ORIGINAL REPORT

Bacterial Analysis of Peri-implantitis and Chronic Periodontitis in Iranian Subjects

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Abstract- Chronic periodontitis (CP) and peri-implantitis (PI) are multifactorial diseases of tooth and implant supporting apparatus. Bacterial invasion and consequent host immune response seem to play a role in relevant pathogenesis. The structural differences between tooth and implant pose preferential biofilm colonization. This study was aimed to compare the prevalence of bacteria in CP and PI. Clinical and radiographic examination performed over 69 individuals referred to Shahid Beheshti Dental School (Tehran, Iran) and four groups categorized: CP (n=22), HP (n=21), PI (n=13) and HI (n=13). The mean age was 45.6 years, 55% of participants were female and 45% were male. Bacterial samples were collected by paper point method and transferred to Institute of Odontology, University of Gothenburg (Gothenburg, Sweden) for checkerboard DNA-DNA hybridization. Kruskal-Wallis and Mann-Whitney U tests were used to compare distribution of bacteria in four groups. Significant differences were observed for *T. forsythia*, *P. intermedia, C. rectus, P. endodontic, P. gingivalis, T. denticola and P. tannerae* ($P<0.05$). The most prevalent bacteria in CP and PI were *T. forsythia* and *P. gingivalis,* respectively. In conclusion, bacterial prevalence differs significantly between tooth and implant. The most prevalent bacteria in Iranian subpopulation do not necessarily bear a resemblance to other populations. The type of implant surface may influence the biofilm. Other studies should be conducted to corroborate these findings.

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Keywords: Oral biofilm, Iran; Peri-implantitis; Chronic periodontitis; Bone, Inflammation; DNA hybridization

Introduction

Dental implants have become today an important treatment option for oral rehabilitation in patients with lost teeth. It would be expected that peri-implant diseases and implant complications may increase by the routine use of dental implants. Consequently, understanding of the etiology, mechanisms, classification, and treatment protocol of peri-implant diseases is necessary for clinicians involved in implant dentistry (1).

According to seventh European workshop on periodontology, at the commencement of peri-implant disease, peri-implant mucositis appears with bleeding on gentle probing (BOP) due to inflammation resides in the mucosa as a response to the bacterial challenge. Peri-implant mucositis may subsequently progress into peri-implantitis (PI) if not treated. Presence of bacteria, bleeding on probing (BOP), deepening of peri-implant pockets, presence of pus and ultimately bone loss are characteristics of PI (2).

Chronic periodontitis (CP) is a progressive disease of dental supporting tissues, which is of infectious nature and characterized similarly by BOP, pathological pocketing, clinical attachment loss (CAL) and bone resorption (3). Both periodontitis and peri-implant may lead to loss of teeth and implants if not treated.

Despite the apparent similarities there are distinct clinical, radiographical and histological differences between the peri-implant lesions compared to periodontitis (2,4), which are explained by the structural dissimilarity (root cementum vs. titanium) that poses a fragile epithelial attachment in the soft tissue interface to the implants (4). Lack of oral hygiene leads to biofilm

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formation on both root and implant surfaces; however it is not fully clear if the same types of bacteria are participating. The plaque formed on the tooth and implant surfaces contains hundreds of different bacterial species and many of them may participate in the progression of the lesions (5). Previously, bacterial analysis has used culture technique and great similarities were found microbiologically between the periodontitis and peri-implant lesions (6,7). Later by using molecular biology methods such as polymerase chain reaction (PCR) (8,9) or quantitative assays such as real time PCR (10-12) more detailed information was reached on the composition of the implant biofilm. Although, the periodontitis associated bacterial species were frequently found also in peri-implantitis cases, the microbial results obtained for the peri-implant lesion varies significantly between studies (13-15). The differences are due to between population differences, variation in methods used, or differences between implant construction and surfaces texture (rough or turned surface). Few studies have in fact compared the microbiota in samples from periodontitis and peri-implant lesions and we do not know the impact of these factors in the establishment and progression of the peri-implant lesions.

The present study was aimed to compare the prevalence and amounts of 10 periodontitis associated bacterial species in periodontitis and peri-implantitis cases in an Iranian population with the use of checkerboard DNA-DNA hybridization technique as described by Dahlén and Leonhardt in 2006 (16).

Materials and Methods

Study subjects

Ethical committee of Shahid Beheshti University of Medical Science approved this double masked, cross-sectional study. Among patients referred to the Periodontology department of Shahid Beheshti Dental School for specialist treatment, 363 persons were clinically screened and enrolled for further investigations. Using our strict inclusion/exclusion criteria resulted in elimination of 294 persons from the study. The exclusion criteria were as follows: Presence of oral diseases except periodontitis, concurrent orthodontic treatment, systemic diseases, local diseases leading to an alteration in immune system, diabetes mellitus, hepatitis, HIV positive, chemotherapy, pregnancy, lactation, all implants with different surfaces except SLA surface, implants used for over denture, implants inserted with additional treatments (e.g. GBR, Sinus floor elevation, etc.) or by inexpert practitioner and chronic usage of anti-inflammatory/ antibiotic drugs.

Finally, a total of 69 periodontally untreated individuals (38 females and 31 males, mean age of 45.6) were selected for this study. They also fulfilled the inclusion criteria of having at least 5 teeth (including implants and excluding 3rd molars) in each quadrant. They were categorized into 4 groups: i) Chronic periodontitis (CP, n=22), ii) Non-periodontitis (HP, n=21) iii), Peri-implantitis (PI, n=13), iv). Non-peri-implantitis (HI, n=13) according to the criteria below for each group. A written informed consent was obtained from each participant.

Parallel-shot x-ray radiographs were taken and clinical examinations (by a periodontal specialist) were performed for all individuals in order to evaluate the periodontal and peri-implant status. Bleeding on probing (BOP), probing pocket depth (PPD) and loss of attachment (from the cemento-enamel junction, CAL) were evaluated in 4 sites per tooth (mesiobuccal, distobuccal, midbuccal and midlingual/palatal) using standard Williams probes (Hu-Freidy, Chicago, IL, USA). CP was defined as presence of BOP, and presence of PPD>4mm and CAL≥3mm in at least 3 teeth in two quadrants. HP was categorized by absence of signs of periodontitis meaning no sites with PPD >4mm and no loss of attachment. The PI cases did not lose their teeth due to periodontitis cases and had implants with SLA surface only. They showed BOP around the implants with or without suppuration, had at least one implant site with PPD>5mm including radiographically crestal bone loss with a minimum of 2 exposed treads (bone loss was measured from implant shoulder to the bottom of resorption) and the implant was in function in more than 12 months. HI cases had no evidence of bone resorption and the PPD was less than 4 mm.

Bacterial sampling and analysis

The paper point method was used for sampling from deepest gingival/periodontal pocket of each tooth or implant. A sterilized medium size paper point (size 40, T.g., UK) was inserted to the bottom of deepest pocket site and kept in place for 15-20 seconds. The soaked paper points were transferred into Eppendorf tubes and sent to the laboratory of Oral Microbiology, Institute of Odontology, Sahlgrenska Academy at University of Gothenburg, Sweden for evaluating the prevalence and amounts of the following bacterial species:

*Porphyromonas gingivalis (*P. g), *Prevotella nigrescens* (P. nig), *Prevotella intermedia* (P. int), *, Treponema denticola* (T. den), *Prevotella tannerae (*P. tan), *Aggregatibacter actinomycetemcomitans* (A. a), *Tannerella forsythia* (T. for), *Porphyromonas endodontalis* (P. end), *Fusobacterium nucleatum* (F. nuc) and *Campylobacter rectus* (C. rec) according to the protocol described elsewhere (16).

Briefly whole genomic DNA probes were prepared using a digoxygenin labeling kit (Roche, Mannheim, Germany). The plaque samples were transferred to 100 µl TE buffer (10 mM Tris HCl, 1mM EDTA, pH 7.6) and denaturated with 100 µl of 0.5 M NaOH. The samples were frozen and thawed 5 times and finally the bacterial cells were mechanically disrupted by vortexing with glass beads for 30 sec. Subsequently the suspensions were boiled for 5 min, neutralized with 800 µl of 5M ammonium acetate, transferred onto nylon membranes (Minislot device, Immunetics, Cambridge, MA, USA) and fixed by UV-light. After 2h of prehybridisation at 42ºC, the DNA probes were allowed to hybridize over night in lanes vertically to the plaque samples using a Miniblotter device (Immunetics) at 42ºC. After a series of stringency washes at 70ºC, hybrids formed between the bacterial DNA and the probes. Hybridizations were detected using phosphatase-conjugated anti-digoxygenin antibodies and the signals were visualized with a chemiluminescent substrate (CDP-Star, Roche, Mannheim, Germany). Evaluation of the number of bacteria in the samples was performed by comparing the obtained signals with the ones generated by pooled standard samples containing 10^6 (high standard) and 10^5 (low standard) cells of each bacterial species. The results were transferred to a scoring system (0-5), where 0 indicated no signals, score 1 visible signals corresponding to $<10^5$, score 2=10^5, score 3>10^5 to $<10^6$, score 4 = 10^6 and score 5 corresponding >10^6 (16).

Data analysis

Data were analyzed using SPSS statistical package version 15. Kruskal–Wallis and Mann-Whitney U tests were used to evaluate and compare the prevalence of bacteria in 4 groups. *P*-value less than 0.05 was considered statistically significant.

Results

Clinical recordings

The clinical findings are summarized in Table 1. The age of participants was ranged between 35-65 years (mean age 45.6). The PI group was slightly but not significantly older (58.3 years) than the CP group (48.3 years). Thirty-eight subjects were female (55%) and 31 (45%) were males. The gender distribution within each group was fairly equal. The mean PPD and attachment/bone loss of the sampling sites of the CP and PI cases was distinctly different from the sites of the HP and HI cases, while the PPD of CP and PI sites show not significant difference (5.95 ± 0.75 and 6.81 ± 0.35 respectively). The attachments loss in the two groups showed also similar values (Table 1).

Bacterial analysis

Kruskal-Wallis test showed a statistical difference (P<0.05) between prevalence of *P. intermedia, P. gingivalis, T. forsythia, C. rectus, P.tannerae, T.denticula* and *P. endonticula* species in all 4 groups. All species demonstrated a higher incidence in the periodontitis sites than in the healthy sites. Nonetheless, only 37.5% of species showed a higher prevalence in the peri-implantitis site than healthy implant sites. A significant difference (P<0.05) between periodontitis and healthy sites was recorded for 70% of species (except *P. nigrescens*, *A. a*. and *F. nucleatum*); however, an insignificant difference between peri-implantitis and healthy implant sites was obtained for all species.

Table 1. Clinical findings within 4 groups.

Objectives / Groups	Age	Sex		Probing pocket Depth (Mean ± SD)	Attachment / bone loss*
		Female	Male		
Chronic Periodontitis (n: 22)	48.3 (20 to 65)	40% (n=9)	60% (n=13)	5.95 ± 0.75	5.34 ± 0.85
Healthy periodontium (n: 21)	33.4 (18 to 60)	62% (n=13)	48% (n=8)	1.83 ± 0.68	0.17 ± 0.11
Peri-implantitis (n: 13)	58.3 (23 to 67)	46% (n=6)	54% (n=7)	6.81 ± 0.52	4.44 ± 1.89
Healthy implant (n: 13)	42.5 (21 to 58)	77% (n=10)	33% (n=3)	3.12 ± 0.35	0.87 ± 0.66

*: Bone loss is related to implants and attachment loss to teeth.

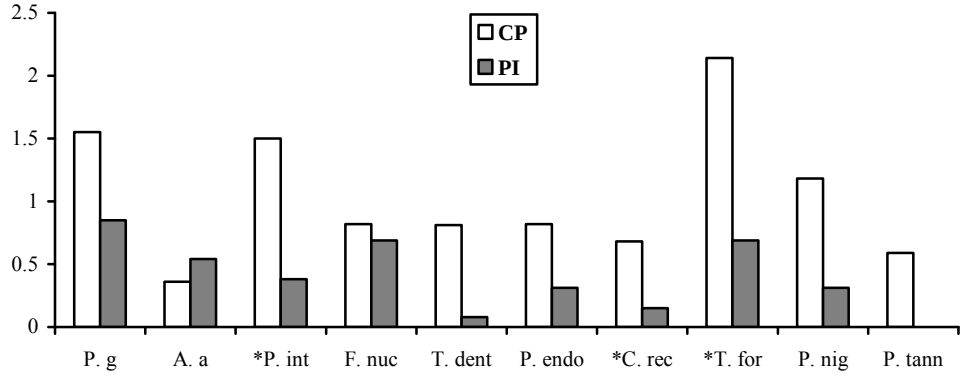

Figure 1. Mean scores of 10 bacteria in individuals with chronic periodontitis (CP) and peri-implantitis (PI).
*: Significant difference; P. int: *Prevotella intermedia,* P. nig: *Prevotella nigrescens,* T. for: *Tannerella forsythia,* A. a: *Aggregatibacter actinomycetemcomitans*, C. rec: *Campylobacter rectus,* P. end: *Porphyromonas endodontalis,* F. nuc: *Fusobacterium nucleatum*, P. g: *Porphyromonas gingivalis,* T. den*: Tannerrella denticola,* P. tan*: prevotella tannerae*

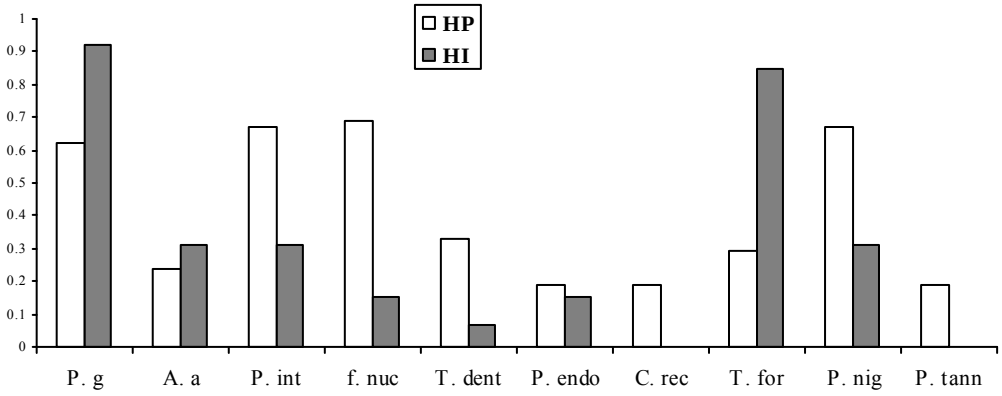

Figure 2. Mean scores of 10 bacteria in individuals with healthy periodontium (HP) and healthy implant (HI).
P. int: *Prevotella intermedia,* P. nig: *Prevotella nigrescens,* T. for: *Tannerella forsythia,* A. a: *Aggregatibacter actinomycetemcomitans*, C. rec: *Campylobacter rectus,* P. end: *Porphyromonas endodontalis,* F. nuc: *Fusobacterium nucleatum*, P. g: *Porphyromonas gingivalis,* T. den*: Tannerrella denticola,* P. tan*: prevotella tannerae*

Table 2. Prevalence (%) of various bacterial species in 4 clinical groups.

Microorganisms	Chronic Periodontitis	Peri-implantitis	Healthy Implant	Healthy Periodontuim
Prevotella intermedia	90.9	30.8	30.8	52.4
Prevotella nigrescens	54.5	23.1	15.4	33.3
Tannerella forsythia	90.9	61.5	46.1	23.8
Aggregatibacter actinomycetemcomitans	27.3	23.1	23.1	23.8
Campylobacter rectus	59.1	15.4	00	19
Porphyromonas endodontalis	54.5	15.4	7.7	19
Fusobacterium nucleatum	72.7	38.5	61.5	76.2
Porphyromonas gingivalis	90.9	53.8	30.8	52.4
Tannerrella denticola	68.2	8.3	7.7	33.3
Prevotella tannerae	40.9	00	00	19

Table 3. Prevalence of bacteria (percentage) based on combined scoring.

	Groups	Score 0	Score 1,2	Score 3,4,5
Prevotella intermedia	CP	9.1	72.7	18.2
	PI	69.2	30.8	0
	HP	47.6	47.7	4.8
	HI	69.2	30.8	0
Prevotella nigrescens	CP	45.5	31.8	22.7
	PI	76.9	23.1	0
	HP	66.7	23.8	9.6
	HI	84.6	7.7	7.7
Tannerella forsythia	CP	9.1	45.5	45.4
	PI	38.5	61.5	0
	HP	76.2	23.8	0
	HI	53.8	46.2	0
Aggregatibacter actinomycetem comitans	CP	72.8	22.7	4.5
	PI	76.9	15.4	7.7
	HP	76.2	23.8	0
	HI	76.9	23.1	0
Campylobacter rectus	CP	40.9	54.6	4.5
	PI	84.6	15.4	0
	HP	81	19	0
	HI	100	0	0
Porphyromonas endodontalis	CP	45.5	50	4.5
	PI	84.6	7.7	7.7
	HP	81	19	0
	HI	92.3	7.7	0
Fusobacterium nucleatum	CP	27.3	72.7	0
	PI	61.5	30.8	7.7
	HP	23.8	76.2	0
	HI	38.5	53.9	7.7
Porphyromonas gingivalis	CP	9.1	72.8	18.1
	PI	46.2	46.2	7.7
	HP	47.6	47.6	4.8
	HI	69.2	7.7	23.1
Tannerrella denticola	CP	31.8	63.7	4.5
	PI	91.7	8.3	0
	HP	66.7	33.3	0
	HI	92.3	7.7	4.5
Prevotella tannerae	CP	59.1	36.4	4.5
	PI	100	0	0
	HP	81	19	0
	HI	100	0	0

CP: chronic periodontitis, HP: healthy periodontium, PI: peri-implantitis, HI: healthy implant

The biofilm accompanying PI and CP are compared in figure 1 based on mean scores. Mann-Whitney test revealed that a significant difference (P<0.05) was obtained for *T. forsythia, P. intermedia* and *C. rectus*.

Figure 2 illustrates the mean scores of bacteria involved in HP and HI. Mann-Whitney test revealed no significant difference between two groups, whereas the prevalence of bacteria did not bear an exact resemblance.

The prevalence of oral biofilm in all groups is compared in Table 2. The abundance of bacteria in healthy implants is of interest. The most periopathogen in CP sites was *T. forsythia*, while the most bacteria in PI sites was *P. gingivalis.*

Table 3 demonstrates the level of bacteria based on combined mean-scores and is of benefit for detecting high/low prevalence of pathogens.

Discussion

It is generally acknowledged that chronic periodontitis and peri-implantitis have a similar nature i.e. bacterial involvement leads to host inflammatory response that in turn may progress into periodontitis and peri-implantitis. Bone resorption and loss of teeth and implants failure if left untreated may be the final stage of CP and PI. A similar bacterial profile of the pathological pocketing in chronic periodontitis and peri-implantitis has thus been suggested. This study compared the prevalence and amounts of 10 periodontitis associated bacterial species in samples from diseased sites of CP and PI Iranian cases with the same methodology and with non-diseased cases as controls. The prevalence and amounts were significantly higher for several species in the diseased sites compared to the healthy controls, however, the CP cases showed significantly higher prevalence and scores than the PI cases for several species e.g. the three red complex bacteria, *P. gingivalis, T. forsythia, T. denticola* as well as *P. intermedia*.

The bacterial profile of deep periodontal pockets of CP cases and in pockets around the implants with significant bone resorption (peri-implatitis) has been evaluated in numerous studies but there is no clear picture obtained on how similar these cases are. Since 1999 a unique definition and classification for CP has been used in literature (17). Nonetheless, there is some controversy among researchers about precise classification of peri-implantitis. The broad range of PI from 8.6% to 56% may results from this disagreement (18-25). The Misch's classification has been convinced some investigators but not all (26). Recently a scoring index for peri-implant status has been presented to help feeling this blank (27).

Few studies have compared the bacterial profile of CP and PI cases that are selected so that they show a similar PPD and bone resorption/attachment loss however there still might be methodological short-comings that can explain the obtained difference. The

dissimilarity of CP and PI are due to many factors such as variation between clinics using different criteria for peri-implantitis, different implant type, different sampling techniques, and different methods for bacterial analysis as well as difference between populations. Although the paper point technique is well established for sampling from deep untreated pockets, there are problems to take the sample in peri-implantitis lesions due to the present supra-construction and presence of exposed threads that both interfere with the paper point to reach the bottom of the lesion to the same extent as in the deep periodontal pocket. This was the main explanation for the apparently many "negative" samples in the study of Charalampakis *et al.* (28).

Another explanation for the obtained differences could be the surface structure of the implant. Amoroso and colleagues concluded that the attachment of *P. gingivalis* to titanium is associated with surface roughness of implant (29). Charalampakis *et al.* also found that the type of implant surface has a strong relation with disease development based on time of functional loading (25). In this study we selected only cases with SLA surfaces and the retention of bacteria and the biofilm would be more similar to the root surface than implants with a deliberate roughness. The bacterial profile in the early plaque formation on titanium and hydroxylapatite shows a striking similarity (30), however there is no information whether the root surface differs from hydroxylapatite surface in this context. It is suggested that the surface structure between the root surface and titanium implants is of minor importance and cannot explain the obtained bacterial differences between the CP and PI lesions. The overall similar profile obtained in samples from non-diseased teeth and implants supports this conclusion.

The overall bacterial profile shows a similar profile that has been obtained with the same sampling technique and with the checkerboard methodology for bacterial evaluation in other populations (31-36). Thus it seems that the red comp.lex bacteria and some other anaerobic species e.g. *P. intermedia, P.nigrescens, F. nucleatum* and *C. rectus* are highly prevalent (>50%) and in significant amounts in both deep periodontal pockets and peri-implantis lesions of the Iranian subjects. *A. actinomycetemcomitans* was low, which is also commonly found in lesion of adults in other populations. *P. tannerae* was not found in the samples from the peri-implant compared to 40.9 % of the periodontal lesions however it should be remembered that we included only 13 peri-implantitis cases in the study. This finding should not be overestimated. In conclusion, within the limitations of this study, our results revealed that both chronic periodontitis and peri-implantitis are associated with anaerobic gram-negative flora including the red complex bacteria, *P. intermedia, F. nucleatum* and *C. rectus*. Obtained significant bacterial differences between chronic periodontitis and peri-implant lesions of similar probing depth and attachment loss is suggested to be due to difficulties to take representative samples due to the surface structure of the implants and clinical presence of supra structure in the peri-implantitis cases

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