Microbial composition and non-surgical periodontal treatment of aggressive periodontitis: Two case reports

Agresif periodontitisin mikrobiyal içeriği ve cerrahi olmayan periodontal tedavisi: İki olgu sunumu

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SUMMARY

The objective of this case report was to characterize the subgingival microbiological profiles of two patients with generalized aggressive periodontitis (GAgP) and to evaluate the clinical outcomes of non-surgical periodontal treatment (NSPT) over a 6-months period. Pooled subgingival samples of two patients who referred to our clinic and diagnosed with GAgP were collected and analyzed for the presence of 300 species/phlotypes using Human Oral Microbe Identification Microarray analysis. NSPT was performed within 3-week period. Clinical parameters were measured at baseline, 3 and 6 months after NSPT. Recall visits were performed every 2 weeks during the first 3 months and every 4 weeks up to 6 months. All samples harboured a total of 61 species and 32 species were common in both patients. First patient had 17 and the other had 12 distinct species. High levels of Filifactor alocis, Porpyromonas gingivalis, Campylobacter concisus and rectus, Fusobacterium nucleatum and Desulfobulbus spp. were detected in both patients while Aggregatibacter actinomycetemcomitans was found in none of them. Six months after NSPT, all clinical parameters were improved in two A. actinomycetemcomitans-negative GAgP patients. In addition to well-recognized periodontal pathogens the presence of high levels of Filifactor alocis and Desulfobulbus spp. seem to be associated with GAgP.

Keywords: Aggressive periodontitis, periodontal treatment, microbial genetics, oligonucleotide microarrays

ÖZET

Bu olgu raporunun amacı iki generalize agresif periodontitis (GAgP) hastasının subgingival mikrobiyolojik profilini belirlemek ve cerrahi olmayan periodontal tedavinin (COPT) 6 aylık klinik sonuçlarını sunmaktır. Kliniğimize başvuran iki GAgP hastasının havuzlama yöntemiyle toplanan subgingival örnekleri, 300 tür/filotipin varlığını belirlemek için Human Oral Microbiome Identificaition Microarray analizi kullanılarak incelendi. COPT 3 hafta içinde uygulandı. Klinik parametreler tedavi öncesi, sonrası 3. ve 6. aylarda kaydedildi. Hastalar, ilk 3 ayda 2 haftada bir, son 3 ayda 4 haftada bir kontrol seanslarına çağırıldı. Tüm örneklerde toplam 61 türe ve iki hastada ortak 32 türe rastlandı. İlk hastada 17, diğer hastada 12 farklı tür tespit edildi. İki hastada da yüksek seviyede Filifactor alocis, Porpyromonas gingivalis, Campylobacter concisus and rectus, Fusobacterium nucleatum and Desulfobulbus spp. saptanırken Aggregatibacter actinomycetemcomitans tespit edilmedi. COPT sonrası altıncı ayda A. actinomycetemcomitans-negatif iki GAgP hastasının tüm klinik parametrelerinde iyileşme olduğu gözlendi. Bilinen periodontal patojenlere ek olarak yüksek seviyedeki Filifactor alocis ve Desulfobulbus spp.'nin varlığının GAgP ile ilişkilendirilebileceği düşünülmüştür.

Anahtar kelimeler: Agresif periodontitis, periodontal tedavi, mikrobiyal genetik, oligonükleotid mikroarray

INTRODUCTION

Generalized aggressive periodontitis (GAgP) is characterized by severe attachment and alveolar bone loss. Subjects with GAgP are mostly under 30 years of age but they may be older.[1] Subgingival bacteria plays a crucial role in the pathogenesis of AgP. In established disease and, particularly, after the spread of periodontal inflammation, the composition of the subgingival microbiota in GAgP might become extremely complicated and generally varies remarkably among individuals. [2, 3] The development of novel, culture-independent molecular techniques and metagenomic analysis allowed the identification of as-yet-unculturable and fastidious organisms in periodontal diseases and added new insights into bacterial communities in periodontal pockets. [4, 5] The role of new and not-yet-cultivable microorganisms in GAgP has emerged in recent years. [3]

Mechanical anti-infective treatment which is composed of oral hygiene instructions, scaling, root planing and removing all local etiological factors is the gold standart in the management of periodontitis. [6, 7] Adjunctive use of systemic or local antimicrobials in non-surgical treatment have been widely used in the treatment of AgP. However, there is still no clear protocols or guidelines about dosage, duration or timing of antibiotic use during non-surgical treatment. [8-12] The irrelevant and irrational use of antimicrobials leads to the emergence, spread and persistence of resistant microorganisms, resulting in prolonged diseases and more importantly, increase risk of death.[13] Moreover, periodontal diseases are biofilm-related, polymicrobial infections showing wide diversity in microbial composition among sites and subjects with similar clinical manifestations.[14] Microbiological testing should be considered for antimicrobial susceptibility of subgingival plaques in biofilms. This evaluation could ensure supplementary information on the susceptibility of periodontal microbiota in GAgP before using antimicrobials.[15, 16] Furthermore, studies demostrated that GAgP responds well to scaling and root planing alone during 6 months. Thus this case report aimed to assess the composition of subgingival pockets and the outcomes of non-surgical periodontal treatment (NSPT) of two patients up to 6 months.

CASE REPORTS

Patient-I:

A 34 year-old male refferred to our clinic with the complaints of spacing between teeth, gingival recession and bad breath. He was systemically healthy, not using any medications and was smoking 3 cigarrettes per day. Familial aggregation of GAgP was denied. Intraoral and radiographic examinations (Fig. 1a-b) revealed teeth #25 and #27 were missing. There was a grade I mobility in teeth #31, #32 and #41. Periodontal examination showed that he had high plaque accumulation as revealed by the plaque index (PI: 2.55). The mean gingival index (GI) was 1.95 and 92% of the sites showed bleeding upon probing (BOP). Moreover, there were generalized and severe bone destruction, with the probing depth (PD) ranging from 2 to 10 mm (mean of 5.22 mm) and clinical attachment level (CAL) ranging from 3 to 11 mm (mean of 7.58 mm) (Table 1). The clinical diagnosis was GAgP.

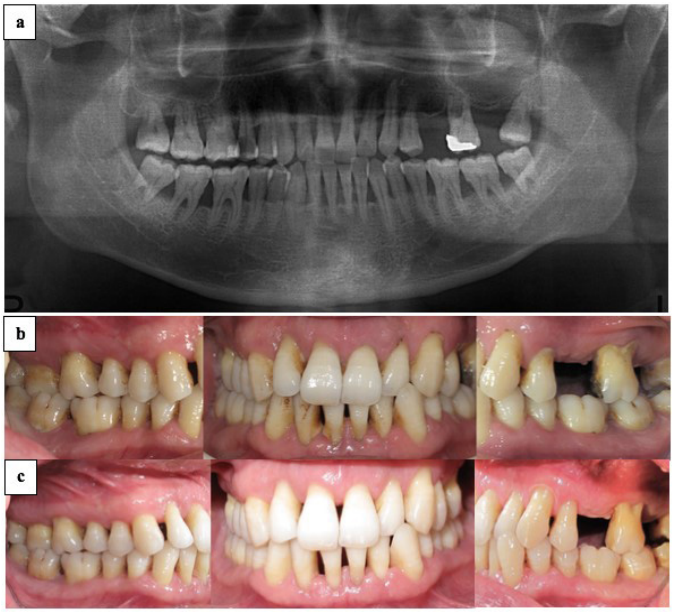

Figure 1. a) Baseline orthopantomography of patient-I. b) Baseline clinical appearance of patient-I. c) Clinical appearance of patient-I after 6 months.

Table 1. Periodontal parameters at baseline and at 3 and 6 months after NSPT.

Periodontal Parameters	Patient-I			Patient-II		
	Baseline	3 months	6 months	Baseline	3 months	6 months
PI	2.55	0.83	0.92	2.39	0.54	0.66
GI	1.96	0.88	1.51	1.98	0.82	1.27
BOP (%)	91.67	47.44	65.38	82.74	51.19	73.81
PD (mm)	5.22	3.93	4.54	5.07	3.27	3.14
PD reduction (mm)		(Δ 0-3) 1.29	(Δ 0-6) 0.68		(Δ 0-3) 1.80	(Δ 0-6) 1.93
CAL (mm)	7.58	6.01	7.29	5.36	4.82	4.92
CAL gain (mm)		(Δ 0-3) 1.57	(Δ 0-6) 0.29		(Δ 0-3) 0.54	(Δ 0-6) 0.44

Patient-II:

A 30 year-old female was refferred to our clinic with a chief complaint of bleeding gums and the feeling of loose teeth. The patient was not using any medications, was systemically healthy and had never smoked. Familial aggregation of GAgP was denied. Grade II mobility was observed in tooth #21. The level of plaque was high with PI 2.39. GI was 1.98 and BOP was detected in 83% of the sites. Periodontal and radiographical examination (Fig. 2a-b) revealed generalized and severe bone destruction with the degree II furcation involment in #16, #26, #36 and #46, with the PD ranging from 2 to 10 mm (mean of 5.07 mm) and CAL ranging from 1 to 10 mm (mean of 5.36 mm) (Table 1). The clinical diagnosis was GAgP.

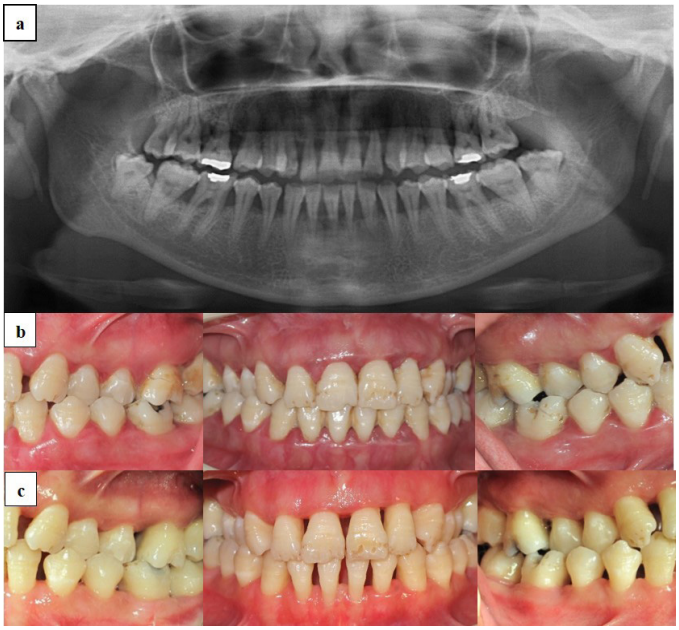

Figure 2. a) Baseline orthopantomography of patient-II. b) Baseline clinical appearance of patient-II. c) Clinical appearance of patient-II after 6 months.

Microbiological sampling

The pooled paper-point subgingival samples were collected at baseline from 8 sites with PD≥5mm (4 multi-rooted and 4 single-rooted teeth) in each quadrants per subject after removal of supragingival biofilm with sterile cotton pallets. Sampling was performed as previously described. [17] A sterile paper point (No. 30, MetaAbsorbent Paperpoints; MetaBiomed Co., Ltd., Chungbuk, Korea) was inserted into each subgingival site for 10 s. The paper points were then pooled into a single empty sterile eppendorf tube and immediately placed at -80˚C prior to PCR amplification.

HOMIM microarray analysis

Purified DNA samples were subjected to Human Oral Microbiome Identification Microarray (HOMIM) analysis, which was performed as described earlier[18]. Briefly, single-stranded PCR products with fluorescent label were captured by highly specific bacterial 16S rRNA probes for around 300 bacterial species printed on a customized aldehyde-coated glass slide. After a thorough validation of all probes, probes with cross-reactions and/or missing reactions were excluded from statistical analysis. HOMIM online tool was used for further analysis and generation of microbial profiles from scanned microarrays (http://bioinformatics.forsyth.org/homim/). Data were normalized by comparing individual signal intensities to the average of signals from universal probes. Positive hybridization signals were categorized into 5 levels, with 1 indicating a signal that was just detectable, to 5 indicating maximum signal intensity.

Microbiological results

Samples from two patients harboured a total of 61 species. Among them 32 species were common in both patients (Fig. 3).

Microorganism/Sample	Patient-1	Patient-2	Total
Buffer	0	0	0%
Negative Control	0	0	0%
Positive Control_16S_Universal_E29	5	5	100%
Bacteroides heparinolyticus_ot784_X18	0	2	50%
Bacteroidetes[G-2] sp clone AU126_ot274_K76	1	0	50%
Bacteroidetes[G-2] sp clone AU126_ot274_X57	2	2	100%
Bacteroidetes[G-1] sp clone _X083_ot272_AA81	0	1	50%
Bacteroidetes[G-1] sp clone _X083_ot272_X17	0	1	50%
Porphyromonas endodontalis and sp clones _F016 and P4GB_100_ot273_285_395_W78	2	2	100%
Porphyromonas gingivalis_ot619_X21	3	4	100%
Porphyromonas gingivalis_ot619_AA93	2	3	100%
Prevotella intermedia_ot643_AB92	1	1	100%
Prevotella intermedia_ot643_AD06	1	1	100%
Prevotella Cluster IV_ot658_693_714_782_AA44	0	1	50%
Tannerella forsythensis_ot613_X56	2	3	100%
Haemophilus parainfluenzae_ot718_W79	1	0	50%
Desulfobulbus sp clone _R004_ot041_K70	2	4	100%
Campylobacter gracilis_ot623_Q04	2	0	50%
Campylobacter gracilis_ot623_X34	2	2	100%
Campylobacter concisus and rectus_ot575_748_T86	3	2	100%
Campylobacter concisus and rectus_ot575_748_X36	4	3	100%
Campylobacter Cluster I_ot580_748_763_T87	2	1	100%
Campylobacter Cluster II_ot580_748_763_X37	2	0	50%
Acidaminococcaceae[G-1] sp clones DM071 and EZ011_ot135_148_W86	1	0	50%
Dialister invisus_ot118_P73	1	0	50%
Dialister pneumosintes_ot736_X78	0	2	50%
Selenomonas dianae_ot139_Q50	0	2	50%
Selenomonas infelix and sp clones EV047 and GT010 and IK004_ot126_479_481_639_O54	2	2	100%
Selenomonas sputigena and sp clone EW051a _ot143_151_AB04	1	1	100%
Selenomonas sputigena and sp clone EW051a_ot143_151_K65	1	0	50%
Selenomonas sp clones DS071 and EW084_ot138_146_Q52	0	2	50%
Parvimonas micra_ot111_L97	2	2	100%
Parvimonas micra_ot111_V05	2	2	100%
Catonella morbi and sp clone BR063_ot164_165_O56	0	1	50%
Lachnospiraceae[G-5] sp clone BB124_ot080_AA65	2	0	50%
Eubacterium[11][G-1] infirmum_ot105_Y45	1	0	50%
Eubacterium[11][G-3] brachy_ot557_AC03	1	0	50%
Eubacterium[11][G-5] saphenum_ot759_AA71	1	1	100%
Eubacterium[11][G-6] nodatum_ot694_Y46	1	2	100%
Filifactor alocis_ot539_AA69	0	3	50%
Filifactor alocis_ot539_AB94	4	5	100%
Filifactor alocis_ot539_AB95	4	5	100%
Peptostreptococcaceae[11][G-4] sp clone MCE10_174 and sp strain PUS9.170_ot103_369_AB49	2	2	100%
Pseudoramibacter alactolyticus_ot538_AB70	0	1	50%
Gemella morbillorum_ot046_K64	1	2	100%
Gemella sanguinis_ot757_AB17	2	2	100%
Streptococcus anginosus_ot543_AB84	0	1	50%
Streptococcus anginosus and gordonii_ot543_622_F49	0	1	50%
Streptococcus anginosus and intermedius_ot543_644_AB82	0	2	50%
Streptococcus cristatus and sp clone BM035_ot058_578_AA47	1	0	50%
Streptococcus anginosus and intermedius_ot543_644_Q62	1	3	100%
Streptococcus constellatus and intermedius_ot576_644_AB77	0	2	50%
Streptococcus constellatus and intermedius_ot576_644_F48	0	3	50%
Streptococcus oralis and sp clones CSMLM037 and EK048_ot064_707_F46	2	1	100%
Streptococcus Cluster III_ot755_758_767_768_Q65	2	2	100%
Fusobacterium nucleatum ss nucleatum and animalis_ot420_698_AE01	3	3	100%
Leptotrichia buccalis and goodfellowii and Sneathia sanguinegens_ot563_837_845_AA45	2	1	100%
Synergistetes[G-3] sp clone BH017_ot360_AD66	1	1	100%
Synergistetes[G-3] Cluster I_ot363_453_452_D70	2	2	100%
Synergistetes[G-3] sp clones BH017 and D084 and JV006_ot360_362_453_AC56	1	1	100%
Treponema maltophilum_ot664_Q01	0	2	50%
Treponema socranskii_ot769_AC38	0	2	50%
Treponema socranskii_ot769_AA63	2	2	100%
Treponema sp clones AT039 and AU076_ot237_242_F89	1	0	50%

Figure 3. Signal intensities ranging from 0 to 5 obtained by HOMIM for detected bacteria.

Patient-I had 12 and patient-II had 17 distinct species. High levels of *F. alocis, Campylobacter concisus and rectus, Porphyromonas gingivalis (P. gingivalis), Fusobacterium nucleatum and animalis (F. nucleatum and animalis) Tannerella forsythensis and Desulfobulbus spp. clones* were detected in high levels whereas *A. actinomycetemcomitans* was found in none of patients. In both patients, *Prevotella intermedia, Porphyromonas endodontalis, Campylobacter gracilis, Parvimonas micra, Selenomonas infelix/ Selenomonas spp.* and *Selenomonas sputigena/ Selenomonas spp, Gamella morbillorum, Gamella sanguinis, Streptococcus anginosus/ Streptococcus intermedius, Streptococcus oralis/ Streptococcus spp., Streptococcus Cluster III, Eubacterium saphenum, Eubacterium nodatum, Peprostreptococcaceae, Treponema socranskii, Leptotrichia buccalis/ Leptotrichia goodfellowii/ Sneathia sanguinegens* and *Synergistetes Cluster I were in low levels.*

Haemophilus parainfluenzae, Acidaminococcaceae spp., Dialister invisus, Lachnospiraceae spp., Eubacterium infirmum, Eubacterium brachy and *Streptococcus cristatus/ Streptococcus spp.* were detected in only patient-I, while *Bacteroides heparinolyticus, Dialister pneumosintes,*

Selenomonas dianae, Catonella morbi/ Catonella spp., Pseudoramibacter alactolyticus, Streptococcus anginosus, Streptococcus anginosus/ Streptococcus gordonii, Streptococcus anginosus/ Streptococcus intermedius, Streptococcus consellatus/ Streptococcus intermedius and *Treponema maltophilum* were detected in only patient-II.

Non-surgical periodontal treatment

After periodontal examination, NSPT consisted of oral hygiene instructions, scaling and root planing with Gracey curettes (Hu-FriedyTM) and ultrasonic devices (Cavitron Bobcat Pro™) (Ultracain® D-S) in a quadrant-wise manner within a 3-week period, and occlusal adjustment were performed. Oral hygiene instructions and supragingival debridement were repeated every 2 weeks during the first 3 months and every 4 weeks during the last 3 months. The clinical measurements including PI, GI, BOP, PD, CAL were also recorded at 3 and 6 months after NSPT.

CLINICAL RESULTS

At 3 and 6 months all the clinical parameters improved compared to baseline in both patients (Table 1). In the patient-I PI reduced from 2.55 to 0.92 at 6 months, GI from 1.96 to to 1.51, BOP from 91.67% to 65.38%. PD reduction was 1.29 mm at 3 months and 0.68 mm at 6 months, CAL gain was 1.57 mm at 3 months and 0.29 mm at 6 months (Fig. 1-c).

In the patient-II PI decreased from 2.39 to 0.66 at 6 months, GI from 1.98 to 1.27, BOP from 82.74% to 73.81%. PD reduction was 1.80 mm at 3 months and 1.93 mm at 6 months while CAL gain was 0.54 mm at 3 months and 0.44 mm at 6 months (Fig. 2-c).

DISCUSSION

In this report we analysed microbiological profiles of GAgP patients at baseline and clinical outcomes of NSPT at 3 and 6 months after treatment. The results of the present study showed that patients harboured high levels of F. alocis and Desulfobulbus spp. in addition to well-recognized red and orange complex periodontal pathogens (*P. gingivalis, T. forsythia, F. nucleatum* and *C. rectus)* whilst none of the patients had *A. actinomycetemcomitans.* Our microbiological results in regard to red and orange complex bacteria resemble with the findings of previous studies in GAgP. [2, 10, 19, 20] Wide heterogeneity exists in the microbiome of GAgP patients. In spite of this discrepancy, microorganisms of the red complex and those of the orange complex are considered as potential pathogens in many studies on GAgP, while A. actinomycetemcomitans does not appear to have such a role in this form of periodontitis. [3, 21] One of the organisms formerly unrecognized is F. alocis which is asaccharolytic, obligate anaerobic rod. In recent years, *F. alocis* has been explored in patients with chronic periodontitis, GAgP and endodontic infections [4]. Presence of *F. alocis* was observed at significantly higher levels in patients with generalized chronic periodontitis and GAgP compared to periodontally healthy ones. [22] Its colonization features and its potential virulence attributes support the suggestion that *F. alocis* should be included as a diagnostic indicator of periodontal disease. [4, 23, 24] In recent years, *Desulfobulbus spp.* were identified in aggressive periodontitis. [22, 25] In line with these studies, high levels of *F. alocis* and *Desulfobulbus spp.* were detected at both patients that had severe periodontal destruction.

Systemic antibiotics are required in *AgP* since the pathogenic bacteria like A. actinomycetemcomitans and P. gingivalis have been discovered to be invasive and mechanical therapy is inadequate to eliminate these microorganisms from periodontal tissue. [26] Several antibiotic regimens have been invastigated as adjuncts to mechanical therapy of GAgP. [10] The criteria for choosing of antibiotics are not clear in GAgP; the choice depends on the case, disease and patient-related factors like patient complience, allergies, and potential side effects and drug interactions with other medications taken. [10, 11, 27] Furthermore, due to concerns about drug resistance and need of antibiotics for more important systemic diseases, adjunctive systemic antimicrobials combined to NSPT has been discussing. [28] In case-I and -II, susbtantial and stable clinical periodontal status were obtained after conventional NSPT and with regular and strict recall appointments. At 6 months after NSPT, clinical measurements indicated distinct improvements with 0.68 and 1.93 mm reduction of PD, 0.29 and 0.44 mm CAL gain, respectively. Studies reported the clinical parameter changes after NSPT alone in GAgP showed PD reduction ranged from 0.60 mm to 2.50 mm for 6 month follow-up [10, 20, 29-32] and CAL gain ranged from 0.20 mm to 0.97 mm for 6 to 12 months follow-up [10, 20, 29, 31-33] which are similar with our findings. In addition, full mouth BOP was decreased at both patients. Oral hygiene levels were acceptable. These clinical outcomes were in accordance with the studies that conventional NSPT was performed alone in GAgP. [19, 34, 35]

NSPT of *A. actinomycetemcomitans-negative* GAgP patients without using any antimicrobials provided good clinical results after 6 months. Patients were prepared for periodontal surgery with optimum oral hygiene levels, decrease of gingival inflammation and reduction of pocket depths. Furthermore, in addition to well-known periodontal pathogens the presence of *F. alocis* and *Desulfobulbus spp.* in high levels were in accordance with the previous studies in the literature, suggesting these microorganisms may be associated with GAgP.

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