P43

Preferential Colonization Patterns of Periodontopathogens in Subgingival Niches of Periodontitis Patients

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Introduction

Chronic periodontitis is an infectious disease resulting from intricate host-parasitic interactions at the soft-hard tissue border of the tooth supporting apparatus (Consensus Report 1996). The role of specific periodontopathogens in this disease process was being investigated dating back to the late 70's (Socransky & Haffajee 1994). At least a dozen putative periodontopathgens were identified (Zambon 1996) while more were being put onto the list when research ability in bacterial identification was pushed beyond the limits of traditional culture techniques (Paster et al 2001). Realizing the possible immense complexity of the microbial etiology of periodontal disease, attention was also being put towards studying the interactions between members of the subgingival microbial biofilm (Socransky et al 1988, Simonson et al 1992). Evidence accumulated in recent years pointed towards certain periodontopathogens tending to co-exist in subgingival niches of subjects with various clinical conditions (Socransky et al 1998, Jin et al 1999, 2002,). Various statistical analysis protocols have been applied to the subgingival microbial data processing (Simonson et al 1992, Cohen 1993, Ali et al 1995, Socransky et al 1998). Many studies employed statistic protocols focusing on two-by-two contingency, correlation or cluster analysis of target species. Configural frequency analysis (CFA) was recommended by Cohen (1993) as the statistical test where configurations of the target microorganisms, present or not, were studied.

The aim of the current study is therefore attempt to utilize CFA in studying the possibility of co-colonization patterns among five periodontopathogens namely, *Actinobacillus actinomycetemcomitans (Aa), Bacteriodes forsythus (Bf), Porphyromonas gingivalis (Pg), Prevotella intermedia (Pi), and Treponema denticola (Td)* in subgingival niches of untreated periodontitis patients.

Material and Methods

Patients management, site selection and sampling

Microbiological samples from 85 subgingival sites of 16 untreated Chinese adults with chronic periodontitis, aged 32-55 years were studied. Related clinical information was published (Jin et al 1999, 2002).

Five to seven non-adjacent sites were sampled from each subject consisting:

- Periodontitis sites - [pocket depth (PD) ≥ 5.0 mm, attachment loss (AL) ≥ 3.0 mm, positive radiographic bone loss (BL)], 3-4 samples,
- Gingivitis sites - [gingival index (GI) > 2, positive bleeding on probing (BOP), PD < 4.0 mm, AL < 1.0 mm, BL (-)], 12 samples and
- Healthy site - [GI < 1, BOP(-), PD < 3.0 mm, AL < 1.0 mm, BL (-)], 1 sample.

Samples were collected using paper points and analyzed for *Aa, Bf, Pg, Pi and Td* using species specific DNA probes (Jin et al. 1999).

Following sampling, each patient received a course of non-surgical periodontal therapy within a 4-week period. Subgingival plaque samples were collected from the same 85 sites one-month after the non-surgical periodontal therapy.

Statistical analysis

The prevalence of *Aa* at baseline and 1-month post-therapy was low (0% and 3.5% respectively) and hence not included in the study. Stuart-Maxwell Chi-square test (Fleiss & Everitt 1997) was used to analyse the change of microbial profile, i.e. presence or absence, of the four target species *Bf, Pg, Pi and Td* alone and in all possible combinations (n = 16, see Table 2) at baseline and one month post non-surgical therapy. Post hoc analysis based on McNemar's statistic with Bonferroni adjustment was carried out. The level of significance chosen was 0.05.

CFA was then applied to the data set (Cohen 1993). The protocol described by von Eye (1990) was followed. In brief, a global K-th order log-linear analysis was performed in order to search for the appropriate statistical types. The first set of marginals fitted describes the model for mutual independence among all variables (the first-order model). The second, third, and fourth models test, respectively, models based on two-, three- and four-way interactions among the variables (Cohen 1993). The statistical types would be selected based on the component of Chi-square *P* value and be put through the corresponding configuration analysis where types and/or antitype were identified.

Results

Many of the *Bf, Pg, Pi and Td* configural type switched to none one month after treatment ($p < 0.001$).

Table 1. Global K-th order log-linear analysis

Marginals fitted	Residual chi-squares df	L	*p*	Component chi-squares df	L	*p*
Baseline						
Bf, Pi, Pg, Td	11	173.490	0.000			
Bf+Pi, Bf+Pg, Bf+Td, Pi+Pg, Pi+Td, Pg+Td	5	17.458	0.004	6	156.032	<0.001
Bf+Pg+Td, Bf+Pi+Td, Pi+Pg+Td	2	0.003	0.955	3	17.455	<0.001
Bf+Pi+Pg+Td	0	0	1	2	0.003	>0.999
One-month post- non-surgical therapy						
Bf, Pi, Pg, Td	11	149.571	0.000			
Bf+Pi, Bf+Pg, Bf+Td, Pi+Pg, Pi+Td, Pg+Td	5	1.993	0.850	6	147.578	<0.001
Bf+Pg+Td, Bf+Pi+Td, Pi+Pg+Td	2	0.003	0.959	3	1.990	<0.75
Bf+Pi+Pg+Td	0	0	1	2	0.003	>0.999

Table 2. First-order configurations (model: *Bf, Pg, Pi, Td*) at baseline

Configurations *Bf*	*Pg*	*Pi*	*Td*	Observed Frequency	Expected Frequency	|z|	*p*	Holm's Criteria *P*	
N	N	N	N	19	1.30	-	0.0000[a]	0.0031	type
Y	N	N	N	0	2.05	-	0.2510[a]		
N	N	Y	N	1	1.53	-	1.0924[a]		
N	Y	N	N	3	3.51	-	1.0643[a]		
N	N	N	Y	1	2.80	-	0.4519[a]		
Y	N	Y	N	0	2.42	-	0.1717[a]		
Y	Y	N	N	4	5.53	0.893	0.3720		
Y	N	N	Y	2	4.41	-	0.3529[a]		
N	Y	Y	N	0	4.14	-	0.0287[a]		
N	N	Y	Y	0	3.30	-	0.0691[a]		
N	Y	N	Y	7	7.54	0.397	0.6916		
Y	Y	Y	N	0	6.52	2.861	0.0042	> 0.0036	
Y	Y	N	Y	3	11.87	2.776	0.0055		
Y	N	Y	Y	0	5.20	2.580	0.0099		
N	Y	Y	Y	2	8.89	2.619	0.0088		
Y	Y	Y	Y	43	14.01	8.475	0.0000	0.0033	type

[a] *p*-level computed using exact binomial

Table 3. First-order configurations (model: *Bf, Pg, Pi, Td*) at one-month post- non-surgical therapy

Configurations *Bf*	*Pg*	*Pi*	*Td*	Observed Frequency	Expected Frequency	|z|	*p*	Holm's Criteria *P*	
N	N	N	N	52	26.00	6.120	0.0000	0.0033	type
Y	N	N	N	0	5.57	2.661	0.0078		
N	N	Y	N	1	10.23	3.077	0.0021	0.0036	antitype
N	Y	N	N	5	14.18	2.671	0.0076	> 0.0038	
N	N	N	Y	1	6.50	2.449	0.0143		
Y	N	Y	N	0	2.19	-	0.8913[a,b]		
Y	Y	N	N	1	3.04	-	0.8121[a,b]		
Y	N	N	Y	1	1.39	-	0.8116[a]		
N	Y	Y	N	9	5.58	1.279	0.2010		
N	N	Y	Y	0	2.56	-	0.9257[a,b]		
N	Y	N	Y	0	3.55	-	0.9734[a,b]		
Y	Y	Y	N	0	1.20	-	0.7014[a,b]		
Y	Y	N	Y	1	0.76	-	0.3530[a]		
Y	N	Y	Y	0	0.55	-	0.8482[a]		
N	Y	Y	Y	2	1.40	-	0.3306[a]		
Y	Y	Y	Y	12	0.30	-	0.0000[a]	0.0031	type

[a] *p*-level computed using exact binomial
[b] one-tail probability

Conclusions

Within the limitations of this study:

i. *Bf, Pg, Pi, Td* colonized periodontal sites in a cluster or not at all;
ii. while proportion of sites with none of these pathogens had markedly increased one month after non-surgical periodontal therapy this cluster-type colonization of *Bf, Pg, Pi* and *Td* persisted.

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