 40 microbial species using the "Checkerboard" DNA–DNA hybridization technique [24], according to procedures previously described [4]. A list of bacterial strains employed for the development of DNA-probes is presented in Table 2. DNA was isolated and purified [25] from American Type Culture Collection (ATCC, Rockville, MD, USA) lyophilized stocks. The specificity and sensitivity of DNA-probes were assessed and the assay was adjusted to a sensitivity of approximately 10^4 cells of each species.

Table 1A: Characteristics of Mestizo subjects.

Mestizos								
	PH Non-T2DM (*n*=41)		CP Non-T2DM (*n*=59)		PH T2DM (*n*=5)		CP T2DM (*n*=10)	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Age (years)***	28.0 ± 1.2	18-60	48.2 ± 1.2	35-75	43.8 ± 2.3	37-51	55.3 ± 2.9	35-70
Gender (% females)	53.7		54.2		20.0		60.0	
Height (cm)	166.9 ± 3.0	143-186	162.1 ± 1.5	139-178	164.2 ± 4.1	150-173	156.6 ± 3.5	143-180
Weight (kg)	68.0 ± 3.6	45-96	71.8 ± 3.0	38-124	77.8 ± 2.7	73-88	68.8 ± 6.2	45-116
Mean body mass index	24.2 ± 1.1	17.9-36.1	26.8 ± 0.8	17.9-43.9	29.3 ± 2.6	24.7-39.1	27.6 ± 1.4	19.7-35.8
% Overweight (BMI: 25.0 to 29.9)	25.0		58.3		80.0		80.0	
% Obesity (BMI: ≥30)	6.3		19.4		40.0		20.0	
Number of missing teeth***	1.5 ± 0.3	0-6	3.7 ± 0.3	0-8	1.8 ± 1.3	0-7	5.0 ± 0.7	1.0-8.0
Mean pocket depth (mm)***	2.1 ± 0.03	1.7-2.6	4.0 ± 0.1	2.3-7.4	2.1 ± 0.1	1.9-2.3	3.4 ± 0.4	2.1-5.8
Mean AL (mm)***, (**)	1.9 ± 0.04	0.9-2.3	4.5 ± 0.2	2.7-9.0	1.3 ± 0.1	1.0-1.5	3.7 ± 0.4	2.2-6.9
Mean sites with AL ≥ 5 mm***, (**)	0±0	0-0	55.4 ± 3.7	11-118	0 ± 0	0-0	37.5 ± 9.1	11-103
% sites with:								
Plaque accumulation	27.3 ± 4.6	0-87.5	46.4 ± 4.4	0-100	89.9 ± 5.2	69.6-98.1	89.1 ± 3.3	61.9-100
Gingival erythema	8.9 ± 2.9	0-78.6	20.4 ± 3.7	0-100	5.2 ± 2.2	0-10.5	37.4 ± 10.5	0-100
Bleeding on probing***	7.6 ± 2.0	0-53.0	45.8 ± 2.9	4.9-100	24.6 ± 1.9	19.1-29.6	60.3 ± 8.7	7.9-95.1
Suppuration***, (**)	0 ± 0	0-0	5.5 ± 1.0	0-37.0	0 ± 0	0-0	20.2 ± 5.2	1.5-47.5

Significance of differences was determined using the Mann-Whitney *U* test: ***$p<0.001$: PH *vs*. CP in non-T2DM. (**)$p<0.01$: PH *vs*. CP in T2DM. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. AL: attachment level. SEM: standard error of the mean. BMI: body mass index).

Table 1B: Characteristics of Otomi-Indian subjects.

Otomi-Indians								
	PH Non-T2DM (*n*=18)		CP Non-T2DM (*n*=8)		PH T2DM (*n*=9)		CP T2DM (*n*=28)	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Age (years)*, (*)	39.2 ± 1.7	29-51	56.0 ± 4.3	38-71	47.2 ± 3.0	36-63	61.0 ± 2.1	38-76
Gender (% females)	72.2		50.0		77.8		71.4	
Height (cm)	154.8 ± 1.7	143-168	152.8 ± 3.0	145-170	157.0 ± 3.4	148-174	149.1 ± 1.5	137-165
Weight (kg)	69.0 ± 2.4	56-92	63.8 ± 4.2	50-83	65.6 ± 4.0	52-91	62.4 ± 2.3	45-88
Mean body mass index	28.8 ± 0.8	23.1-35.7	27.3 ± 1.7	23.1-37.9	26.4 ± 0.8	23.1-31.1	28.1 ± 1.0	20.8-45.5

% Overweight (BMI: 25.0 to 29.9)	88.2		62.5		55.6		67.9	
% Obesity (BMI: ≥30)	35.3		12.5		11.1		25.0	
Number of missing teeth	0.8 ± 0.2	0-3	1.3 ± 0.5	0-4	2.1 ± 0.6	0-5	3.4 ± 0.5	0-8
Mean pocket depth (mm)$^{**,(*)}$	2.4 ± 0.1	2.0-3.2	3.2 ± 0.1	2.8-4.0	2.4 ± 0.1	1.9-3.1	3.1 ± 0.1	1.9-4.5
Mean AL (mm)$^{**,(***)}$	1.8 ± 0.1	1.1-2.4	3.1 ± 0.2	2.3-4.0	1.5 ± 0.1	1.2-2.1	3.4 ± 0.2	2.0-5.5
Mean sites with AL ≥5 mm$^{***,(***)}$	0 ± 0	0-0	14.4 ± 2.7	8-28	0 ± 0	0-0	35.4 ± 5.1	9-101
% sites with:								
Plaque accumulation	88.3 ± 2.7	60.3-100	90.8 ± 4.0	67.3-100	84.3 ± 5.8	48.8-100	94.2 ± 1.7	68.3-100
Gingival erythema	44.9 ± 6.1	0-80.4	59.2 ± 8.4	14.0-90.7	32.6 ± 11.3	4.3-100	33.9 ± 3.1	0-61.4
Bleeding on probing	36.3 ± 4.3	16.0-83.9	46.9 ± 10.2	0-97.5	33.5 ± 5.6	9.4-62.0	57.4 ± 4.2	6.7-100
Suppuration$^{(**)}$	0 ± 0	0-0	0.6 ± 0.3	0-2.1	0 ± 0	0-0	17.7 ± 2.9	0-68.8

Significance of differences was determined by paired comparisons using the Mann-Whitney *U* test: $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$: PH *vs.* CP in non-T2DM. $^{(*)}p<0.05$, $^{(**)}p<0.01$, $^{(***)}p<0.001$: PH *vs.* CP in T2DM. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. AL: attachment level. SEM: standard error of the mean. BMI: body mass index).

Table 2: Reference strains employed for the development of DNA-probes.

Species	ATCC	Complex	Species	ATCC	Complex
Actinomyces georgiae	49285	*Actinomyces*	*Neisseria mucosa*	19696	Other
Actinomyces israelii	12102	*Actinomyces*	*Parvimonas micra*	33270	Orange
Actinomyces naeslundii	12104	*Actinomyces*	*Porphyromonas endodontalis*	35406	Other
Actinomyces odontolyticus	17929	Purple	*Porphyromonas gingivalis*	33277	Red
Actinomyces viscosus	43146	*Actinomyces*	*Prevotella intermedia*	25611	Orange
Aggregatibacter actinomycetemcomitans	*	Ungrouped	*Prevotella melaninogenica*	25845	Other
Campylobacter gracilis	33236	Orange	*Prevotella nigrescens*	33563	Orange
Campylobacter rectus	33238	Orange	*Propionibacterium acnes*	6919	Other
Campylobacter showae	51146	Orange	*Selenomonas artemidis*	43528	Other
Capnocytophaga gingivalis	33624	Green	*Selenomonas noxia*	43541	Ungrouped
Capnocytophaga ochracea	27872	Green	*Streptococcus anginosus*	33397	Yellow
Capnocytophaga sputigena	33612	Green	*Streptococcus constellatus*	27823	Orange
Corynebacterium matruchotii	14266	Other	*Streptococcus gordonii*	10558	Yellow
Eikenella corrodens	23834	Green	*Streptococcus intermedius*	27335	Yellow
Eubacterium saburreum	33271	Other	*Streptococcus mitis*	49456	Yellow
Eubacterium sulci	35585	Other	*Streptococcus oralis*	35037	Yellow
Fusobacterium nucleatum	**	Orange	*Streptococcus sanguinis*	10556	Yellow
Fusobacterium periodonticum	33693	Orange	*Tannerella forsythia*	43037	Red
Gemella morbillorum	27824	Other	*Treponema denticola*	35405	Red
Leptotrichia buccalis	14201	Other	*Veillonella parvula*	10790	Purple

*Serotypes a (43717) and b (43718) were combined to generate a single DNA-probe. **Subspecies *nucleatum* (25586), *polymorphum* (10953) and *vincentii* (49256) were combined to generate a single DNA-probe. (ATCC: American Type Culture Collection, Rockville, MD. USA. Complex: species were grouped similarly to the descriptions of microbial complexes in subgingival plaque [26,27] with the following exception: *C. matruchotii*, *E. saburreum*, *E. sulci*, *G. morbillorum*, *L. buccalis*, *N. mucosa*, *P. endodontalis*, *P. melaninogenica*, *P. acnes* and *S. artemidis* were grouped as "Other").

Statistical Analysis

Microbial data obtained were the absolute counts of each of the 40 test-species from up to 28 subgingival plaque samples per subject. The analyses compared the composition of subgingival plaque between clinical groups, expressed as mean levels (DNA-probe count), prevalence (% sites colonized) and proportion (% total DNA-probe count) ± standard error of the mean (SEM) of individual test species. The proportion of microbial complexes was also determined by grouping the test species as similarly as possible to previous descriptions of microbial complexes in subgingival plaque [26,27]. Exceptions are noted in Table 2. Each data type was computed for individual species and for microbial complexes in every sample, averaged within a subject and then across subjects in each group. Significance of differences in microbiological parameters was sought by paired comparisons between Mestizos and OI, separately for diabetic groups with either PH or CP, as well as for periodontal groups separately for non-T2DM and T2DM using the Mann-Whitney *U* test. The significance of differences in microbial profiles was also sought between subjects with different results in BCD and diabetic clinical parameters. All values of significance were adjusted for multiple comparisons, as previously described [28].

Results

All the test-species were detected in both Mestizos and OI with non-T2DM, T2DM, PH and CP; however, significant differences were observed in the subgingival microbial composition between several specific groups. Figure 1 summarizes the mean total levels of bacterial species (total DNA-probe counts $x10^5$ ± SEM) in all subject groups. OI harbored higher mean total levels than Mestizos in non-T2DM subjects with both PH and CP; however, the difference was statistically significant only in subjects with PH (CP: Mestizo 115.8 ± 12.1 *vs*. OI 224.4 ± 28.3, *NS*. PH: Mestizo 73.8 ± 10.7 *vs*. OI 268.3 ± 36.8, $p<0.001$). In T2DM subjects, no significant differences were detected between Mestizos and OI with either PH or CP. On the other hand, T2DM subjects exhibited significantly higher mean total levels than non-T2DM in Mestizos with both PH and CP (PH: Non-T2DM 73.8 ± 10.7 *vs*. T2DM 307.4 ± 100.6, $p<0.05$. CP: Non-T2DM 115.8 ± 12.1 *vs*. T2DM 388.4 ± 57.9, $p<0.001$). In OI, no significant differences were detected between non-T2DM and T2DM subjects with either PH or CP. Also, while in Mestizos, subjects with CP tended to have higher mean total level than those with PH in both diabetic groups, no statistically significant differences were observed between PH and CP in either OI or Mestizos with T2DM or non-T2DM. The mean proportions (% total DNA-probe count) of microbial complexes in each clinical group are summarized in Figure 2. In PH subjects, OI exhibited higher proportions of red-complex species ($p<0.001$) and of the species in the "ungrouped-complex" *A. actinomycetemcomitans* and *Selenomonas noxia* ($p<0.01$), as well as a significantly lower proportion of *Actinomyces* sp. ($p<0.001$) than Mestizos with both T2DM and non-T2DM; however, the differences were statistically significant only for the non-T2DM groups after adjusting for multiple comparisons. In CP subjects, no significant differences were detected between Mestizos and OI with either T2DM or non-T2DM. On the other hand, in subjects with CP, T2DM individuals exhibited significantly higher proportions than non-T2DM of the *Streptococcus* sp. grouped in the yellow-complex in both Mestizos ($p<0.05$) and OI ($p<0.01$), as well as lower proportions of red-complex species (Mestizos: *NS*. OI: $p<0.001$). In subjects with PH, no significant differences were detected between non-T2DM and T2DM subjects in either the Mestizo or OI groups. Also, in Mestizos, subjects with CP exhibited higher proportions of red-complex species ($p<0.001$) and of the "ungrouped-complex" ($p<0.05$), as well as a significantly lower proportion of *Actinomyces* sp. ($p<0.01$) than those with PH, in both T2DM and non-T2DM groups; however, the differences were statistically significant only in non-T2DM individuals. The only statistically significant difference between CP and PH subject in T2DM Mestizos was a higher proportion of purple-complex species ($p<0.05$). No statistically significant differences were observed between PH and CP in OI with either T2DM or non-T2DM.

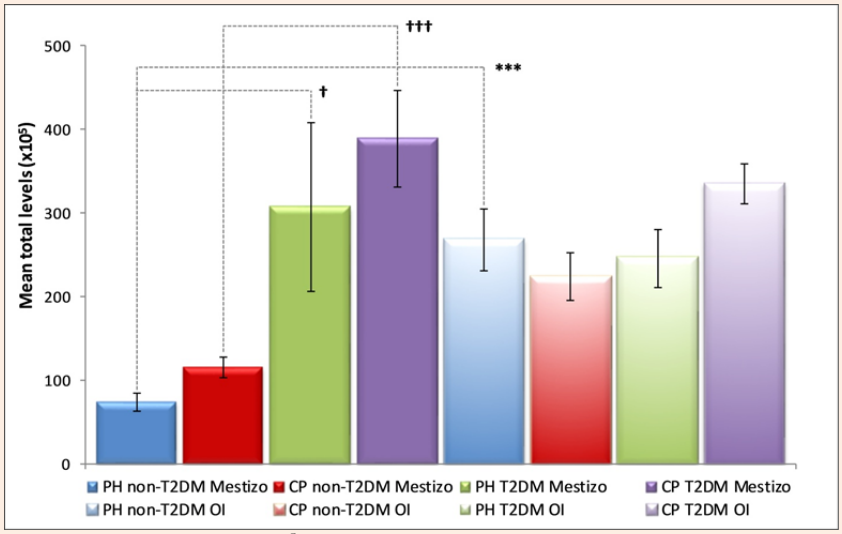

Figure 1: Mean total levels (total DNA-probe counts $x10^5$ ± SEM) of bacterial species in subgingival plaque samples from 115 Mestizo and 63 OI subjects grouped as non-T2DM and T2DM with either PH or CP. Total levels of the 40 bacterial test species were computed in every sample, averaged within a subject and then across subjects in each group. Significance of differences was determined by paired comparisons using the Mann-Whitney *U* test. $^{***}p<0.001$: Mestizo *vs*. OI. $^{\dagger}p<0.05$, $^{\dagger\dagger\dagger}p<0.001$: non-T2DM *vs*. T2DM. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. OI: Otomi-Indians).

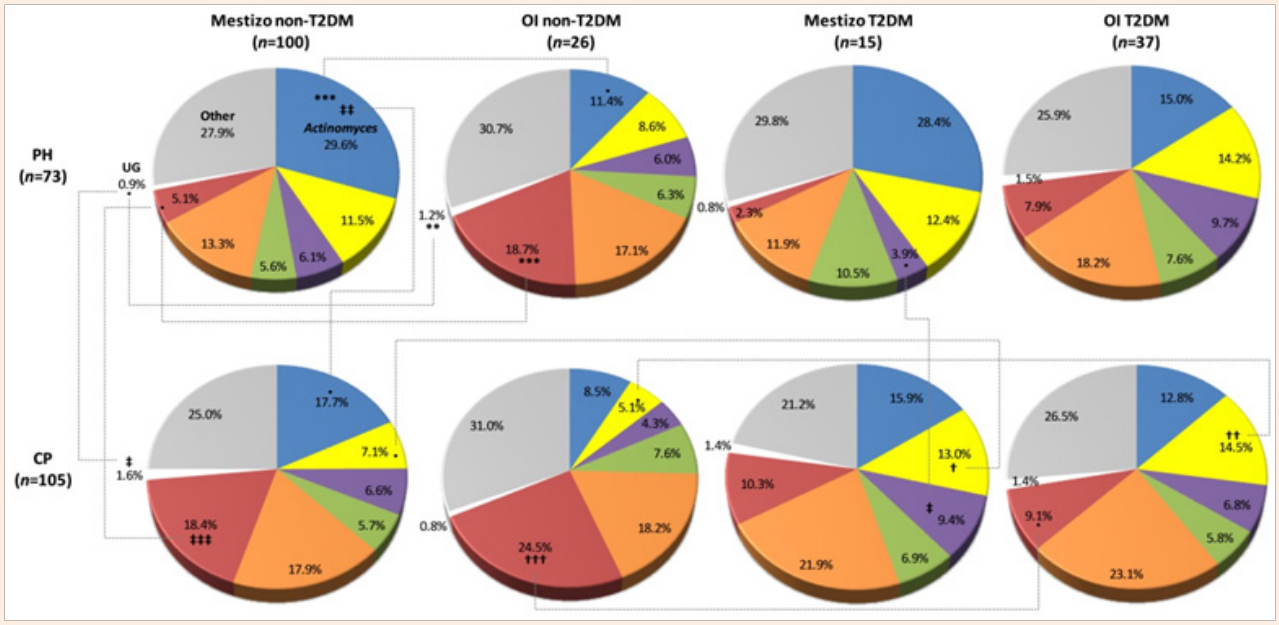

Figure 2: Mean proportion (% total DNA-probe count) of microbial complexes in subgingival plaque samples from 178 subjects. The proportion of species in each microbial complex was computed in every sample, averaged within a subject and then across subjects in each group. Taxa were grouped as similarly as possible to the descriptions of microbial complexes in subgingival plaque [26,27] (exceptions are noted in Table 2). Significance of differences was determined by paired comparisons using the Mann-Whitney *U* test after adjusting for multiple comparisons, as previously described [28]. $^{***}p$<0.01, $^{***}p$<0.001: Mestizo *vs.* OI. $^{\dagger}p$<0.05, $^{\dagger\dagger}p$<0.01, $^{\dagger\dagger\dagger}p$<0.001: non-T2DM *vs.* T2DM. $^{\ddagger}p$<0.05, $^{\ddagger\ddagger}p$<0.01, $^{\ddagger\ddagger\ddagger}p$<0.001: PH *vs.* CP. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. OI: Otomi-Indians. UG: Ungrouped).

The mean prevalence (% sites colonized) and proportion (% total DNA-probe count) of the 40 individual test-species are summarized in Figures 3 and 4, respectively. In non-T2DM subjects with PH, OI exhibited a significantly higher mean prevalence than Mestizos of 29 species, 10 of which also presented larger mean proportions. Such species included several putative and known periodontal pathogens of the orange and red-complexes, namely *Fusobacterium periodonticum* (Prevalence: Mestizo 24.7% ± 4.2% *vs.* OI 72.8% ± 5.8%, p<0.001. Proportion: Mestizo 1.3% ± 0.3% *vs.* OI 2.8% ± 0.4%, p<0.05), *Prevotella nigrescens* (Prevalence: Mestizo 30.5% ± 4.9% *vs.* OI 85.7% ± 4.3%, p<0.001. Proportion: Mestizo 2.1% ± 0.6% *vs.* OI 3.8% ± 0.4%, p<0.05), *P. gingivalis* (Prevalence: Mestizo 25.3% ± 3.7% *vs.* OI 78.3% ± 4.1%, p<0.001. Proportion: Mestizo 1.6% ± 0.7% *vs.* OI 4.3% ± 1.1%, p<0.001), *T. forsythia* (Prevalence: Mestizo 25.5% ± 3.9% vs. OI 68.2% ± 4.5%, p<0.001. Proportion: Mestizo 2.0% ± 0.7% *vs.* OI 11.7% ± 2.0%, p<0.001) and *T. denticola* (Prevalence: Mestizo 24.6% ± 4.1% *vs.* OI 83.2% ± 3.4%, p<0.001. Proportion: Mestizo 1.4% ± 0.3% *vs.* OI 2.8% ± 0.4%, p<0.01), as well as *Gemella morbillorum* (Prevalence: p<0.001. Proportion: p<0.01), *Neisseria mucosa* (Prevalence: p<0.001. Proportion: p<0.01), *Porphyromonas endodontalis* (Prevalence: p<0.001. Proportion: p<0.05), *Prevotella melaninogenica* (Prevalence: p<0.01. Proportion: p<0.01) and *Selenomonas artemidis* (Prevalence: p<0.001. Proportion: p<0.05). In non-T2DM subjects with CP, only *Streptococcus gordonii* exhibited a significantly higher prevalence in OI than in Mestizos (Mestizo 35.3% ± 3.8% *vs.* OI 75.1% ± 5.7%, p<0.05). None of the test-species were significantly more prevalent or presented higher proportions in Mestizos than in OI in either one of the diabetic or periodontal groups, except for *Actinomyces viscosus* which presented a higher proportion in the non-T2DM PH group (Mestizo 10.0% ± 1.6% *vs.* OI 2.2% ± 0.3%, p<0.05). Furthermore, no significant differences between Mestizos and OI in either the prevalence or proportion of any of the test-species were detected in T2DM with PH or CP.

The most important differences in the prevalence of individual species between non-T2DM and T2DM subjects were detected in Mestizos with CP. In this group, 28 of the 40 test-species were significantly more prevalent in T2DM, 5 of such species also exhibited significantly higher proportions in T2DM individuals: *Capnocytophaga ochracea* (Prevalence: non-T2DM 29.1% ± 3.5% *vs.* T2DM 76.9% ± 6.1%, p<0.01. Proportion: non-T2DM 0.4% ± 0.1% *vs.* T2DM 1.5% ± 0.4%, p<0.05), *S. artemidis* (Prevalence: non-T2DM 26.4% ± 3.2% *vs.* T2DM 76.2% ± 7.6%, p<0.01. Proportion: non-T2DM 0.9% ± 0.3% *vs.* T2DM 1.1% ± 0.2%, p<0.05), *Streptococcus constellatus* (Prevalence: non-T2DM 36.6% ± 4.1% *vs.* T2DM 91.5% ± 2.1%, p<0.01. Proportion: non-T2DM 1.5% ± 0.4% *vs.* T2DM 2.8% ± 0.5%, p<0.05), *Streptococcus intermedius* (Prevalence: non-T2DM 37.0% ± 3.5% *vs.* T2DM 91.3% ± 3.6%, p<0.001. Proportion: non-T2DM 0.8% ± 0.2% *vs.* T2DM 2.0% ± 0.3%, p<0.01) and *Streptococcus oralis* (Prevalence: non-T2DM 32.3% ± 3.4% *vs.* T2DM 90.9% ± 5.4%, p<0.001. Proportion: non-T2DM 0.8% ± 0.2% *vs.* T2DM 2.5% ± 0.4%, p<0.01). In Mestizos with PH, the only significant differences observed between T2DM and non-T2DM was a higher prevalence in T2DM subjects of *Eikenella corrodens* (non-T2DM 44.4% ± 4.2% *vs.* T2DM 97.0% ± 2.2%, p<0.05) and *F. periodonticum* (non-T2DM 24.7% ± 4.2% *vs.* T2DM 85.5% ± 6.2%, p<0.05). No significant differences were

detected in the proportion of any test-species in the same study group. In OI, the subgingival microbiota of T2DM, as compared to that of non-T2DM subjects, was characterized primarily by an elevated prevalence and/or proportion of a number of *Streptococcus* sp., including higher prevalence and proportion of *S. anginosus* in CP subjects (Prevalence: non-T2DM 22.9% ± 4.2% *vs*. T2DM 70.3% ± 4.9%, $p<0.01$. Proportion: non-T2DM 0.3% ± 0.1% *vs*. T2DM 1.9% ± 0.2%, $p<0.01$), larger proportions of *Streptococcus sanguinis* in subjects with both PH and CP (PH: non-T2DM 0.7% ± 0.1% *vs*. T2DM 3.1% ± 1.0%, $p<0.05$. CP: non-T2DM 0.6% ± 0.2% *vs*. T2DM 2.7% ± 0.4%, $p<0.05$) and a higher proportion of *S. oralis* in PH subjects (non-T2DM 0.9% ± 0.1% *vs*. T2DM 2.8% ± 0.5%, $p<0.05$). Interestingly, CP OI individuals with T2DM, exhibited significantly lower proportions than those with non-T2DM of *T. forsythia* (non-T2DM 14.1% ± 2.2% *vs*. T2DM 3.4% ± 0.6%, $p<0.01$).

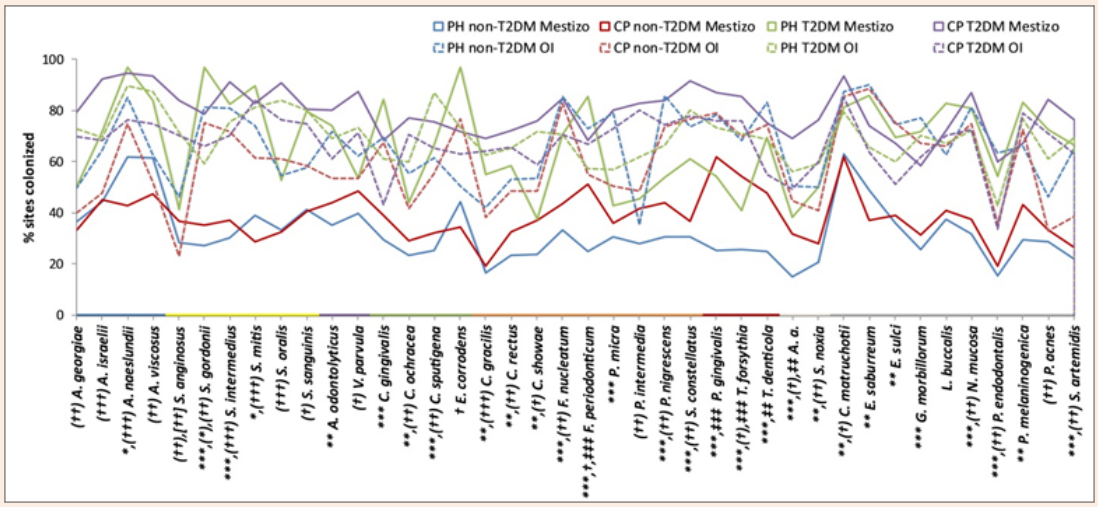

Figure 3: Mean prevalence (% sites colonized) of 40 individual test species in subgingival plaque samples from 115 Mestizo and 63 OI subjects grouped as non-T2DM and T2DM with either PH or CP. The presence of each species was determined in every sample, averaged within a subject and then across subjects in each group. Taxa were arranged similarly to the descriptions of microbial complexes in subgingival plaque [26,27] (exceptions are noted in Table 2) and are presented alphabetically within each complex. Significance of differences was determined by paired comparisons using the Mann-Whitney *U* test after adjusting for multiple comparisons, as previously described [28]. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$: Mestizo *vs*. OI in non-T2DM with PH. $^{\dagger}p<0.05$: non-T2DM *vs*. T2DM in Mestizos with PH. $^{(\dagger)}p<0.05$, $^{(\dagger\dagger)}p<0.01$, $^{(\dagger\dagger\dagger)}p<0.001$: non-T2DM *vs*. T2DM in Mestizos with CP. $^{[\dagger\dagger]}p<0.01$: non-T2DM *vs*. T2DM in OI with CP. $^{\ddagger\ddagger}p<0.01$, $^{\ddagger\ddagger\ddagger}p<0.001$: PH *vs*. CP in non-T2DM Mestizos. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. OI: Otomi-Indians).

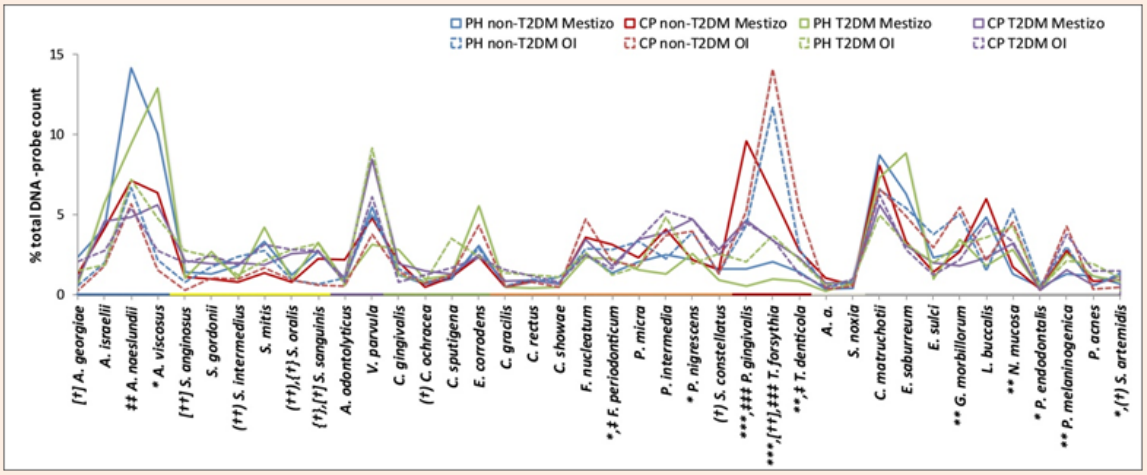

Figure 4: Mean proportion (% total DNA-probe count) of 40 individual test species in subgingival plaque samples from 115 Mestizo and 63 OI subjects grouped as non-T2DM and T2DM with either PH or CP. The proportion of each species was computed in every sample, averaged within a subject and then across subjects in each group. Taxa were arranged similarly to the descriptions of microbial complexes in subgingival plaque [26,27] (exceptions are noted in Table 2) and are presented alphabetically within each complex. Significance of differences was determined by paired comparisons using the Mann-Whitney *U* test after adjusting for multiple comparisons, as previously described [28]. $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$: Mestizo *vs*. OI in non-T2DM with PH. $^{(\dagger)}p<0.05$, $^{(\dagger\dagger)}p<0.01$: non-T2DM *vs*. T2DM in Mestizos with CP. $^{(\dagger)}p<0.05$: non-T2DM *vs*. T2DM in OI with PH. $^{[\dagger]}p<0.05$, $^{[\dagger\dagger]}p<0.01$: non-T2DM *vs*. T2DM in OI with CP. $^{\ddagger}p<0.05$, $^{\ddagger\ddagger}p<0.01$, $^{\ddagger\ddagger\ddagger}p<0.001$: PH *vs*. CP in non-T2DM Mestizos. (Non-T2DM: non-diabetic. T2DM: type 2 diabetes. PH: periodontal health. CP: chronic periodontitis. OI: Otomi-Indians).

In Mestizos with non-T2DM, CP subjects exhibited a significantly higher prevalence and a larger proportion than PH individuals of the putative and known periodontal pathogens: *F. periodonticum* (Prevalence: PH 24.7% ± 4.2% *vs*. CP 51.3% ± 3.8%, $p<0.001$. Proportion: PH 1.3% ± 0.3% *vs*. CP 3.1% ± 0.7%, $p<0.05$), *P. gingivalis* (Prevalence: PH 25.3% ± 3.7% *vs*. CP 61.9% ± 3.9%, $p<0.001$. Proportion: PH 1.6% ± 0.7% *vs*. CP 9.6% ± 1.2%, $p<0.001$), *T. forsythia* (Prevalence: PH 25.5% ± 3.9% *vs*. CP 54.1% ± 3.4%, $p<0.001$. Proportion: PH 2.0% ± 0.7% *vs*. CP 6.2% ± 0.9%, $p<0.001$) and *T. denticola* (Prevalence: PH 24.6% ± 4.1% *vs*. CP 47.7% ± 4.1%, $p<0.01$. Proportion: PH 1.4% ± 0.3% *vs*. CP 2.7% ± 0.4%, $p<0.05$). Additionally, in this group (non-T2DM Mestizos) there was a significantly higher prevalence of *A. actinomycetemcomitans* in subjects with CP than in PH (PH 14.9% ± 2.9% *vs*. CP 31.8% ± 3.0%, $p<0.01$) and a significantly smaller proportion of *Actinomyces naeslundii* (PH 14.2% ± 1.7% *vs*. CP 7.1% ± 1.5%, $p<0.01$). In OI with either T2DM or non-T2DM and in T2DM Mestizos, there were no statistically significant differences in the prevalence or proportion of any of the 40 test-species between PH and CP.

In the analyses of BCD and diabetic clinical parameters of T2DM subjects, no statistically significant differences were detected in any of the variables examined between Mestizos and OI individuals with either PH or CP. Mestizos and OI exhibited mean values of HbA_{1c} between 8.0% ± 0.4% and 9.2% ± 0.9% (PH: Mestizo 8.2% ± 1.2% *vs*. OI 8.1% ± 0.5%, *NS*. CP: Mestizo 9.2% ± 0.9% *vs*. OI 8.0% ± 0.4%, *NS*). There were no statistically significant differences in either the levels, prevalence or proportion of any of the 40 test-species between study groups associated with the results of either of the BCD parameters evaluated, including HbA_{1c}, TL, TG, TC, HDL and LDL (data not show). However, T2DM subjects with obesity (Mestizos and OI with PH and CP, BMI≥30), exhibited significant increases in a number of bacterial species when compared to individuals with a BMI<30, including *S. intermedius* (Levels: $p<0.01$. Proportion: $p<0.05$), *T. denticola* (Levels: $p<0.05$) and *N. mucosa* (Levels: $p<0.05$) (data not shown).

Discussion

The present study compared the microbial composition of 4,512 subgingival plaque samples (up to 28 samples per subject, mean 25.3 ± 2.4 standard deviation, range 20 to 28 samples per subject) in 178 systemically healthy and T2DM Mexican subjects of two ethnic groups: Mestizos and Otomi-Indians (OI) exhibiting either chronic periodontitis or periodontal health, in order to provide a more comprehensive picture of the subgingival microbial profiles associated with the etiology and progression of disease, as well as of the profiles that may characterize both type 2 diabetes and periodontal disease in these groups, to ultimately be able to contribute with this information to the implementation of more specific and effective preventive and therapeutic clinical periodontal strategies in benefit of such populations for which there is, to date, only scarce information in the scientific literature. All the species evaluated were detected in both Mestizo and OI subjects, regardless of their periodontal or diabetic status. However, significant differences were observed between ethnic groups. Notably, non-T2DM OI individuals with both periodontal conditions, but particularly those with PH, exhibited a subgingival microbial profile which was distinctive from the microbiota identified in Mestizo subjects. The subgingival microbiota of non-T2DM OI subjects was characterized by high proportions and prevalence of known periodontal pathogens of the red-complex, namely *P. gingivalis*, *T. denticola* and *T. forsythia*, together with low proportions of the *Actinomyces* sp. grouped in the blue-complex. The previous general microbial profile was characteristic of OI individuals with both PH and CP, but was only detected in systemically healthy subjects and not in T2DM individuals, thus it cannot be attributed to the systemic changes associated with diabetes. Numerous previous studies have reported that a similar microbial profile with elevated levels, proportions and/or prevalence of red-complex species and low numbers of *Actinomyces* sp. was characteristic of CP, and it is generally considered as the distinguishing microbiological trait that separates periodontally healthy subjects and sites from those with periodontal disease in a wide range of ethnic groups and geographical locations [4-31]. Furthermore, periodontally healthy non-T2DM OI, exhibited higher levels and proportions of one of the most important periodontal pathogens, *T. forsythia*, than previously reported in the scientific literature in other populations with periodontal disease. A study of populations from Sweden, the United States, Brazil and Chile, indicated that the proportion of *T. forsythia* in CP subjects ranged from 6.2% to 8.5%, with the highest proportion reported in individuals from Chile [29]. Our research group, previously reported in two studies that *T. forsythia* was among the species that exhibited the highest levels and proportion in CP Mexican Mestizos (Levels: 3.5 ± 0.6 to 5.6 ± 1.5 $x10^5$. Proportion: 4.3% ± 0.7% to 5.0% ± 0.9%, Mean ± SEM) [3,4]; however, in the present study, the non-T2DM OI population exhibited much higher levels and proportion of the same species, even in periodontally healthy subjects, than in any of the previously cited reports (Levels: 32.9 ± 5.3 $x10^5$, data not shown. Proportion: 11.7% ± 2.0%). Compared to previous reports and to the Mestizo population included in the present study, non-T2DM periodontally healthy OI subjects also exhibited unusually high levels, prevalence and/or proportions of other putative and known periodontal pathogens such as *P. gingivalis*, *T. denticola*, *P. nigrescens* and *F. periodonticum* that were, in most cases, higher than those detected in CP individuals of other ethnic origins. It is concerning that periodontally healthy OI individuals exhibit a similar subgingival microbial profile to those reported previously in subjects with periodontitis. This could be indicative of a higher intrinsic risk for periodontal disease, not related to T2DM, in the Otomi-Indian population of Mexico. Thus, further risk-assessment studies of this ethnic group are warranted.

In terms of the comparisons of the subgingival microbial composition between non-T2DM and T2DM subjects, we determined that although there were particular differences in the microbiota associated with T2DM between Mestizos and OI, it was possible to identify a common subgingival profile that was specifically associated with T2DM in CP subjects from both ethnic groups. The profile was characterized primarily by significantly elevated proportions in T2DM subjects, as compared to non-T2DM individuals, of all *Streptococcus* sp. grouped in the yellow and orange-complexes and a lower proportion of the periodontal pathogens in the red-complex, particularly of *T. forsythia* which

exhibited a significantly lower proportion ($p<0.01$) in CP OI individuals with T2DM than in non-T2DM subjects. Individually, a number of putative periodontal pathogens such as *Campylobacter gracilis*, *Campylobacter rectus*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *P. nigrescens*, as well as *C. ochracea*, *S. artemidis* and the 7 species of the genus *Streptococcus* evaluated (*S. intermedius*, *S. oralis*, *S. constellatus*, *S. anginosus*, *S. gordonii*, *S. mitis* & *S. sanguinis*), presented significantly higher levels, prevalence and/or proportion in T2DM than in non-T2DM subjects with CP. This microbial profile dominated the subgingival microbial composition of T2DM in both Mestizo and OI subjects with CP, and while OI individuals with PH tended to have a similar microbiota, the differences between T2DM and non-T2DM were not statistically significant in the latter group. Furthermore, under the parameters of the present study, a distinctive subgingival microbial profile of T2DM could not be determined in periodontally-healthy Mestizos, thus it would appear that such a profile may be related to T2DM specifically in subjects with CP. It is interesting that a similar subgingival profile, dominated by *Streptococcus* sp. and putative periodontal pathogens, with low numbers and/or proportions of classical periodontal pathogens, has been previously described in the scientific literature associated with specific subsets of patients with refractory periodontitis [32,33,34]. While a direct comparison of the results of such studies with the present investigation cannot be made, because the subjects included in the cited studies did not have T2DM, the authors indicated that the presences of this unusual virulent periodontal microbial profile could be the result of various host factors, including immune disorders or genetic predisposition [33,34]. Other previous reports in the scientific literature have also proposed that local and systemic host factors may affect the composition of the colonizing subgingival microbiota [30]. We suggest that T2DM could be one of such factors that may determine changes in the subgingival microbial composition associated with CP. Our results are in accord with previous studies that have also reported high levels of several *Streptococcus* sp., including *S. oralis*, *S. intermedius* and *S. sanguinis* [35] and a low prevalence of red-complex species [17,18] in T2DM subjects; however, they do not coincide with other publications that have reported a low prevalence of putative and known periodontal pathogens in T2DM subjects [14-16]. In our comparative analyses between subjects with PH and CP, we determined that non-T2DM Mestizos with CP harbored a subgingival microbiota that was similar to that previously described in numerous studies, which is currently recognized as the microbiota most frequently associated with periodontal disease, particularly with chronic periodontitis [26-31], consisting primarily of increases in the numbers and/or proportions of red-complex species and of decreases in *Actinomyces* sp., as compared to periodontally healthy individuals. In terms of individual species, non-T2DM Mestizos with CP presented higher prevalence and proportions of *P. gingivalis*, *T. denticola*, *T. forsythia* and *F. periodonticum*, as well as a higher prevalence of *A. actinomycetemcomitans* and a significantly lower proportion of *A. naeslundii* when compared to PH individuals. Such findings were in accord with the only previously published studies of the Mexican population [3,4]. However, the same microbial profile was not characteristic of CP in Mestizos or OI with T2DM or in non-T2DM OI individuals. No significant differences were detected between PH and CP in OI individuals with either T2DM or non-T2DM, and the only difference detected in T2DM Mestizos was a significant increase in the proportion of purple-complex species in subjects with CP, which was in accord with a previously published study [36]. In another recent study, researchers analyzed subgingival samples from subjects with different systemic conditions including T2DM and chronic kidney disease. They reported two microbial clusters, one associated with the common periodontitis profile (high red-complex & low *Actinomyces*) and the second one associated with periodontal health. The latter cluster presented higher proportions of a mixture of health-associated species such as members of the genera *Actinomyces* and *Capnocytophaga* and of putative pathogen like *Prevotella* sp. in subjects with PH [37].

An unexpected finding was the lack of significant microbial differences associated with any of the BCD parameters evaluated in T2DM subjects. Under the conditions of the present study, no significant subgingival microbial characteristics seemed to be related with the level of HbA_{1c}, TL, TG, TC, HDL or LDL in either Mestizo or OI individuals with T2DM. Nevertheless, in the analyses of the subgingival microbiota associated with the clinical parameters related to diabetes, subjects with obesity exhibited higher levels and proportions of *S. intermedius*, as well as higher levels of *T. denticola* and *N. mucosa*, than subjects with a BMI of lower than 30%. Previous studies have also reported higher proportions of periodontal pathogens such as *T. forsythia* in obese subjects with BMIs greater than 35%. Taken together, the previous data suggests that individuals with obesity might have a higher predisposition to periodontal diseases due to changes in their subgingival microbial profiles [38].

Conclusion

Significant microbiological differences were detected between ethnic groups. The subgingival microbiota of non-T2DM OI subjects was characterized by high proportions and prevalence of known periodontal pathogens and by low proportions of the *Actinomyces* sp., a profile that was similar to the subgingival microbiota often associated with periodontal disease in systemically healthy individuals. The previous finding suggests that OI individuals may have a higher risk for periodontal disease, not related to T2DM. On the other hand, we identified a common subgingival profile specifically associated with T2DM in CP subjects from both ethnic groups, which was characterized by significantly elevated proportions of putative periodontal pathogens and *Streptococcus* sp., as well as by lower proportion of red-complex species in T2DM subjects as compared to non-T2DM individuals. However, under the parameters of the present study, a distinctive subgingival microbial profile of T2DM could not be identified in periodontally-healthy subjects, suggesting that such a profile may be related to T2DM specifically in subjects with CP. Interestingly, while non-T2DM Mestizos with CP harbored the expected subgingival microbiota associated with periodontal diseases, no significant differences were detected between PH and CP in OI individuals with either T2DM or non-T2DM. Lastly, no subgingival microbial characteristics were found to be related with the level of any of the BCD parameters evaluated, including HbA_{1c}, TL, TG, TC, HDL or LDL in either Mestizo or OI individuals with T2DM,

however, our findings suggest that individuals with obesity might have a higher predisposition to periodontal diseases due to specific changes in the subgingival microbiota.

Conflict of Interest

The authors declare that there is no conflict of interest in this study.

Source of Funding

This study was supported in part by grant IN213006 from the General Direction of Faculty Affairs, National University of Mexico (PAPIIT. DGAPA. UNAM) and by the School of Dentistry of UNAM.

Acknowledgments

The authors acknowledge the collaboration of Alejandro Monroy-Alquicirez and Sara-Ideny Lucio-Arreola (UAEH), and wish to thank Dr. Arturo Fernández-Pedrero, Dean of the School of Dentistry (UNAM), for his support throughout the development of this study.

References

1. Haffajee AD, Bogren A, Hasturk H, Feres M, Lopez NJ, et al. (2004) Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 31(11): 996-1002.
2. Sanz M, Van Winkelhoff AJ, Herrera D, Dellemijn-Kippuw N, Simon R, et al. (2000) 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(5): 383-392.
3. Ximenez Fyvie LA, Almaguer-Flores A, Jacobo-Soto V, Lara-Cordoba M, Moreno-Borjas JY, et al. (2006) Subgingival microbiota of periodontally untreated Mexican subjects with generalized aggressive periodontitis. J Clin Periodontol 33(12): 869-877.
4. Ximenez Fyvie LA, Almaguer-Flores A, Jacobo-Soto V, Lara-Cordoba M, Sanchez-Vargas LO, et al. (2006) Description of the subgingival microbiota of periodontally untreated Mexican subjects: chronic periodontitis and periodontal health. J Periodontol 77(3): 460-471.
5. Gaetti Jardim E, Pereira MF, Vieira EM, Schweitzer CM, Okamoto AC, et al. (2015) Occurrence of periodontal pathogens in ethnic groups from a native Brazilian reservation. Arch Oral Biol 60(6): 959-965.
6. Dowsett SA, Kowolik MJ, Archila LA, Eckert GJ, LeBlanc DJ (2002) Subgingival microbiota of indigenous Indians of Central America. J Clin Periodontol 29(2): 159-167.
7. Navarrete-Linares F (2008) Los pueblos indígenas de México. Comisión Nacional para el Desarrollo de los Pueblos Indígenas.
8. Alvarado-Osuna C, Milian-Suazo F, Valles-Sanchez V (2001) Prevalence of diabetes mellitus and hyperlipidemia among Otomi indians. Salud Publica Mex 43(5): 459-463.
9. Emrich LJ, Shlossman M, Genco RJ (1991) Periodontal disease in non-insulin-dependent diabetes mellitus. J Periodontol 62(2): 123-131.
10. Isordia-Salas I, Santiago-German D, Rodriguez-Navarro H, Almaráz-Delgado M, Leaños-Miranda A, et al. (2012) Prevalence of metabolic syndrome components in an urban Mexican sample: comparison between two classifications. Exp Diabetes Res 202540.
11. Villalpando S, De la Cruz V, Rojas R, Shamah-Levy T, Avila MA, et al. (2010) Prevalence and distribution of type 2 diabetes mellitus in Mexican adult population: a probabilistic survey. Salud Publica Mex 52 Suppl 1: S19-S26.
12. Gutierrez-Hermosillo H, Diaz de Leon-Gonzalez E, Perez-Cortez P, Cobos-Aguilar H, Gutierrez-Hermosillo V, et al. (2012) Prevalence of type 2 diabetes mellitus in elderly patients and associated factors in a general hospital of the north of Mexico. Gac Med Mex 148(1): 14-18.
13. Contreras A, Moreno SM, Jaramillo A, Pelaez M, Duque A, et al. (2015) Periodontal microbiology in Latin America. Periodontol 2000 67(1): 58-86.
14. Ojima M, Takeda M, Yoshioka H, Nomura M, Tanaka N, et al. (2005) Relationship of periodontal bacterium genotypic variations with periodontitis in type 2 diabetic patients. Diabetes Care 28(2): 433-434.
15. Ebersole JL, Holt SC, Hansard R, Novak MJ (2008) Microbiologic and immunologic characteristics of periodontal disease in Hispanic americans with type 2 diabetes. J Periodontol 79(4): 637-646.
16. Li C, Liu J, Tan L, Yu N, Lin L, et al. (2013) The sociodemographic characteristics, periodontal health status, and subgingival microbiota of patients with chronic periodontitis and type 2 diabetes mellitus: a case-control study in a Chinese population. J Periodontol 84(8): 1058-1066.
17. Casarin RC, Barbagallo A, Meulman T, Santos VR, Sallum EA, et al. (2013) Subgingival biodiversity in subjects with uncontrolled type-2 diabetes and chronic periodontitis. J Periodontal Res 48(1): 30-36.
18. Castrillon CA, Hincapie JP, Yepes FL, et al. Occurrence of red complex microorganisms and Aggregatibacter actinomycetemcomitans in patients with diabetes. J Investig Clin Dent 20(2): 141-144.
19. Field CA, Gidley MD, Preshaw PM, Jakubovics N (2012) Investigation and quantification of key periodontal pathogens in patients with type 2 diabetes. J Periodontal Res 47(4): 470-478.
20. Abusleme L, Dupuy AK, Dutzan N, Silva N, Burleson JA, et al. (2013) The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J 7(5): 1016-1025.
21. Zhou M, Rong R, Munro D, Zhu C, Gao X, et al. (2013) Investigation of the effect of type 2 diabetes mellitus on subgingival plaque microbiota by high-throughput 16S rDNA pyrosequencing. PLoS One 8(4): e61516.
22. Haffajee AD, Socransky SS, Goodson JM (1983) Clinical parameters as predictors of destructive periodontal disease activity. J Clin Periodontol 10(3): 257-265.
23. Feinberg AP, Vogelstein B (1983) A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132(1): 6-13.
24. Socransky SS, Smith C, Martin L, Paster BJ, Dewhirst FE, et al. (1994) "Checkerboard" DNA-DNA hybridization. Biotechniques 17(4): 788-792.
25. Smith GL, Socransky SS, Smith CM (1989) Rapid method for the purification of DNA from subgingival microorganisms. Oral Microbiol Immunol 4(1): 47-51.
26. Socransky SS, Haffajee AD, Cugini MA, Smith C, Kent RL (1998) Microbial complexes in subgingival plaque. J Clin Periodontol 25(2): 134-144.

27. Socransky SS, Haffajee AD (2005) Periodontal microbial ecology. Periodontol 2000. 38(1): 135-187.

28. Socransky SS, Haffajee AD, Smith C, Dibart S (1991) Relation of counts of microbial species to clinical status at the sampled site. J Clin Periodontol 18(10): 766-775.

29. Haffajee A, Bogren A, Hasturk H, Feres M, Lopez N, Socransky S (2004) Subgingival microbiota of chronic periodontitis subjects from different geographic locations. J Clin Periodontol 31(11): 996-1002.

30. Haffajee A, Socransky S (2005) Microbiology of periodontal diseases: introduction. Periodontol 2000 38(1): 9-12.

31. Socransky SS, Haffajee AD (1992) The bacterial etiology of destructive periodontal disease: current concepts. J Periodontol 63(4 suppl): 322-331.

32. Colombo AP, Bennet S, Cotton SL, Goodson JM, Kent R, et al. (2012) Impact of periodontal therapy on the subgingival microbiota of severe periodontitis: comparison between good responders and individuals with refractory periodontitis using the human oral microbe identification microarray. J Periodontol 83(10): 1279-1287.

33. Colombo AP, Boches SK, Cotton SL, Goodson JM, Kent R, et al. (2009) Comparisons of subgingival microbial profiles of refractory periodontitis, severe periodontitis, and periodontal health using the human oral microbe identification microarray. J Periodontol 80(9): 1421-1432.

34. Socransky SS, Smith C, Haffajee AD (2002) Subgingival microbial profiles in refractory periodontal disease. J Clin Periodontol 29(3): 260-268.

35. Hintao J, Teanpaisan R, Chongsuvivatwong V, Ratarasan C, Dahlen G (2007) The microbiological profiles of saliva, supragingival and subgingival plaque and dental caries in adults with and without type 2 diabetes mellitus. Oral Microbiol Immunol 22(3): 175-181.

36. Demmer RT, Jacobs DR, Singh R, Zuk A4, Rosenbaum M, et al. (2015) Periodontal Bacteria and Prediabetes Prevalence in ORIGINS: The Oral Infections, Glucose Intolerance, and Insulin Resistance Study. Journal of dental research 94(9 supp): 201S-211S.

37. Hong BY, Furtado Araujo MV, Strausbaugh LD, Terzi E, Ioannidou E, Diaz PI (2015) Microbiome profiles in periodontitis in relation to host and disease characteristics. PLoS One 10: e0127077.

38. Haffajee AD, Socransky SS (2009) Relation of body mass index, periodontitis and Tannerella forsythia. J Clin Periodontol 36(2): 89-99.

Citation: Rodriguez-Hernandez AP, Marquez-Corona ML, Pontigo-Loyola AP, Ximenez-Fyvie LA (2017) Subgingival Microbiota of an Indigenous Mexican Population with Chronic Periodontitis and T2DM. J Dent Health Oral Disord Ther 7(2): 00238. DOI: 10.15406/jdhodt.2017.07.00238