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Subgingival microbiota of periodontally untreated Mexican subjects with generalized aggressive periodontitis

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Abstract

Background and Aim: Specific microbial profiles that may distinguish between generalized aggressive-periodontitis (GAgP) and generalized chronic-periodontitis (GCP) have, to date, not been described. The purpose of the present study was to describe the subgingival microbial composition of Mexican subjects with GAgP and compare it with that of individuals with GCP and periodontal health (PH).

Material and Methods: Seventy-seven subjects with GAgP ($n = 19$), GCP ($n = 39$) and PH ($n = 19$) were selected. Clinical measurements included plaque accumulation, gingival erythema, bleeding on probing, suppuration, pocket depth and attachment level. Up to 28 subgingival plaque samples were obtained from each subject and analysed using the checkerboard DNA–DNA hybridization technique.

Results: GAgP and GCP subjects harboured significantly higher levels and/or proportion of *Porphyromonas gingivalis*, *Tannerella forsythia* (levels: $p < 0.001$, proportion: $p < 0.01$), *Prevotella nigrescens* ($p < 0.05$ levels) and ''red'' complex species ($p < 0.001$ proportion) than PH subjects. All GAgP subjects were carriers of *P. gingivalis* and *P. nigrescens*. No significant differences in any of the 40 microbial species tested were detected between GAgP and GCP subjects.

Conclusions: Our results revealed that the microbial differences between GAgP and GCP subjects were only discrete and none of the bacterial species tested seemed to specifically differentiate the subgingival microbial profile of either periodontitis group.

Key words: aggressive periodontitis; dental plaque; DNA probes; periodontal microbiota; subgingival plaque

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In the 1999 International Workshop for a Classification of Periodontal Diseases and Conditions, the terms ''early-onset periodontitis'' and ''adult periodontitis'' were substituted for ''aggressive periodontitis'' (AgP) and ''chronic periodontitis'' (CP), respectively. Such changes were due primarily to considerable uncertainty about setting age limits and using age as a primary classification criterion (Armitage 1999). Currently, AgP and CP are recognized as two distinct forms of periodontal disease. AgP is said to be characterized by familial aggregation as well as by rapid attachment loss and bone destruction in otherwise healthy individuals. CP, on the other hand, has been defined as an infectious disease leading to slowly or moderately progressive loss of attachment and bone, which is most prevalent in adults but may occur in children and adolescents. According to the 1999 classification of periodontal diseases, both AgP and CP can be further characterized by extent and severity. Thus, localized and generalized forms, as well as slight, moderate and severe forms of each disease are recognized. Microbiological criteria were not mentioned in the current classification as primary features separating AgP from CP. However, an elevated proportion of *Actinobacillus actinomycetemcomitans* and, in some populations, of *Porphyromonas gingivalis*, was recognized as one of the secondary features that are generally, but not universally present in AgP.

Various studies have indicated that elevated proportions and/or prevalence of specific subgingival microorganisms such as *A. actinomycetemcomitans* may distinguish subjects with localized aggressive periodontitis (LAgP) from those with the generalized forms of both chronic (GCP) and aggressive periodontitis (GAgP) (Zambon et al. 1983a; Tanner 1992; Muller et al. 1993; Lopez et al. 1996; Tinoco et al. 1997). However, whether or not specific subgingival microbial profiles can distinguish between individuals with GCP and GAgP, remains controversial. While a number of studies have suggested significant microbial differences between GCP and GAgP subjects (Dogan et al. 2003; Darby et al. 2005), others have reported only discrete variations in the microbial profile of such periodontitis groups (Mombelli et al. 2002; Lee et al. 2003). For example, Dogan et al. 2003 evaluated by cultural methods the prevalence and proportion of six periodontal pathogens in 69 Turkish subjects with LAgP, GAgP, GCP and periodontal health (PH). Their findings suggested that while *A. actinomycetemcomitans* was not over-represented in the AgP groups, a larger percentage of GCP subjects were colonized by *Campylobacter rectus* and *Tannerella forsythia* than individuals with either LAgP or GAgP. In contrast, a different study compared the prevalence of seven putative periodontal pathogens in 156 diseased sites from AgP and 116 sites from CP Korean subjects, and reported no significant difference between clinical groups (Lee et al. 2003). Whether such discrepancies are due to microbial variations between subjects from different populations around the world or to difficulties in accurately grouping individuals into distinct clinical categories remains to be determined.

To our knowledge, no studies have been published in which the subgingival microbiota of Mexican subjects with GAgP has been described. The purpose of the present study was to determine the microbial composition of subgingival plaque samples from periodontally untreated Mexican subjects with GAgP, and to compare it with that of individuals with GCP and PH using the checkerboard DNA–DNA hybridization technique.

Material and Methods

Subject population

The present study received approval from the Ethics Committee for Human Studies of the Division of Postgraduate Studies and Research of the School of Dentistry of the National University of Mexico (UNAM). All subjects were asked to sign informed-consent forms, with which they acknowledged their willingness to participate.

Nineteen subjects with GAgP, 39 with GCP and 19 with PH were included in the study ($n = 77$ subjects). Subjects were recruited from the population of individuals that sought consults and/or treatment at the Periodontology Department of the Division of Postgraduate Studies and Research of the School of Dentistry of UNAM in Mexico city from February of 1999 to February of 2004. Every subject that fit the entry criteria was included in the study. All of the subjects selected were currently non-smokers, who had not received any form of periodontal therapy in the past other than professional supragingival plaque removal and had $\geqslant 20$ natural teeth (excluding third molars). All subjects were born and lived in Mexico, and were of Mexican descent, i.e. both of their parents and $\geqslant 2$ of their grandparents were born and lived in Mexico. Subjects included in the periodontitis clinical groups had $\geqslant 18$ sites with attachment level $\geqslant 5$ mm. GAgP and GCP subjects were 12–29 and >35 years of age, respectively. PH subjects had less than three sites with attachment level of 4 mm, no sites with attachment level $\geqslant 5$ mm, and were $\geqslant 22$ years of age. Exclusion criteria included pregnancy, lactation, antibiotic therapy in the previous 3 months and any systemic condition which could influence the course of periodontal disease such as diabetes, HIV/AIDS or autoimmune diseases.

Clinical monitoring and sample collection

Clinical measurements were taken at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) at all teeth excluding third molars (a maximum of 168 sites per subject) as previously described (Haffajee et al. 1983). Clinical assessment included plaque accumulation (0/1, undetected/detected), gingival erythema (0/1), bleeding on probing (0/1), suppuration (0/1), pocket depth and attachment level. Pocket depth and attachment level measurements were taken twice by the same examiner and the average of the pair of measurements was used for analysis. Such measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA). Table 1 summarizes the clinical features of the 77 subjects included in the study.

Samples of subgingival plaque were obtained from the mesiobuccal site of up to 28 teeth in each subject. After drying and isolation with cotton rolls, supragingival plaque was removed from the sampled sites and subgingival samples were taken with individual sterile Gracey curettes (Hu-Friedy). The samples were placed in individual tubes containing 150 μl of TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.6). Samples were dispersed and 100 μl of 0.5 M NaOH were added to each tube. All samples were stored at -20°C until processing.

Microbial assessment

Digoxigenin-labelled whole-genomic DNA probes were prepared and samples were processed individually for the detection and enumeration of 40 microbial species using the checkerboard DNA–DNA hybridization technique (Socransky et al. 1994), following the procedures previously described (Ximenez-Fyvie et al. 2006). Table 2 presents a list of the 40 bacterial strains employed for the preparation of DNA probes. Before the microbial detection in clinical samples, the specificity and sensitivity of DNA probes were assessed by hybridizing each DNA probe against individual pure cultures of all of the test species adjusted to 10^4, 10^5, 10^6 and 10^7 cells. The sensitivity of the assay was set to allow the detection of approximately 10^4 cells of a given species by adjusting the concentration of each individual DNA probe.

Statistical analysis

Microbiological data available for each subject were the absolute counts of each of the 40 test species from up to 28 subgingival plaque samples (mean = 25.6 samples per subject, total = 1971 samples analysed). The analyses compared the composition of subgingival plaque samples between the three clinical groups. The data are presented as mean $\pm$ standard error of the mean (SEM) levels (DNA probe counts $\times 10^5$) and proportion (percentage of the total DNA probe count). In order to compare the levels and proportion of every bacterial species, each type of data were recorded at each site, averaged within a subject and then across subjects in each clinical group. The percentage of carriers

Table 1. Clinical characteristics of GAgP, GCP and periodontally healthy subjects included in each clinical group

Clinical characteristic	GAgP ($n = 19$)		GCP ($n = 39$)		Health ($n = 19$)	
	Mean ± SEM	Range	Mean ± SEM	Range	Mean ± SEM	Range
Age (years)[†‡‖#]	21.5 ± 1.2	12–29	48.3 ± 1.7	35–75	27.8 ± 1.4	22–51
Number of missing teeth[†‡‖]	1.1 ± 0.4	0–7	3.8 ± 0.3	0–8	0.8 ± 0.3	0–4
Gender (% females)[¶]	84.2		64.1		42.1	
Mean pocket depth (mm, full mouth)[†‖**]	3.9 ± 0.2	2.6–6.1	4 ± 0.2	2.8–7.4	2 ± 0.03	1.7–2.3
Mean attachment level (mm, full mouth, AL)[†‖**]	3.9 ± 0.2	2.6–5.9	4.6 ± 0.2	3.1–9	2 ± 0.03	1.7–2.3
Number of sites with AL $\geqslant$ 5 mm[†‖**]	44.9 ± 4.6	18–80	56.8 ± 4.6	8–118	0 ± 0	0–0
Percent sites with:						
Plaque accumulation[† ‖]	38.3 ± 8.4	0–100	51.7 ± 5.6	0–100	12.2 ± 3.8	0–72
Gingival erythema[*§¶]	26.5 ± 8.2	0–100	26 ± 5	0–100	3.8 ± 2.3	0–38
Bleeding on probing[† ‖ **]	44.4 ± 4.8	13.1–95.5	48.8 ± 3.7	4.5–100	2.8 ± 1.2	0–22.7
Suppuration[† ‖**]	5.3 ± 1.4	0–22	6.8 ± 1.5	0–37	0 ± 0	0–0

[*] $p < 0.01$ and
[†] $p < 0.001$ Kruskal–Wallis test between the three clinical groups.
[‡] $p < 0.001$ Mann–Whitney test between GAgP and GCP subjects.
[§] $p < 0.01$ and
[‖] $p < 0.001$ Mann–Whitney test between GCP and healthy subjects.
[¶] $p < 0.05$,
[#] $p < 0.01$ and
[**] $p < 0.001$ Mann–Whitney test between GAgP and healthy subjects.
GAgP, generalized aggressive periodontitis; GCP, generalized chronic periodontitis.

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

Species	Strain[*]	Complex[†]	Species	Strain[*]	Complex[†]
Actinobacillus actinomycetemcomitans	[‡]	Ungrouped	*Peptostreptococcus micros*	33270	Orange
Actinomyces georgiae	49285	*Actinomyces*	*Neisseria mucosa*	19696	Other
Actinomyces israelii	12102	*Actinomyces*	*Porphyromonas endodontalis*	35406	Other
Actinomyces naeslundii stp. 1	12104	*Actinomyces*	*Porphyromonas gingivalis*	33277	Red
Actinomyces odontolyticus	17929	Purple	*Prevotella intermedia*	25611	Orange
Actinomyces viscosus	43146	*Actinomyces*	*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

[*] American Type Culture Collection, Rockville, MD.
[†] Strains were grouped according to the description of microbial complexes in subgingival plaque (Socransky et al. 1998) with the following exceptions: *A. georgiae, A. israelii, A. naeslundii* 1 and *A. viscosus* were grouped as ''*Actinomyces*''; *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''.
[‡] DNA from serotypes a (43717) and b (43718) was combined to generate a single DNA probe.
[§] DNA from subspecies *nucleatum* (25586), *polymorphum* (10953) and *vincentii* (49256) was combined to generate a single DNA probe.

was computed by determining the presence or absence of every species in each sample. Subjects in which a given species was detected in at least one sample, were considered carriers of that particular microorganism. Percentages for each microbial species tested were determined on the basis of the total number of subjects in each clinical group. The proportion of groups of microorganisms was determined for PH and periodontitis subjects by grouping the 40 test species as similarly as possible to the description of subgingival microbial complexes (Socransky et al. 1998). Significance of differences between the three clinical groups and between GAgP and GCP in the levels, proportion and percentage of carriers of each species or microbial complex was determined using the Kruskal–Wallis and Mann–Whitney

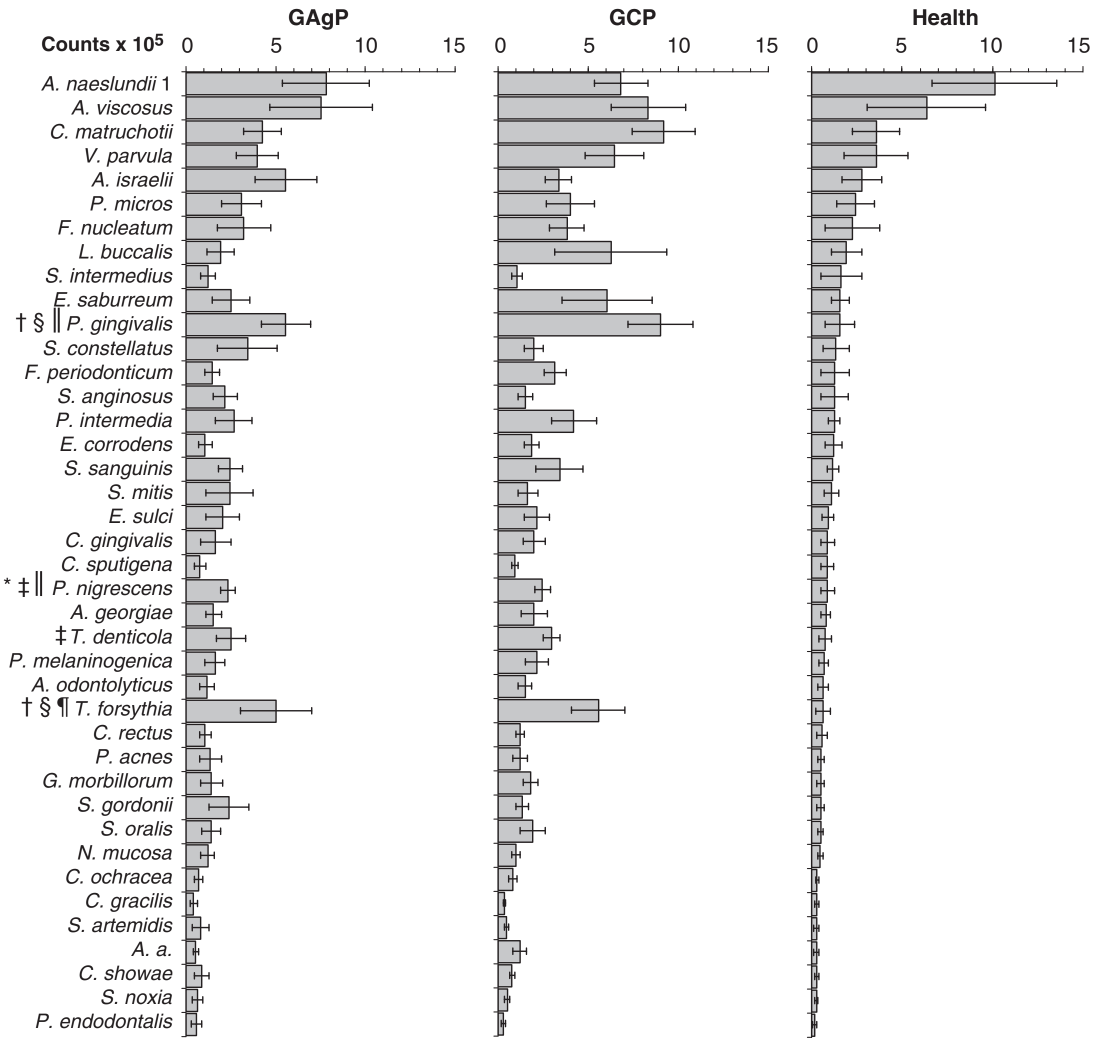

Fig. 1. Bar charts of the mean levels (DNA probe count $\times 10^5 \pm$ SEM) of each of the 40 test species in 1971 subgingival plaque samples from 19 generalized aggressive periodontitis (GAgP), 39 generalized chronic periodontitis (GCP) and 19 periodontally healthy subjects. The levels of each species were computed in each sample, averaged within a subject and then across subjects in each clinical group. The data are presented in decreasing order based on the levels detected in periodontally healthy subjects.$^{*}p<0.05$ and $^{\dagger}p<0.001$ Kruskal–Wallis test between the three clinical groups. $^{\ddagger}p<0.05$ and $^{\S}p<0.001$ Mann–Whitney test between GCP and healthy subjects. $^{\|}p<0.05$ and $^{\P}p<0.01$ Mann–Whitney test between GAgP and healthy subjects. Differences between GAgP and GCP subjects were not statistically significant for any of the species tested after adjusting for multiple comparisons.

tests, respectively, after adjusting for multiple comparisons as previously described (Socransky et al. 1991).

Results

Figure 1 summarizes the mean levels ($\times 10^5 \pm$ SEM) of the 40 individual test species in 1971 subgingival plaque samples from GAgP, GCP and PH subjects. All of the species tested were detected in subjects from the three clinical groups. *Actinomyces naeslundii* 1, *A. viscosus*, *Corynebacterium matruchotii*, *Peptostreptococcus micros* and *Veillonella parvula* were the species that presented the highest mean levels in all three clinical groups. PH subjects harboured higher mean levels of only *A. naeslundii* 1 and *Streptococcus intermedius* than subjects in either periodontitis group. GAgP subjects harboured higher mean levels of *A. israelii*, *Campylobacter showae*, *Neisseria mucosa*, *P. endodontalis*, *Propionibacterium acnes*, both *Selenomonas* spp. and all *Streptococcus* spp. tested except *S. sanguinis* and *S. oralis*, than GCP subjects. However, the levels of most of the microbial species tested in both periodontitis groups, tended to be very similar. *A. actinomycetemcomitans* was among the species detected in the lowest levels in all clinical groups (GAgP = $0.5 \pm 0.2 \times 10^5$; GCP = $1.2 \pm 0.4 \times 10^5$; PH = $0.3 \pm 0.1 \times 10^5$). The differences between the three clinical groups and between GAgP and healthy subjects, were only statistically significant for *P. gingivalis* (GAgP = $5.6 \pm 1.4 \times 10^5$; GCP = $9 \pm 1.8 \times 10^5$; PH = $1.6 \pm 0.8 \times 10^5$; $p<0.001$ and $p<0.05$, respectively), *P. nigrescens* (GAgP = $2.3 \pm 0.4 \times 10^5$; GCP = $2.5 \pm 0.4 \times 10^5$; PH = $0.9 \pm 0.4 \times 10^5$; $p<0.05$) and *T. forsythia* (GAgP = $5 \pm 2 \times 10^5$; GCP = $5.6 \pm 1.5 \times 10^5$; PH = $0.6 \pm 0.4 \times 10^5$; $p<0.001$ and $p<0.01$,

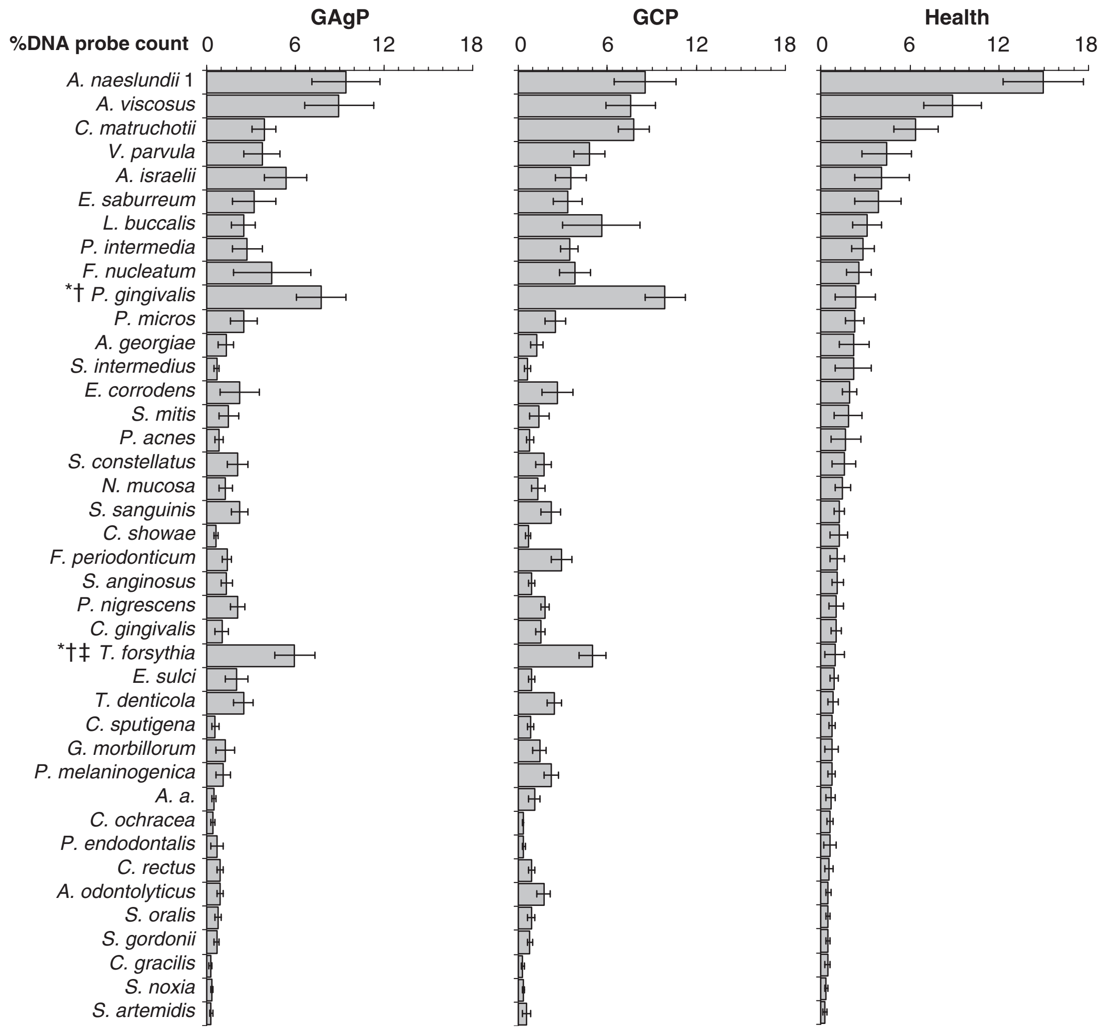

Fig. 2. Bar charts of the mean proportion (% of the total DNA probe count ± SEM) of each of the 40 test species in 1971 subgingival plaque samples from 19 generalized aggressive periodontitis (GAgP), 39 generalized chronic periodontitis (GCP) and 19 periodontally healthy subjects. The proportion of each species was computed in each sample, averaged within a subject and then across subjects in each clinical group. The data are presented in decreasing order based on the proportions detected in periodontally healthy subjects.*$p<0.01$ Kruskal–Wallis test between the three clinical groups. †$p<0.001$ Mann–Whitney test between GCP and healthy subjects. ‡$p<0.05$ Mann–Whitney test between GAgP and healthy subjects. Differences between GAgP and GCP subjects were not statistically significant for any of the species tested after adjusting for multiple comparisons.

respectively). Comparing GCP and healthy subjects, the mean levels of *P. gingivalis* ($p<0.001$), *P. nigrescens* ($p<0.05$), *T. forsythia* ($p<0.001$) and *Treponema denticola* (GCP = $3 \pm 0.5 \times 10^5$; PH = $0.8 \pm 0.3 \times 10^5$; $p<0.05$) were also significantly different. The differences between GAgP and GCP subjects were not statistically significant for any of the species tested.

The mean proportion (± SEM) of individual species in each clinical group is summarized in Fig. 2. Samples from PH subjects harboured larger proportions of 11 of the 40 test species, including *A. georgiae*, *A. naeslundii* 1, *Capnocytophaga ochracea*, *N. mucosa*, *P. acnes* and *S. intermedius*, than those from either periodontitis group. The proportion of a number of putative and recognized periodontal pathogens, on the other hand, was higher in both GAgP and GCP subjects than in healthy individuals. Such species included *C. rectus*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *F. periodonticum*, *P. micros*, *P. gingivalis*, *P. melaninogenica*, *P. nigrescens*, *T. forsythia* and *T. denticola*. It was notable, that *A. actinomycetemcomitans* was among the species that represented the lowest proportion in samples from both GAgP and PH subjects ($0.5 \pm 0.1\%$ and $0.7 \pm 0.3\%$, respectively). *F. periodonticum*, *P. gingivalis* and *P. intermedia*, were among the species that represented higher mean proportions in samples from GCP subjects, while *P. nigrescens*, *T. forsythia* and *T. denticola*, were among those that represented higher proportions in samples from subjects in the GAgP group. The differences between the three clinical groups, as well as between GCP and healthy subjects, were statistically significant for *P. gingivalis* (GAgP = $7.7 \pm 1.7\%$; GCP = $9.9 \pm 1.4\%$; PH = $2.3 \pm 1.4\%$; $p<0.01$ and $p<0.001$, respectively) and *T. forsythia* (GAgP = $5.9 \pm 1.4\%$; GCP = $5 \pm 0.9\%$; PH = $0.9 \pm 0.6\%$; $p<0.01$ and $p<0.01$, respectively). The differences in the mean proportion of species between GAgP and healthy subjects, were only statistically significant

Table 3. Percentage of carriers of individual species in subjects with generalized aggressive periodontitis (GAgP), generalized chronic periodontitis (GCP) and periodontal health

Species	GAgP	GCP	Health	Species	GAgP	GCP	Health
Actinobacillus actinomycetemcomitans	94.7	89.7	73.7	*Peptostreptococcus micros*	94.1	85.7	88.2
Actinomyces georgiae	100	87.2	89.5	*Neisseria mucosa*	94.7	89.7	100
Actinomyces israelii	94.7	100	93.8	*Porphyromonas endodontalis*	89.5	84.2	68.4
Actinomyces naeslundii stp. 1	100	94.6	100	*Porphyromonas gingivalis*	100	100	89.5
Actinomyces odontolyticus	94.7	94.4	87.5	*Prevotella intermedia*	77.8	94.6	72.2
Actinomyces viscosus	100	97.1	94.4	*Prevotella melaninogenica*	89.5	92.1	72.2
Campylobacter gracilis	73.7	84.2	77.8	*Prevotella nigrescens*	100	94.9	72.2
Campylobacter rectus	89.5	97.4	84.2	*Propionibacterium acnes*	84.2	82.1	84.2
Campylobacter showae	89.5	92.3	78.9	*Selenomonas. artemidis*	89.5	81.1	64.7
Capnocytophaga gingivalis	84.2	88.2	82.4	*Selenomonas noxia*	89.5	87.2	88.9
Capnocytophaga ochracea	89.5	92.1	89.5	*Streptococcus anginosus*	100	82.1	89.5
Capnocytophaga sputigena	73.7	92.1	89.5	*Streptococcus constellatus*	100	87.2	94.1
Corynebacterium matruchotii	94.4	97.3	100	*Streptococcus gordonii*	94.7	91.9	68.4
Eikenella corrodens	94.7	84.6	88.9	*Streptococcus intermedius*	84.2	92.3	78.9
Eubacterium saburreum	94.7	81.8	88.9	*Streptococcus mitis*	83.3	81.1	94.1
Eubacterium sulci	94.7	78.9	100	*Streptococcus oralis*	94.7	94.6	84.2
Fusobacterium nucleatum	100	92.1	88.2	*Streptococcus sanguinis*	78.9	89.7	89.5
Fusobacterium periodonticum	94.4	97.1	88.2	*Tannerella forsythia*	94.4	97.4	89.5
Gemella morbillorum	84.2	82.1	73.7	*Treponema denticola*	89.5	94.6	78.9
Leptotrichia buccalis	84.2	93.9	88.9	*Veillonella parvula*	100	92.3	83.3

Subjects in which a given species was detected in at least one sample, were considered carriers of that particular microorganism. Percentages were determined based on the total number of subjects in each clinical group. No significant differences between the three clinical groups (Kruskal–Wallis test), GAgP and GCP, GCP and health or GAgP and health (Mann–Whitney test) were found after adjusting for multiple comparisons.

for *T. forsythia* ($p < 0.05$), and no significant differences were detected between GAgP and GCP subjects for any of the microorganisms tested, after adjusting for multiple comparisons.

Table 3 presents the percentage of carriers of each individual test species in the three clinical groups. 73.7–100% of GAgP, 78.9– 100% of GCP and 64.7–100% of PH subjects were carriers of each of the microorganisms tested. Twenty-two of the 40 test species (55%) in both the GAgP and GCP groups, were detected in 90% or more of subjects. A number of such species included important periodontal pathogens like *P. gingivalis*, *T. forsythia* and *P. nigrescens*. In contrast, only eight of the 40 test species (20%), in the PH group, were detected in 90% or more of subjects. In healthy subjects, none of such species were putative or recognized periodontal pathogens. It was interesting that all GAgP and GCP subjects were carriers of *P. gingivalis*, and that *P. nigrescens* was also detected in every subject included in the GAgP group. GAgP subjects were more frequently carriers of *E. corrodens*, *F. nucleatum*, *P. micros*, *P. nigrescens* and other species, than subjects included in the other two clinical groups. On the other hand, all *Campylobacter* spp., *F. periodonticum*, *P. intermedia*, *T. forsythia* and *T. denticola* were among the species that were most frequently detected in GCP subjects. A larger percentage of healthy subjects were carrier of *C. matruchotii*, *Eubacterium sulci*, *N. mucosa* and *S. mitis* than subjects from either periodontitis group. Although both the levels and proportion of *A. actinomycetemcomitans* were low in GAgP subjects, a larger percentage of individuals (94.7%) were colonized by this particular microorganism than either GCP (89.7%) or PH (73.7%) subjects. The differences in the percentage of carriers of all of the species tested, were not statistically significant between the three clinical groups, GAgP and GCP subjects or between either periodontitis groups and healthy subjects.

The mean proportion of eight microbial groups in subjects from each clinical category is summarized in Fig. 3. The areas of the pies, were adjusted to reflect the mean total levels (mean total DNA probe count) of species in each clinical category (GAgP = $93.3 \pm 18.4 \times 10^5$; GCP = $110.7 \pm 16.7 \times 10^5$; PH = $55.3 \pm 16.9 \times 10^5$. $p < 0.01$ between all clinical groups and GCP *versus* PH. $p < 0.05$ between GAgP and PH subjects. Not significant between the GAgP and GCP groups). The most striking difference in the proportion of groups of microorganisms between PH and periodontitis subjects was a significant increase in the proportion of ''red'' complex species observed in subjects included in either periodontitis group ($p < 0.001$ between the three groups and GCP *versus* PH, $p < 0.01$ GAgP *versus* PH). Additionally, the proportion of species included in the *Actinomyces* group was substantially lower in periodontitis subjects and in particular, in GCP individuals. The differences in the mean proportion of microbial groups, between all clinical groups, GCP and healthy subjects, as well as between GAgP and healthy subjects were only significant for the ''red'' complex. No significant differences in the proportion of either one of the eight microbial groups were detected between GAgP and GCP subjects.

Discussion

The present study compared the subgingival microbial composition of 77 currently non-smoking Mexican subjects with no previous history of periodontal therapy that were either periodontally healthy or presented two different forms of periodontal disease (GAgP and GCP). All of the species detected in both periodontitis groups, were also present in PH subjects and the percentage of healthy and periodontitis carriers of all of the species tested was not

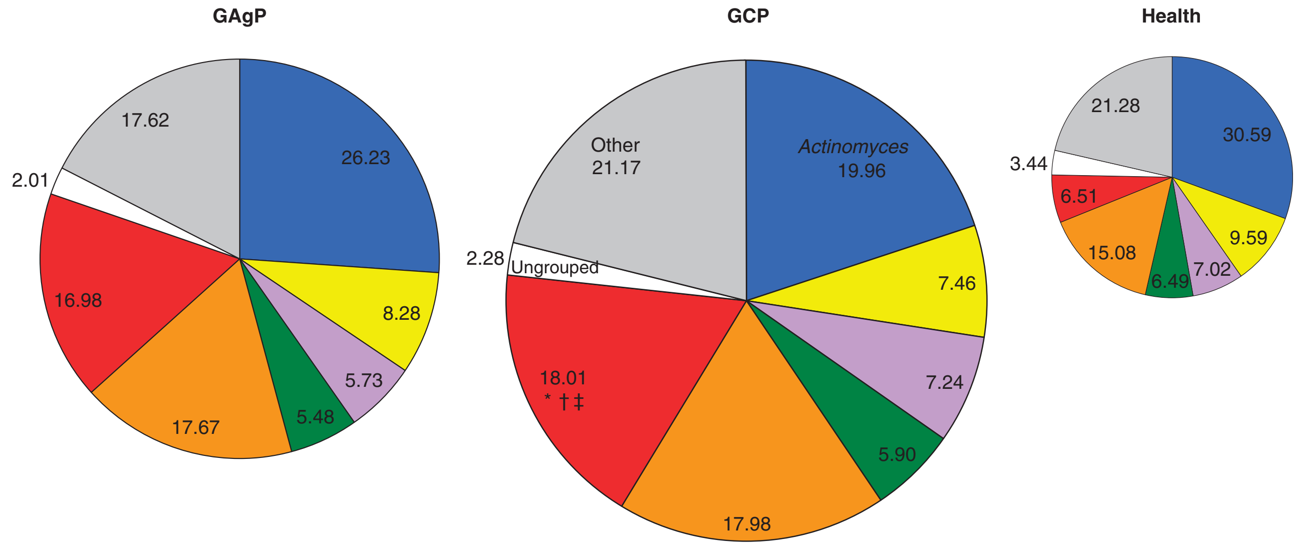

Fig. 3. Pie charts of the mean proportion (% of the total DNA probe count) of microbial groups in 1971 subgingival plaque samples from 19 generalized aggressive periodontitis (GAgP), 39 generalized chronic periodontitis (GCP) and 19 periodontally healthy subjects. The species were organized into 8 microbial groups based on the description of subgingival microbial complexes (Socransky et al. 1998) (exceptions are noted in Table 2). The areas of the pies were adjusted to reflect the mean total levels of species in each clinical group.*$p<0.001$ Kruskal–Wallis test between the three clinical groups. †$p<0.001$ Mann–Whitney test between GCP and healthy subjects. ‡$p<0.01$ Mann–Whitney test between GAgP and healthy subjects. Differences between GAgP and GCP subjects were not statistically significant for any of the species tested after adjusting for multiple comparisons.

significantly different between clinical groups. Certain microbial species, including *A. naeslundii* 1, *A. viscosus*, *C. matruchotii* and *V. parvula* dominated in levels and proportion the subgingival microbiota of both periodontitis and healthy subjects. The levels and proportion of *P. gingivalis*, *T. forsythia* and ''red'' complex species as a groups, on the other hand, were dominant only in samples from GAgP and GCP subjects. Low levels and proportion of *A. actinomycetemcomitans*, that were not significantly different between clinical groups, were detected irrespective of the periodontal condition of subjects. Taken together, our results indicated that in the Mexican population, there were significant differences in the microbiota of subgingival plaque samples between periodontitis and PH subjects. The microbial differences between GAgP and GCP subjects, however, were only discrete and not statistically significant in terms of the levels, proportion or prevalence of any of the species or groups of microorganisms evaluated.

Our findings are in accord with the results of previous studies that have suggested that *P. gingivalis* and *T. forsythia* are important pathogenic species in both GAgP and GCP subjects, but have failed to determine significant microbial differences between individuals with either one of these forms of periodontal disease (Mombelli et al. 2002; Lee et al. 2003; Takeuchi et al. 2003). Mombelli et al. 2002 systematically reviewed 33 cross-sectional and longitudinal studies that provided microbiological data from both CP and AgP subjects, to determine if the presence or absence of five periodontal pathogens could distinguish between individuals with either clinical condition. They concluded that the presence or absence of *A. actinomycetemcomitans*, *P. gingivalis*, *P. intermedia*, *T. forsythia* or *C. rectus* could not discriminate between subjects with CP and AgP. Takeuchi et al. 2003 employed polymerase chain reaction to determine the prevalence and culture to evaluate the relative proportion of seven subgingival species in samples from 93 Japanese subjects with LAgP, GAgP, GCP and PH. A significantly higher percentage of GAgP and GCP subjects were carriers of *C. rectus*, *P. gingivalis*, *T. forsythia* and *T. denticola* than PH subjects. The proportion of *A. actinomycetemcomitans*, *P. gingivalis* and *T. forsythia*, however, was similar in all periodontitis groups.

A. actinomycetemcomitans has been associated with cases of aggressively progressing periodontitis in children, adolescents and adults (Zambon 1985; Moore 1987; Slots & Listgarten 1988; Preus et al. 1994). However, its role in GAgP is still unclear. Our results revealed that neither the levels, proportion nor prevalence of *A. actinomycetemcomitans*, which were generally low in all clinical groups, varied significantly between GAgP, GCP and healthy subjects. Thus, in Mexican subjects, *A. actinomycetemcomitans* did not appear to play a distinct role in GAgP. Other studies have also reported low prevalence and proportion of *A. actinomycetemcomitans* in GAgP subjects from Japan (Ishikawa et al. 2002; Takeuchi et al. 2003), Brazil (Trevilatto et al. 2002), Indonesia (Timmerman et al. 2001) and Greece (Kamma & Baehni 2003; Kamma et al. 2004). A number of reports, however, have suggested that different serotypes of *A. actinomycetemcomitans* could be associated with various forms of periodontal disease in geographically distinct populations (Zambon et al. 1983b; Asikainen et al. 1991; Holtta et al. 1994; Haubek et al. 1995; Gmur & Baehni 1997; Socransky et al. 1999). A possible confounder in our findings with respect to *A. actinomycetemcomitans* was the inability to discriminate between different serotypes. Separate whole-genomic DNA probes for serotypes a and b of *A. actinomycetemcomitans* were tested in preliminary studies to determine the sensitivity and specificity of the DNA probes used in our ''checkerboard'' assay (data not shown). Significant cross-reactions between these two particular DNA probes, however, made it difficult to distinguish between serotypes in clinical samples. Therefore a single DNA probe was generated which did not exhibit cross-reactions with the other test species but could not distinguish between serotypes.

The current classification of periodontal diseases and conditions describes GAgP and GCP as two different forms of disease (Armitage 1999), and while it is in fact reasonable that GAgP and GCP represent distinct entities, in cross-sectional studies, separating GAgP and GCP subjects into non-overlapping groups is a difficult challenge. The classification emphasizes that the diagnosis of such forms of periodontal disease should not be based on age or knowledge of the rate of disease progression. However, AgP was described as presenting rapid attachment loss and bone destruction, usually in persons under 30 years of age with a pronounced episodic nature of the destruction. CP was described as most prevalent in adults with a slow to moderate rate of progression. All of such features continue to be, to a certain extent, age-dependant and require knowledge of the rate of disease progression. Because in cross-sectional studies there are no reliable means of determining the actual time of disease initiation, rate of progression or even disease activity, subject classification is primarily based on the clinical measurements observed at a given point in time. Thus, while it is highly unlikely that GAgP will be misdiagnosed when only subjects under the age of 30 years exhibiting severe and extensive periodontal destructions are included in such groups, it is impossible to ascertain what proportion of the individuals included in GCP groups are actually GAgP subjects that were evaluated after the age of 30. While a certain amount of such overlap cannot entirely be ruled out in the present study, a conscious effort was made to minimize it, e.g., we established an age gap between GAgP and GCP subjects. Individuals of up to 29 years of age were included in the GAgP group and only subjects that were 35 years of age or more were selected for the GCP group.

The subgingival microbiota of both GAgP and GCP Mexican subjects, in contrast to PH subjects, was characterized by significant increases in the levels and/or proportion of certain periodontal pathogens, including *P. gingivalis*, *T. forsythia*, *T. denticola* and *P. nigrescens*. However, significant microbiological differences between GAgP and GCP subjects could not be determined and none of the 40 bacterial species tested seemed to specifically characterize the subgingival microbial profiles of either periodontitis group. Thus, we conclude that in Mexican individuals, changes in the levels, proportion or prevalence of specific microbial species, cannot be used to accurately differentiate between subjects with GAgP and GCP. Our results warrant further research of possible non-microbial determinants in the pathogenesis of GAgP and GCP in the Mexican population such as genetic and immunological factors that may be specifically involved in these particular forms of periodontal disease.

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Clinical Relevance

Scientific rationale for study: Various studies have indicated that elevated proportions and/or prevalence of specific subgingival microorganisms may distinguish subjects with localized AgP from those with the GCP and GAgP. However, whether or not specific subgingival microbial profiles can distinguish between individuals with GCP and GAgP, remains to be determined.

Principal findings: No significant differences in the levels, proportion or prevalence of any of the 40 microbial species tested were detected between GAgP and GCP subjects.

Practical implications: Our results suggested that in Mexican individuals, specific microbial profiles cannot accurately differentiate between subjects with GAgP and GCP.