한국인 만성 치주염 환자에서 치주질환 원인균의 동정

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Identification of putative periodontal pathogens in Korean chronic periodontitis patients

Jeong-Ho Yun[1], Jung-Eun Park[2], Doo-il Kim[2], Syng-Ill Lee[3], Seong-Ho Choi[4], Kyoo-Sung Cho[4], Dae-Sil Lee[2*]

1. Department of Dentistry, College of Medicine, Kwandong University, Myongji Hospital
2. Systems Microbiology Research Center, Korea Research Institute of Bioscience and Biotechnology
3. Department of Oral Biology, Brain Korea 21 Project and Oral Science Research Center, College of Dentistry, Yonsei University
4. Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University

ABSTRACT

Purpose: Specific bacteria are believed to play an important role in chronic periodontitis. Although extensive microbial analyses have been performed from subgingival plaque samples of periodontitis patients, systemic analysis of subingival microbiota has not been carried out in a Korean population so far. The purpose of this study was to investigate the prevalence of 29 putative periodontal pathogens in Korean chronic periodontitis patients and evaluate which pathogens are more associated with Korean chronic periodontitis.

Material and Methods: A total of 86 subgingival plaque samples were taken from 15 chronic periodontits(CP) patients and 13 periodontally healthy subjects in Korea. CP samples were obtained from the deepest periodontal pocket (>3 mm probing depth[PD]) and the most shallow periodontal probing site (3 mm PD) in anterior tooth and posterior tooth, respectively, of each patient. Samples in healthy subjects were obtained from 1 anterior tooth and 1 posterior tooth. Polymerase chain reaction (PCR) of 16S ribosomal DNA (rDNA) of subgingival plaque bacteria was performed. Detection frequencies(% prevalence) of 29 putative periodontal pathogens were investigated as bacterium-positive sites/total sites

Results: With the exception of *Olsenella profuse* and *Prevotella nigrescens*, the sites of diseased patients generally showed higher prevalence than the healthy sites of healthy subjects for all bacteria analyzed. *Tanerella forsythensis (B.forsythus)*, *Campylobacter rectus, Filifactor alocis*, *Fusobacterium nucleatum*, *Porphyromonas endodontalis* and *Porphyromonas gingivalis* were detected in more than 80% of sites with deep probing depths in CP patients. In comparison between the sites (deep or shallow PD) of CP patients and the healthy sites of healthy subjects, there was statistically significant difference($P <0.05$) of prevalence in *T.forsythensis* (*B.forsythus*), *C.rectus*, *Dialister invisus*, *F.alocis*, *P.gingivalis* and *Treponema denticola*.

Conclusion: Our results demonstrate that the four putative periodontal pathogens, *T.forsythensis* (*B.forsythus*), *C.rectus*, *P.gingivalis* and *F.alocis* are closely related with CP patients in the Korean population. (*J Korean Acad Periodontol 2008;38:143-152*)

KEY WORDS: Periodontal pathogen; chronic periodontitis; Korean; polymerase chain reaction.

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500   *Actinobacillus actino-mycetemcomitans, Porphyromonas gingivalis, Tanerella forsythensis(Bacteroides forsythus), Prevotella intermedia, Treponema denticola, Fusobacterium nucleatum, Micromonas micros(Peptostreptococcus micros), Eikenella corrodens, Campylobacter rectus*

1).

,   *T.forsythensis*, *P.gingivalis*, *T.denticola*

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7).   ,

2).

,   biofilm   .

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P.gingivalis, *T.denticola*

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13

.   ,   16S   rRNA

Polymerase chain reaction(PCR)

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T.forsythensis, *P.gingivalis*, *T.denticola*

,   1. Subject selection

(prevalence)   4).

(Localized aggressive periodontitis)   28

5),   .

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(IRB)   (06-022).

,   ,

5,6).   ,   ,

.   (informed consent)   .

24   35   13   13   .

,   ,

10)   (chronic periodontitis)   (PD>3mm)   1   1

33   63   15   .   .   ,

4mm   (PD 3mm)   1

4   , 3mm   1   .

4   11).   1

,   3mm   (Table 1).

.   ,

3   cotton pellet   ,

.   cotton roll   ,

,   paper point(#30, Sure-endo®, Sure Dent Co., Korea)

.   ,   10   .

20   Paper point   200   phosphate buffered sal-

.   Table 1   ine(PBS, Gibco BRL, Life Technologies, Grand Island,

.   NY, USA)   ,   -20

.   ,   29

2. Clinical measurements

PCR

(Table 2).

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6   (,   ,   ,   ## 4. Microbial assessment

,   ,   )   .

color coded probe(2mm   ,   12mm)   DNA   200

.   30   400   PBS   .   600

/   .   QIAamp DNA Mini kit(Qiagen, Germany)

buccal swab spin protocol12)   Genomic

3. Collection of subgingival plaque sample

DNA   .   oligo-

nucleotide primer(Bioneer Corp., Korea)

86   15   13-32)   16S rRNA

Table 1. *Demographic Data and Mean Clinical Parameter of Sampling sites*

	CP Patients		Healthy Subjects
Number of Subjects	15		13
Gender (Male:Female)	8:7		7:6
Age (mean±SD;mm)	46.3±8.13		29.8±3.83
Sampling Sites	Sites Showing the Deepest PD (*n*=30)	Sites Showing the most shallow PD (*n*=30)	Healthy Sites (*n*=26)
PD (mean±SD;mm)	6.37±2.30	2.50±0.51	2.58±0.50

CP, chronic periodontitis; PD, probing depth.

, PCR

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, Treponema socranskii   16S rRNA
PCR   ,
phosphoglucokinase site   primer   .
29   phospho-
glucokinase   ORF
. , A. actinomycetemcomitans   ATCC29524
16S rDNA   , strain poly-
saccharide antigen gene cluster(serotype a-f)
(Table 2).

PCR   AccuPower® PCR PreMix
(Bioneer Corp., Korea)   , thermal cycler
(Applied biosystems, CA, USA)   30   PCR cycle
. 94   30   denaturation, 55
30   annealing, 72   30   extension
, cycle 10   extension
. PCR product   sequencing
DNA   .
20   10   0.8% agarose gel   30
(0.04 M Tris-acetate, 0.01 M EDTA, pH 8.0,
100 V)   UV detector   ,
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5. Statistical analysis

(%)   (Table 3).

Chi-square test
Fisher's exact test   .
(PD > 3 mm)
(PD   3 mm)
McNemar's test   .
$P < 0.05$.

T.forsythensis(B.forsythus),   C.rectus,   F.nucleatum,
P.gingivalis
80%   ,
F.alocis, P.endodontalis   80%
. , O.profuse, P.nigrescens
27

. ,
A. actinomycetemcomitans
(13.3%)   (Table 3).

, T. for-
sythensis(B.forsythus),   C.rectus,   D.invisus,   F.alocis,
P.gingivalis, T.denticola   (P < 0.05)
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(P
< 0.05)   .
A.parvulum, T.forsythensis(B.forsythus), C.gracilis, C.rectus,
D.invisus, F.alocis, F.nucleatum, O.profuse, M.micros
(P.micros),   P.endodontalis,   P.gingivalis,   P.intermedia,
T.denticola, C.gingivalis, T.socranski
. ,

(P < 0.05)   , Actinobaculum
. ,
A. actinomycetemcomitans
(Table 3).

Table 2. *Putative Periodontopathogenic Bacteria and Specific Primer Sequences*

Bacteria Strains	Primer Sequence(5'→3')	Product Size(bp)
A.actinomycetemcomitans serotype a[13]	F: GCAATGATGTATTGTCTTCTTTTGGA R: CTTCAGTTGAATGGGGATTGACTAAAAC	428
A.actinomycetemcomitans serotype b[13,14]	F: CGGAAATGGAATGCTTGC R: CTGAGGAAGCCTAGCAAT	298
A.actinomycetemcomitans serotype c[13,15]	F: AATGACTGCTGTCGGAGT R: CGCTGAAGGTAATGTCAG	559
A.actinomycetemcomitans serotype d[14,16]	F: TTACCAGGTGTCTAGTCGGA R: GGCTCCTGACAACATTGGAT	690
A.actinomycetemcomitans serotype e[13,17]	F: CGTAAGCAGAAGAATAGTAAACGT R: AATAACGATGGCACATCAGACTTT	211
A.actinomycetemcomitans serotype f[18]	F: CCTTTATCAATCCAGACAGC R: AAAACTTCTCTTCGGGAATG	232
A.actinomycetemcomitans[19] ATCC29524	F: CTCAGAGATGGGTTTGTGCC R: AGATTCACTCCCCATCGCTG	273
Actinobaculum oral clone EL030[20,21]	F: GCCTACCAAGGCGTCGACGGGTAGCCGGCC R: GGTGGGGATGACGTCAAATCATCATGCCCC	877
Atopobium parvulum[22] ATCC33793	F: AGAGTTTGATCCTGGCTCAG R: TGCGGCACGGAAGAAATACTCCCC	827
Tanerella forsythensis (B.forsythus)[23] ATCC43037	F: GCGTATGTAACCTGCCCGCA R: TGCTTCAGTGTCAGTTATACCT	641
Tanerella forsythensis (B.forsythus)[24] ATCC700191	F: AAAACAGGGGTTCCGCATGG R: TTCACCGCGGACTTAACAGC	425
Tanerella forsythensis (B.forsythus)[25] ATCC700198	F: TACAGGGGAATAAAATGAGATACG R: ACGTCATCCCCACCTTCCTC	745
Campylobacter gracilis[20,21] *ATCC33236*	F: CAACCCGCTTAGCGAGGTTACGCATAAGCG R: CGGTTAGCATCGTCGTGTTCCAAAAACGGA	420
Campylobacter rectus[23] ATCC33238	F: TTTCGGAGCGTAAACTCCTTTTC R: TTTCTGCAAGCAGACACTCTT	598
Dialister invisus E7.25[22,26] (=CCUG 47026=DSM 15470)	F: CAGAAATGCGGAGTTCTTCTTCG R: CCCGGGAACGTATTCACCG	381
Dialister pneumosintes[27] ATCC51894	F: TTCTAAGCATCGCATGGTGC R: GATTTCGCTTCTCTTTGTTG	1106
Filifactor alocis[28] ATCC35896	F: CAGGTGGTTTAACAAGTTAGTGG R: CTAAGTTGTCCTTAGCTGTCTCG	594
Fusobacterium nucleatum subsp. nucleatum[29] ATCC25586	F: AGAGTTTGATCCTGGCTCAG R: GTCATCGTGCACACAGAATTGCTG	360
Olsenella profuse[20,21]	F: GATGAACGCTGGCGGCGCGCCTAACACATG R: GCCCCCGGATTTGACTCCCGACCTAGCAGG	510
Micromonas micros (P.micros)[29] ATCC33270	F: AGAGTTTGATCCTGGCTCAG R: ATATCATGCGATTCTGTGGTCTC	207
Porphyromonas endodontalis[30] ATCC35406	F: GCTGCAGCTCAACTGTAGTC R: CCGCTTCATGTCACCATGTC	672
Porphyromonas gingivalis[23] ATCC33277D	F: AGGCAGCTTGCCATACTGCG R: ACTGTTAGCAACTACCGATGT	404
Prevotella intermedia[31] ATCC25611	F: CGTGGACCAAAGATTCATCGGTGGA R: CCGCTTTACTCCCCAACAAA	259
Prevotella intermedia[31] ATCC15032	F: CCTAATACCCGATGTTGTCCACA R: AAGGAGTCAACATCTCTGTATCC	855
Prevotella nigrescens[23] ATCC33563	F: ATGAAACAAAGGTTTTCCGGTAAG R: CCCACGTCTCTGTGGGCTGCGA	804
Pseudoramibacter alactolyticus[32] ATCC23263	F: CGAATAAGTCAGTGCCGG R: CTTCGCTTCCCTTTGTTCAG	421
Treponema denticola[23] ATCC33521	F: TAATACCGAATGTGCTCATTTACAT R: TCAAAGAAGCATTCCCTCTTCTTCTTA	316
Capnocytophaga gingivalis[29] ATCC33624	F: AGAGTTTGATCCTGGCTCGA R: GGACGCATGCCCATCTTTCACCACCGC	227
Treponema socranskii subsp. socranskii[20,21] ATCC35536	F: GAAAGAGCTTTCGACTTACG R: ATCGGATCGAGATACTTCAC	230

Table 3. Prevalence of 29 Putative Periodontopathogenic Bacteria in Chronic Periodontitis Patients and Periodontally Healthy Subjects

Bacteria strains	% Prevalence		
	CP patients (*n*=15)		Healthy subjects (*n*=13)
	Deep PD sites (*n*=30)	Shallow PD sites (*n*=30)	Healthy sites (*n*=26)
A.actinomycetemcomitans serotype a	0	0	0
A.actinomycetemcomitans serotype b	3.3	10	0
A.actinomycetemcomitans serotype c	13.3	6.7	0
A.actinomycetemcomitans serotype d	3.3	3.3	0
A.actinomycetemcomitans serotype e	3.3	3.3	0
A.actinomycetemcomitans serotype f	0	0	0
A.actinomycetemcomitans ATCC29524	10	6.7	3.8
Actinobaculum oral clone EL030	26.7*	16.7	0
Atopobium parvulum ATCC33793	23.3*‡	10	0
Tanerella forsythensis (B.forsythus) ATCC43037	83.3*‡	40†	3.8
Tanerella forsythensis (B.forsythus) ATCC700191	76.7*‡	46.7†	11.5
Tanerella forsythensis (B.forsythus) ATCC700198	90.0*‡	36.7†	3.8
Campylobacter gracilis ATCC33236	73.3*‡	36.7	26.9
Campylobacter rectus ATCC33238	80.0*‡	43.3†	11.5
Dialister invisus E7.25 (=CCUG 47026=DSM 15470)	53.3*‡	26.7†	0
Dialister pneumosintes ATCC51894	13.3	16.7	11.5
Filifactor alocis ATCC35896	83.3*‡	36.7†	3.8
Fusobacterium nucleatum subsp. nucleatum ATCC25586	80.0*‡	43.3	30.8
Olsenella profuse	26.7*‡	13.3	15.4
Micromonas micros (P.micros) ATCC33270	53.3*‡	26.7	19.2
Porphyromonas endodontalis ATCC35406	86.7*‡	43.3	23.1
Porphyromonas gingivalis ATCC33277D	80.0*‡	43.3†	3.8
Prevotella intermedia ATCC25611	50.0*‡	23.3	15.4
Prevotella intermedia ATCC15032	63.3*‡	33.3	11.5
Prevotella nigrescens ATCC33563	13.3	6.7	15.4
Pseudoramibacter alactolyticus ATCC23263	30.0	26.7	19.2
Treponema denticola ATCC33521	70.0*‡	43.3†	3.8
Capnocytophaga gingivalis ATCC33624	56.7*‡	33.3	23.1
Treponema socranskii subsp. socranskii ATCC35536	53.3*‡	16.7	7.7

CP, chronic periodontitis; PD, probing depth.

% Prevalence is given as bacterium-positive sites/total sites (%).

* Statistically significant difference between deep PD sites of CP patients and healthy sites of healthy subjects (*P*<0.05)

† Statistically significant difference between shallow PD sites of CP patients and healthy sites of healthy subjects (*P*<0.05)

‡ Statistically significant difference between deep PD sites and shallow PD sites of CP patients (P<0.05)

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26,35). ,

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, , F. alocis

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36). ,

. , F. alocis

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. , probe, polymerase chain

reaction(PCR) 1). PCR .

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33).

16S rRNA PCR .

29 , , ,

, .

T. forsythensis(B. forsythus), ,

C. rectus, F. alocis, F. nucleatum, P. endodontalis, P. gingivalis

80% , . real- time

PCR

, T. forsythensis(B. forsythus),

C. rectus, D. invisus, F. alocis, P. gingivalis, T. denticola 37,38),

(P< 0.05) . ,

,

, 37).

T. forsythensis(B. forsythus), P. gingivalis, T. denticola, T. forsythensis

C. rectus, P. gingivalis, F. alocis ,

, 37).

. , T. forsythensis(B. forsythus), C. rectus,

P. gingivalis ,

1),

T. forsythensis

P. gingivalis 34). F. alocis . ,

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,

7   6mm

, *Fusobacterium sp.*, *Treponema sp.*, *P. gingivalis*, *B. forsythus*   96%

M. micros(P. micros),
A. actinomycetemcomitans, *P. intermedia*

[8].

T. forsythensis,
F. nucleatum, *P. gingivalis*   80%

, *M. micros(P. micros)*(53.3%), *P. intermedia*
(50.0/63.3%)

A. actinomycetemcomitans

(13.3%)   .   ,   ,

,

A. actinomycetemcomitans

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[35],

,

.   ,

A. actinomycetemcomitans

[39-41],   ,

[11].   , *A. actinomycetemcomitans*

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[41].   ,

[8].   ,

A. actinomycetemcomitans

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[8]

(PD   3mm)

[8,42,43]

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[42].   ,

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[8].

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,   *T. forsythensis*
(B. forsythus), *C. rectus*, *P. gingivalis*, *F. alocis*

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