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ORIGINAL ARTICLE

Confounding and interaction effect of *Treponema denticola* salivary carriage in chronic periodontitis

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AIM: To evaluate the salivary carriage of *Treponema denticola* and its association with demographic variables in the etiopathogenesis of chronic periodontitis.

SUBJECTS AND METHODS: Ninety-seven chronic periodontitis (CP) patients and a control group of 51 healthy subjects (HC) were selected. Periodontal status was assessed by criteria based on probing depth, attachment loss, extent, and severity of periodontal breakdown. A polymerase chain reaction method was used to determine the occurrence of *T. denticola* in saliva samples. Risk indicators for CP were assessed individually and adjusted for confounding and/or interaction using a logistic regression model.

RESULTS: Although univariate analysis revealed a positive association of age ≥30 years, smoking, and salivary carriage of *T. denticola* with CP, after logistic regression analysis, the association between age ≥30 years/smoking and CP persisted, whereas salivary carriage of *T. denticola* failed to achieve statistical significance. An interaction effect was significantly detected between these three variables.

CONCLUSION: Although salivary carriage of *T. denticola* may be a risk indicator for CP, its pathogenicity should not be exclusively endorsed to its detection in saliva, but it might be associated with the synergistic biological interaction of the bacterium with some demographic characteristics of the susceptible host.

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Introduction

Periodontal diseases are progressive and destructive inflammatory conditions of the tooth-supporting tissues. Chronic periodontitis is initiated and maintained by a very complex microbial biofilm that forms on the surfaces of teeth in close proximity to their supporting tissues, however several demographic and behavioral characteristics, such as race, age, gender, and smoking, as well as socioeconomic status, have been identified to affect the distribution, severity, and extension of the disease (Albandar, 2002; Calsina *et al*, 2002; Hyman and Reid, 2003; Susin *et al*, 2004a,b; Krustrup and Petersen, 2006).

It is well known that periodontitis-associated organisms colonize not only subgingival but also supragingival sites and are continuously washed into saliva by gingival crevicular fluid (Umeda *et al*, 1998; Darout *et al*, 2002; Könönen *et al*, 2007). However, the role of salivary periodontal pathogens in pathogenesis of periodontitis is yet controversial. Whereas various studies have found that chronic periodontitis is associated with salivary carriage of periodontal pathogens (Von Troil-Lindén *et al*, 1995; Takeuchi *et al*, 2001; Könönen *et al*, 2007), only limited information is available on those variables that could influence this association as potential confounders and their biological interactions (Könönen *et al*, 2007). In general terms, confounding is defined as a distortion of the association between a risk variable and a disease brought about by other extraneous or independent risk variables (Rothman and Greenland, 1998). In contrast, interaction occurs when there is a difference in the biologic effect of a risk variable according to the presence or absence of another factor (Katz, 2003; Jose *et al*, 2008).

It has been postulated that the proportion of pathogens increases in saliva due to deteriorating periodontal status (Von Troil-Lindén *et al*, 1995), indicating that a subject with advanced periodontitis may serve as a potential source of pathogens to his/her close contacts (Könönen *et al*, 2007). Thus, saliva might appear to be a major source for bacterial transmission and cross-infection

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(Leblebicioglu *et al*, 2009). Despite of being a carrier of periodontal pathogens, which is accepted as a risk indicator for future periodontal diseases for susceptible host (Kinane and Bartold, 2007; Kulekci *et al*, 2008), the pathogenicity of these microbial species cannot be entirely attributed to the detection of their presence in saliva, but it is rather connected with the interaction that the bacteria, organized in the subgingival plaque, develop with the host organism (Squeri *et al*, 2006).

Amongst periodontopathic bacteria, *Treponema denticola* has been predominantly associated with the incidence and severity of human periodontal disease (Loesche and Grossman, 2001) and is considered one of the main etiological agents of periodontitis (Ellen and Galimanas, 2005). This aerotolerant, motile, and anaerobic spirochete (Paster *et al*, 2001) dwells in a complex and diverse microbial community within the oral cavity and as such is highly specialized to survive within this milieu (Seshadri *et al*, 2004), being especially prevalent in the anaerobic gingival crevice (Paster *et al*, 2001). Virulence for this bacterium is conferred by a number of microbiological properties such as lysis and damage of periodontal cells, adhesion to epithelia and fibroblasts, coaggregation to biofilm-bridging organisms, high motility, and production of proteins and polypeptides interacting with the host (Sela, 2001).

Experimental evidence suggests that whole saliva is even superior to pooled subgingival samples to detect *T. denticola* in the oral cavity (Umeda *et al*, 1998; Mager *et al*, 2003a,b). As it is difficult to isolate and identify spirochetes from clinical plaque samples, currently, the 16S rRNA-based polymerase chain reaction (PCR) method continues to be one of the most sensitive, rapid, and cost-effective methods for determining the prevalence of such microorganisms (Sakamoto *et al*, 1999, 2001). Although it is not possible to correlate with absolute certainty of the periodontal disease to specific pathogens, the etiopathogenic role of these bacteria cannot be ignored (Squeri *et al*, 2006). This study aimed to evaluate the salivary carriage of *T. denticola*, the relationship with clinical characteristics, and the possible associations with demographic variables in the etiopathogenesis of chronic periodontitis.

Materials and methods

Subject selection and inclusion/exclusion criteria

This cross-sectional study was performed with a convenience sample of 148 consecutive patients attending the Graduate Periodontics Clinic at the University of Antioquia between August 2005 and February 2009. All participants provided written informed consent prior to their enrolment into the study. The study conformed to the ethical guidelines of the Helsinki Declaration and was evaluated and approved by the Institutional Research Ethics Board (CIFO). Participants were privately interviewed to obtain medical and demographic information and were given a clinical screening for oral pathology and a periodontal examination. The information that was gathered included the subject's gender, age, and tobacco use. A subject was considered a non-smoker if he or she had never smoked, or had stopped smoking more than 5 years previous to the date of examination.

All clinical periodontal measurements were performed by a single calibrated investigator (AM). The clinical parameters recorded, including duplicate measurements of probing depth (PD) and clinical attachment level (CAL), were measured at six surfaces of all teeth (midbuccally, midlingually, and proximally both buccally and lingually) to the nearest mm, using a manual periodontal probe (PCP UNC 15, Hu-Friedy, Chicago, IL, USA). The interval between readings 1 and 2 was 7 days. PD was defined as the distance from the coronal-most margin of the free gingival to the most apical penetration of periodontal probe. As a reference for CAL measurement, the cemento-enamel junction (CEJ) or restorative margin (RM), if the CEJ was not visible, was used. CAL was estimated either by adding PD measurement and the location of gingival margin in relation to CEJ and/or RM (in cases with gingival recession), or by subtracting from the PD measurement the distance from the gingival margin to CEJ and/or RM when gingival margin was positioned coronal to CEJ and/or RM. Following previously defined criteria (Armitage, 1999), these measurements were used to calculate the extent and severity of periodontitis based on the percentage of tooth sites having PD $\geq$4 mm along with CAL $\geq$2 mm (extent) and the average value of attachment loss of the diseased sites (severity). All calculations were based on data derived from the full-mouth examination.

Subjects for this study comprised 97 chronic periodontitis (CP) and 51 healthy controls (HC). These patients were classified as follows: *CP*, subjects having a minimum of 20 remaining teeth, with periodontal disease as evidenced by at least four tooth sites with PD $\geq$4 mm and CAL $\geq$2 mm (Lindhe *et al*, 1999); HC, subjects with no evidence of pocket depth $>$3 mm and no clinical gingival inflammation (not more than 10% of sites with bleeding on probing and absence of gingival redness/edema), but could have attachment loss or gingival recession due to mechanical trauma. Exclusion criteria included pregnancy; any systemic condition that could affect the host's periodontal status (e.g., diabetes mellitus, immunological disorders, AIDS) or that would require drugs for monitoring or treatment procedures (e.g., heart conditions, joint replacements); use of antibiotics and/or anti-inflammatory drugs within the last 3 months; and professional cleaning or periodontal treatment within the last 6 months. Therefore, a critical differential diagnosis between CP and aggressive periodontitis (AgP) was established on the basis of a rapid attachment loss and bone destruction, as well as the presence of inconsistent amounts of microbial deposits regarding to the severity of periodontal destruction according to parameters approved for AgP (Lang *et al*, 1999).

Saliva collection and processing

About 10 ml of unstimulated whole saliva was collected from each subject into a 50 ml sterile plastic

centrifuge tube (Greiner Bio-one®, Frickenhausen, Germany) before breakfast intake and any dental hygiene procedure. No antiseptic mouth rinse was used before collection. Samples were processed at the same day, as reported by Umeda *et al*, 1998. Briefly, the tubes were vigorously dispersed using a vortex (IKA® Vibrofix, Staufen, Germany) for 10 s. Immediately, 0.5 ml of whole saliva was diluted 1:2 with sterile distilled water and collected by centrifugation at 9800 rpm for 5 min in a microcentrifuge (Biofuge fresco/Heraeus®, Kendro Laboratory Products, Langenselbold, Germany). The resulting pellet was washed four more times with sterile water and then reconstituted with 0.5 ml water. Washed samples were kept at −70°C until processing. Remaining saliva was stored under same conditions for further research purposes.

DNA extraction

The saliva specimens were prepared for PCR according to the procedure of Sánchez-Jiménez and Cardona-Castro, 2004. Briefly, 200 μl of washed saliva were centrifuged at 10 000 rpm for 5 min. The pellet was mixed with 200 μl of 10 mM Tris/HCl, pH 8, and 1 mM ethylenediamine tetraacetic acid (EDTA) and centrifuged at 12 000 rpm for 6 min. This procedure was performed twice. Then, the pellet was mixed with 200 μl distilled water and centrifuged for 1 min at 12 000 rpm, and 30 μl distilled water was added to the pellet followed by incubation at 100°C for 20 min. Supernatant was used for PCR analysis.

Electrophoresis-based signal detection of PCR

Conventional PCR detection of *T. denticola* was performed in duplicate using species-specific primers as previously described (Ashimoto *et al*, 1996) with some modifications. Briefly, 10 μl of DNA sample was added to 50 μl of PCR mixture containing, 5 μl 10× PCR buffer (Promega, Madison, WI, USA), 0.2 mM of each of deoxyribonucleotides (Promega), 1.0 μM of each primer (IDT®, Coralville, IA,USA), 2 mM $MgCl_2$ (Promega), and 1.25 U of *Taq* DNA polymerase (Promega). A pair of primers (5′-TAA TAC CGA ATG TGC TCA TTT ACA T-3′ and 5′-TCA AAG AAG CAT TCC CTC TTC TTC TTA-3′) targeted 16S rRNA genes, amplifying a fragment of 316 bp length (base positions 193–508). PCR amplification was carried out in a 96-well thermal cycler (Mastercycler® gradient, Eppendorf, Hamburg, Germany). Samples were initially denatured at 95°C for 2 min, followed by 36 cycles of 95°C for 30 sc, 60°C for 1 min, 72°C for 1 min, and a final extension step of 72°C for 2 min. The specificity of the primer pair has been thoroughly proven in other studies (Ashimoto *et al*, 1996; Sakamoto *et al*, 2001; Takeuchi *et al*, 2001; Seshadri *et al*, 2004).

PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with 0.5 μg/ml ethidium bromide, visualized in a BioRad Gel Doc 2000 System® (BioRad Laboratories, Athens, OH, USA), and digitized using the Quantity One® software package (BioRad Laboratories). As molecular size marker, 1 kb DNA ladder (Promega) was used. Positive and negative controls were included in each PCR assay. *T. denticola* ATCC 35405 genomic DNA was used as a positive control, which was kindly provided by Professor SJ Norris (University of Texas at Houston, USA). For negative control, DNA sample was replaced by sterile water. The sensitivity of the PCR assay and testing for possible inhibition of PCR reaction by saliva components was previously described (Martínez-Pabón *et al*, 2008).

Statistical analysis

Data collected were analyzed using Statistical Package for the Social Sciences (SPSS) 16.0® (SPSS, Chicago, IL, USA). Intra-observer reproducibility for both clinical measures and PCR assay was determined through double evaluations for each specific test performed by the same observers in five patients randomly selected using a computer-generated randomization code. The Cohen's kappa statistic (κ) was used to evaluate the reproducibility of categorical variables, and the Intraclass Correlation Coefficient (ICC) was selected when variables were quantitative. A value >0.6 was considered high reproducibility, a value >0.8 represented very high reproducibility, and a value >0.9 represented excellent reproducibility.

All parameters were tested for normal distribution using the Kolmogorov-Smirnov test. All variables were summarized according to the clinical groups for all subjects. As the results for each group did not follow a normal distribution, the variables were analyzed using the non-parametric methods: Pearson's chi-square test (χ^2) for categorical variables, Mann–Whitney *U*-test for the comparison between a dichotomous and an ordinal variable, and Spearman's rank correlation coefficient to describe the relationship between quantitative variables. Furthermore, the odds ratio (OR) calculation with 95% confidence interval was applied to examine the association between risk variables and chronic periodontitis. In this sense, while demographic data were analyzed and categorized focusing on gender (male *vs* female), age strata (i.e., <30 years *vs* ≥ 30 years), and tobacco use (i.e., non-smoking *vs* smoking), PCR findings were stratified taking in account the detection frequency of *T. denticola* (i.e., negative *vs* positive). The significance of the association was determined by the Pearson's chi-square test (χ^2). A positive association existed when the OR was >2 and the confidence range did not include 1.0. Finally, to test the strength and independence of the association of each significant risk variable with chronic periodontitis, a binary logistic regression model was used. In this analysis, each candidate risk variable was evaluated after adjusting for other candidate risk variable and for potential confounding. In the stepwise logistic regression analysis, $P < 0.05$ was used as the entry criterion while $P > 0.10$ was the removal criterion. The Hosmer–Lemeshow goodness-of-fit test statistic was fixed at $P > 0.05$. Significance for all tests was established at a *P*-value <0.05.

Results

Reproducibility of clinical measurements
Intra-observer reproducibility was excellent for both PD (ICC = 0.999, $P < 0.001$) and CAL (ICC = 0.995, $P < 0.001$) scores in each series of measures recorded per patient by the same examiner. Likewise, intra-observer agreement was excellent for PCR assay ($\kappa = 1.00$).

Clinical profile of the patients
Demographic and clinical characteristics of the study groups are displayed in Table 1. Although females comprised about 67% of the patients studied, there were no significant differences between men and women in the study group compared with healthy controls ($P > 0.05$, χ^2). The age of the subjects ranged between 20 and 72 years, being the age in the CP group significantly greater than in healthy subjects ($P < 0.001$, Mann–Whitney *U*-test). Likewise, patients in the CP group were significantly more smokers ($P < 0.001$, χ^2) than healthy controls. As also shown in Table 1, median values of PD and CAL scores in healthy subjects were significantly lower than those of periodontitis patients ($P < 0.001$, Mann–Whitney *U*-test). CP group comprised patients with different extent of the periodontal involvement, which varied from relatively localized (58 subjects, median value of extent 12.82%, range 2.98–30.0%) to generalized (39 subjects, median value of extent 42.53%, range 30.86–75.0%) form of the disease. Likewise, three patients showed a slight chronic periodontitis (median value of severity 2.42 mm, range 2.42–2.85 mm), 38 patients had a moderate chronic periodontitis (median value of severity 4.25 mm, range 3.0–4.95 mm), and 56 patients had severe periodontal breakdown (median value of severity 5.64 mm, range 5.0–7.74 mm). Also as a result, the combined proportion of 'extent' and 'severity' parameters was 3.1% (three cases) for localized slight CP, 28.9% (28 cases) for localized moderate CP, 27.8% (27 cases) for localized severe CP, 10.3% (10 cases) for generalized moderate CP, and 29.9% (29 cases) for generalized severe CP. Moreover, Spearman correlation analysis (based on both healthy and diseased groups) showed significant positive correlations ($P < 0.001$) between patient's age and PD ($r_s = 0.335$), CAL ($r_s = 0.559$), extent ($r_s = 0.346$), and severity ($r_s = 0.566$) of periodontal damage. It was also noteworthy that all of the clinical measurements were significantly greater for smokers compared with non-smokers subjects ($P < 0.02$, data not shown).

Relationship of Treponema denticola detection with diagnosis and clinical parameters
Although detection frequency of *T. denticola* was significantly higher ($P = 0.044$, χ^2) in saliva samples from CP cases (41 out of 97, 42.3%) than those from healthy patients (13 out of 51, 25.5%), irrespective of their periodontal health status, no significant differences ($P > 0.05$) regarding gender, age strata, and tobacco use were observed. Even so, median values of PD, CAL, extent, and severity of periodontal involvement were significantly greater ($P < 0.05$, Mann–Whitney *U*-test) for *T. denticola*-positive subjects compared with *T. denticola*-negative subjects (Figure 1).

Analysis of risk variables for association with chronic periodontitis
The results of the univariate analysis of candidate risk variables for association with chronic periodontitis are presented in Table 2. The OR of chronic periodontitis was significantly increased ($P < 0.05$, χ^2) for older patients (≥30 years of age), smokers, and *T. denticola*-positive detection. Based on the univariate analysis, significant risk variables associated with CP were fitted into a binary logistic regression model to examine the

Table 1 Demographic and clinical characteristics of study subjects

Characteristics		*Clinical groups*		
		Healthy controls (n = 51)	*Chronic periodontitis (n = 97)*	P-*value*
Gender[a]	Male	17 (11.5)	32 (21.6)	0.966[d]
	Female	34 (23.0)	65 (43.9)	
Age (years)[b]		30 (21–72)	46 (20–66)	<0.001[e]
Tobacco use[a]	Non-smoking	48 (32.4)	67 (45.3)	<0.001[d]
	Smoking	3 (2.0)	30 (20.3)	
PD score (mm)[b,c]		1.90 (1.24–2.51)	2.93 (1.43–6.66)	<0.001[e]
CAL score (mm)[b,c]		0.86 (0.02–3.60)	3.22 (1.19–7.70)	<0.001[e]
Extent[f] of periodontitis[a]	Localized (≤30%)		58 (59.8)	
	Generalized (>30%)	0	39 (40.2)	
Severity[g] of periodontitis[a]	Slight (1–2 mm)		3 (3.1)	
	Moderate (3–4 mm)	0	38 (39.2)	
	Severe (≥5 mm)		56 (57.7)	

[a]Values are given as *n* (%) of subjects.
[b]Values are given as median (range).
[c]Data based on measurements obtained from the entire dentition.
[d]Two-sided Pearson's chi-square test (χ^2).
[e]Two-sided Mann–Whitney *U*-test.
[f]Percentage of periodontal pockets ≥4 mm deep and attachment loss ≥2 mm.
[g]Data based on average value of CAL of the affected tooth sites.

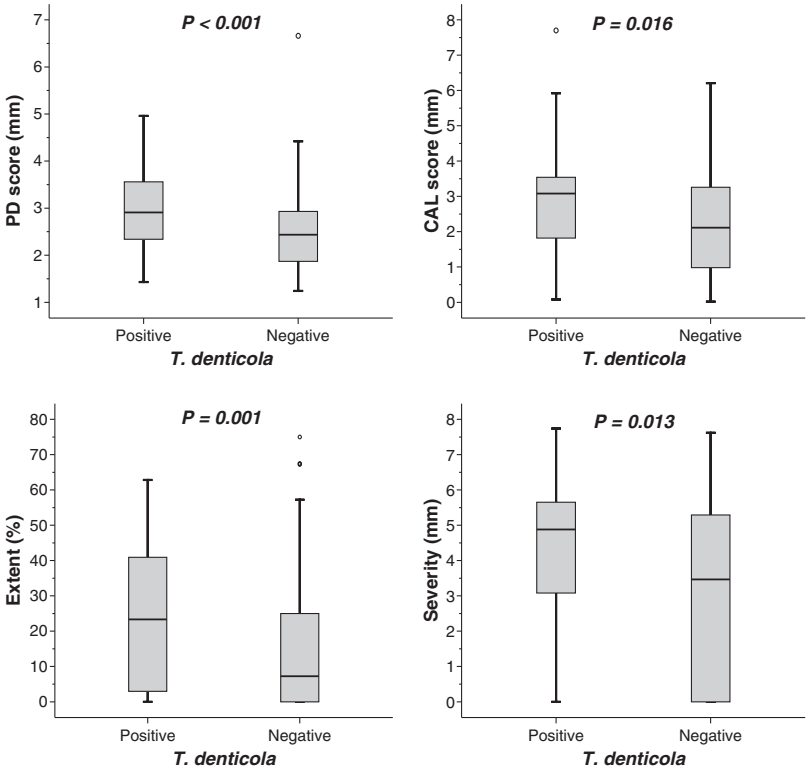

Figure 1 Quantitative comparison of clinical measurements obtained from *Treponema denticola*-positive and negative subjects as detected in saliva samples. The box represents the first and third quartiles (rectangular boxes); the line within the box is the median; vertical bars show the 95% confidence interval. Little circles above these levels represent atypical values that were plotted separately. All differences were statistically significant at level $P < 0.02$

robustness and independence of the relationship and to assess for the presence of confounding and/or interaction. This final model with adjusted OR is presented in Table 3. There was no confounding of the relationship between patient's age ≥30 years and smoking habit regarding chronic periodontitis. In these cases, each relationship remained associated with diseased status ($P < 0.05$, Wald test) when adjusted for variables individually. In other words, patient's age ≥30 years remained a robust risk indicator for chronic periodontitis when adjusted for smoking habit and *T. denticola*-positive detection. Likewise, smoking habit remained significantly associated with chronic periodontitis when adjusted for all other variables. Conversely, although chronic periodontitis status was positively associated with *T. denticola*-positive detection in the univariate analysis, confounding of the association was evident after adjustment for covariates as this association failed to achieve statistical significance. Finally, pairwise significant interactions included patient age ≥30 years/smoking habit (adjusted OR, 9.45; 95% CI, 2.15–41.59; $P = 0.003$, Wald test), patient age ≥30 years/*T. denticola*-positive detection (adjusted OR, 4.83; 95% CI, 1.88–12.42; $P = 0.001$), and *T. denticola*-positive detection/smoking habit (adjusted OR, 9.15; 95% CI, 1.17–71.37; $P = 0.035$).

Table 2 Univariate analysis of risk variables for association with chronic periodontitis

Risk variables	*No. cases*[a]	*OR (95% CI)*[b]	P-*value*[c]
Gender			
Male[d]	32 (33.0)	1	0.966
Female	65 (67.0)	1.01 (0.49–2.08)	
Age strata, year			
Age <30[d]	8 (8.2)	1	<0.001
Age ≥30	89 (91.8)	9.89 (3.98–24.53)	
Tobacco use			
Non-smoking[d]	67 (69.1)	1	<0.001
Smoking	30 (30.9)	7.16 (2.06–24.84)	
Treponema denticola detection			
Negative[d]	56 (57.7)	1	0.044
Positive	41 (42.3)	2.14 (1.01–4.52)	

[a]The values are given as *n* (%) of subjects within diagnosis.
[b]Odds ratio (95% confidence interval).
[c]Two-sided Pearson's chi-square test (χ^2).
[d]Reference group.

Discussion

Multi-factorial etiology of chronic periodontitis has been analyzed in numerous studies including risk factors, risk indicators, and risk predictors as explanatory variables

Table 3 Binary logistic regression analysis: significant risk variables associated with chronic periodontitis

Risk variable	*Unadjusted OR (95% CI)*[a]	*Adjusted OR (95% CI)*[a]	P-*value*[b]
Patient age ≥30 years	9.89 (3.98–24.53)	10.13 (3.78–27.12)	<0.001
Smoking habit	7.16 (2.06–24.84)	6.46 (1.70–24.63)	0.006
Salivary *Treponema denticola*-positive subjects	2.14 (1.01–4.52)	1.91 (0.80–4.52)	0.143

[a]Odds ratio (95% confidence interval).
[b]Wald test.
Hosmer and Lemeshow goodness-of-fit test: $P = 0.914$.

(Albandar, 2002; Hyman and Reid, 2003; Cortelli *et al*, 2008). A risk factor is any characteristic or behavior with an association to a particular disease, identified in a longitudinal study design. On the contrary, risk indicator describes a possible factor associated with a disease, which is identified from case-control or cross-sectional studies. Alternatively, risk predictor usually refers to a risk variable associated with an increased probability of disease in the future (Cortelli *et al*, 2008).

In addition, the association between a risk variable and the disease may be confounded by other factors. Confounding occurs when the association between a risk variable and an outcome are strongly associated with the effect of a third variable (Rothman and Greenland, 1998). Also, the disease may result from the interaction between risk factors. Interaction (effect modification) is concerned with the way in which two or more potential risk factors act together (Katz, 2003; Jose *et al*, 2008). In a regression analysis, confounding variables can usually be adequately handled by including them in the model, if there is overlap in the confounding variables amongst the exposed and unexposed population (Hyman, 2006). In this study, a full-mouth examination was used to evaluate whether salivary carriage of *T. denticola* and some risk indicators possess a true risk-modifying effect regarding to the clinical manifestations of chronic periodontitis. Consequently, the CP subjects of this study represent a stratified sample of patients exhibiting different levels of periodontal breakdown. As expected, clinical and demographic characteristics of study subjects showed statistically significant differences in age, tobacco use, and PD and CAL scores according to parameters agreed for CP (Lindhe *et al*, 1999). Furthermore, CP patients showed a predominantly generalized and severe form of the disease (Armitage, 1999).

It is well known that chronic periodontitis have cumulative characteristics so that sequels are more easily identified in older age groups (Hyman and Reid, 2003). In this study, the correlation between age and the clinical parameters was statistically significant. These results are consistent with other studies which found that periodontitis severity increased as age increased (Susin *et al*, 2004a; Krustrup and Petersen, 2006). Likewise, it has been shown that smokers have more bone loss, attachment loss, and deeper periodontal pockets than non-smokers. Moreover, smokers are more susceptible to advanced and severe forms of periodontal disease than non-smokers (Haber *et al*, 1993; Calsina *et al*, 2002). In agreement with the former, in this study, smokers had greater periodontal destruction than non-smokers.

Saliva is known to reflect the total bacterial profile of an individual (Tamura *et al*, 2006) and previous studies had indicated that periodontopathic bacteria are more consistently detected in this diagnostic fluid than in subgingival plaque samples (Umeda *et al*, 1998; Sakamoto *et al*, 2001). It is worth mentioning that several of the known periodontal pathogens, including *T. denticola,* have similar percentages of DNA count in saliva compared with supra and subgingival tooth surfaces (Mager *et al*, 2003b). Nevertheless, whereas it has been demonstrated that saliva is equal to or better than subgingival plaque for detecting and quantifying periodontopathic bacteria in the oral cavity (Umeda *et al*, 1998; Sakamoto *et al*, 2001; Tamura *et al*, 2006), only partial information is available regarding salivary carriage of *T. denticola* and its association with periodontal status (Takeuchi *et al*, 2001; Könönen *et al*, 2007; Martínez-Pabón *et al*, 2008; Leblebicioglu *et al*, 2009).

Treponemes have been thoroughly associated with periodontitis, and *T. denticola* especially has been suggested as a reliable diagnostic marker (Socransky *et al*, 1998). In a mixed bacterial community within the oral cavity, it is likely that *T. denticola* has its virulence enhanced or it can enhance the virulence of other species in the consortium (Yao *et al*, 1996; Kesavalu *et al*, 1998). A noteworthy feature of this study was the relatively high frequency (25.5%) of salivary carriage of *T. denticola* found in healthy controls. This finding concurs with previous reports that found high detection frequencies of putative periodontal pathogens in saliva of periodontally healthy subjects (Takeuchi *et al*, 2001; Mager *et al*, 2003b; Tamura *et al*, 2006; Könönen *et al*, 2007; Leblebicioglu *et al*, 2009). However, the reasons for such high prevalence are unclear. It is possible that control subjects who had participated in the screening procedures were not a representative sample of the general population. On the other hand, this high prevalence might be attributable to transfer mechanisms including horizontal/vertical transmissions as well as cross-infections (Von Troil-Lindén *et al*, 1996; Greenstein and Lamster, 1997). Given that a pathogen may need to be in numbers sufficient to exceed a particular threshold to initiate disease, the transfer of organisms does not necessarily result on colonization or infection of the host (Leblebicioglu *et al*, 2009). Consequently, it is quite probable that saliva might act as reservoir so that *T. denticola* colonizes these sites contributing to the onset of periodontitis when other factors such as high levels of one or more specific pathogens, low levels of beneficial species, and environmental/genetic features associated with a susceptible host are also present (Page and Kornman, 1997).

The salivary levels of periodontal pathogens reflect the periodontal status of the patient, and saliva and oral mucous membranes may serve as a source for re-infection of the periodontium after treatment (Von Troil-Lindén *et al*, 1995; Smoot *et al*, 2005; Leblebicioglu *et al*, 2009). In this study population, the clinical signs of periodontal disease appeared to be linked to the salivary carriage of *T. denticola*, as statistically significant elevations were seen in the median values of PD, CAL, extent, and severity of periodontitis in *T. denticola* salivary carriers compared with non-carriers subjects. These findings are consistent with those described by others (Simonson *et al*, 1988; Takeuchi *et al*, 2001) whose different detection techniques use observed a positive association between presence of *T. denticola* and periodontal tissue destruction. Conversely, not only gender but also age strata as well as tobacco use proved to have a very little impact on the salivary carriage of *T. denticola*. Other authors have also reported lack of

association between salivary carriage of *T. denticola* concerning gender (Könönen *et al*, 2007; Leblebicioglu *et al*, 2009), tobacco use (Mager *et al*, 2003b) and age strata (Könönen *et al*, 2007). From the data presented, it seems that the carriage of *T. denticola* in saliva might be more related to the degree of periodontal breakdown than to the smoking habit, gender, and age strata.

It is well known that complex interactions between host defense mechanisms and the bacterial flora influence the equilibrium between bacterial aggression and host protection, and thus determine whether periodontal breakdown occurs (Socransky and Haffajee, 1997). Although this study confirmed the association of age ≥30 years, smoking habit, and salivary carriage of *T. denticola* with CP in univariate analysis, confounding of the association between salivary carriage of *T. denticola* and CP was evident after adjustment for covariates, as this association weakened considerably. This confounding on the risk of CP was because of the lack of relationship of salivary carriage of *T. denticola* regarding age strata and tobacco use in this study population. Notwithstanding, an interaction effect defined as departure from additivity of their absolute effects was significantly detected between these three variables. Despite this phenomenon has already been documented in the literature (Hyman, 2006; Ylöstalo and Knuuttila, 2006), the observations of this study underscore the necessity to adjust for a confounding effect between covariates when studying their individual effects on the risk of CP. Confirmation of these findings and further research into the underlying mechanisms responsible for these interactions might lead to selective therapies aimed at preventing this destructive disease. In other words, the risk that originate from salivary carriage of *T. denticola* might imply an insufficient local immune response or decreased functional capacity to prevent colonization or infection due to age-related changes, as well as factors that promote episodes of periodontal disease activity, such as smoking (Beck *et al*, 2005). Consequently, it is quite probable that the effects on the vascular and immune systems (Johnson and Slach, 2001), along with the decreased oxygen tension which occurs in the oral cavities of smokers (Kenney *et al*, 1975), may promote the colonization or infection by *T. denticola* contributing to the onset of periodontitis.

Several limitations were associated with this study. First, it is a single center and may have limited generalizability to other centers. In particular, more variability in patient populations could potentially yield different risk indicators. Second, for some subgroups of candidate risk variables, sample sizes were too small to produce interpretable results and might not represent landmark data to evaluate the association of candidate risk variables with CP. Third, although the significant risk variables were adjusted for the confounding effects of other variables, the potential for uncontrolled confounding exists. An analysis with more patients would have greater statistical power and precision.

In summary, the findings when considered within the limitations of this study suggest that although salivary carriage of *T. denticola* may be a risk indicator for CP, its pathogenicity should not be exclusively endorsed to its detection in saliva, but it might be associated with the synergistic biological interaction of the bacterium with some demographic characteristics of the susceptible host.

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Authors contributions

Martínez-Pabón MC handled the study concepts and design, carried out the end-point PCR assays, acquisition and analysis of data, and manuscript preparation. Martínez-Gaviria A participated in the recording of clinical data, analysis of the results, as well as was involved in drafting the manuscript. Isaza-Guzmán DM conceived the study, participated in its design and coordination, carried out the end-point PCR assays, and helped drafting the manuscript. Muskus-López CE gave advice and content expertise with the PCR assays throughout the evolution of the study and reviewed the manuscript for important intellectual content. Tobón-Arroyave SI elaborated the conception and participated in the design of the study, accomplished the analysis and interpretation of data, supervised the interpretation of the data, and critically evaluated and supplemented the manuscript. All the authors read and approved the final manuscript.

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