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Tracking of plasma antibodies against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* during 15 years

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***Background*:** Plasma antibody measurements of antibody levels to periodontal pathogens may be used to support diagnosis, disease activity, classification, and prognosis of periodontitis.

***Objective*:** The aim of this study was to investigate the long-term stability of plasma antibody levels against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*.

***Design*:** Plasma immunoglobulin G (IgG) antibody levels against the pathogens were analyzed annually during 15 years from 21 voluntary subjects, whose periodontal status was not known at the point of selection. The total number of plasma samples was 315. In connection of the last sampling, the clinical and radiographic periodontal status was examined. Pooled bacterial samples from periodontal pockets, as well as salivary samples were collected for *A. actinomycetemcomitans* and *P. gingivalis* detection, and antibody determinations, respectively. According to the clinical status, six subjects had periodontitis, whereas 15 did not.

***Results*:** Plasma IgG-class antibody levels to periodontal pathogens remained extremely stable during the 15-year period and no significant ($p > 0.05$) intra-individual variations were observed. Retrospectively, the average plasma IgG antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* were 1.6–2.3 ($p < 0.05$) and 1.4–1.7 ($p < 0.05$) fold higher in the subjects with periodontitis than those without, respectively, during the whole 15-year tracking. As expected, at the time of the periodontal examination the plasma and salivary IgG antibody levels were associated both with periodontitis and bacterium-positivity.

***Conclusions*:** Plasma IgG levels against *A. actinomycetemcomitans* and *P. gingivalis* are extremely stable during 15 years both in subjects with and without periodontitis.

Keywords: *Aggregatibacter actinomycetemcomitans*; *longitudinal studies*; *periodontitis*; *plasma*; *oral infections*; *Porphyromonas gingivalis*; *saliva*

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Major periodontal pathogens include *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. The association between *P. gingivalis* and periodontitis is stronger even though a carrier-state of the species may exist (1). *A. actinomycetemcomitans* has a dual role in the oral flora: it has been associated with aggressive forms of periodontitis (2), but the bacterium can also be found in the oral cavity of healthy individuals (3). The bacterial load in periodontal pockets leads to the activation of host-bacterial interactions. To establish an infection, the pathogen must overcome numerous surface barriers, such as epithelium and mucus, and also the innate immunity and adaptive immunity (4). Periodontal pathogens stimulate B cell activation mainly through classical antigen-pecific immune response. *A. actinomycetemcomitans* and

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P. gingivalis are capable of inducing high antibody response that can in fact exceed those found in many other pathological bacterial infections (5).

Plasma and saliva antibody measurements have been used to diagnose periodontitis, estimate its activity, classification and prognosis, and success of treatment (5). Several studies have reported elevated levels of plasma immunoglobulin G (IgG) against periodontal pathogens especially in patients with aggressive periodontitis (6–9). Immunoglobulin A (IgA) is the predominant immunoglobulin in saliva, and salivary IgA plays a role in the local immune defense system. In addition to the plasma-derived IgG and IgA, certain subclasses of IgG and IgA can be produced locally in the periodontal pockets (10). Antibody production, especially that of IgG and IgA, is considered to have a protective role in the pathogenesis of periodontitis (11, 12). It has also been suggested that antibodies may not necessarily need to decrease periodontal pathogen loads in order to exert a protective effect, but they may neutralize toxins and proteolytic enzymes as well as promote opsonization, and complement activation (11).

The stability of IgG-class antibody levels has been investigated in a few follow-up studies. The follow-up time in those studies has varied from two to three years (13, 14). IgG-class antibody levels showed long-term stability; no decrease has been reported after periodontal treatment (6, 14) or treatment has lowered the IgG levels only temporarily (15).

Earlier studies have mainly focused on the relationship between antibody levels and severity of periodontitis or between bacterial presence and their homologous antibodies. The aim in this study was therefore to investigate the long-term stability of plasma antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* in plasma samples analyzed annually from the same individuals during 15 years.

Material and methods

Subject sample

This report is based on the 15-year collection of blood samples from a rural population in Leppävirta, Savolax, Finland, whose selenium status was followed by annual blood sampling. The original sample included 26 men and 19 women in 1985 (16). The local public health-care center recruited subjects. Volunteers for the periodontal examination were recruited by a questionnaire, and 9 men and 12 women participated. During the 15 years, the subjects had had dental check-ups by their general practitioners and none of them had previously been treated by a periodontologist. However, they were instructed to seek periodontal treatment after the examination if necessary. The characteristics of the participants at the end of the study are shown in Table 1. All the participating subjects gave their informed consent.

Clinical and radiographic examination

The subjects were examined with respect to visible plaque and bleeding on probing (17), probing depth (periodontal pockets ≥ 5 mm), and clinical attachment loss at six sites per tooth, at all present teeth (Table 1). One dentist (JA) performed all the clinical examinations. WHO probe (LM Dental LM 550B Si, LM-instruments Oy/Ab, Pargas, Finland) was used in the examinations. Subjects were categorized into those with and without periodontitis according to the following criteria: subjects with periodontitis had at least one site with a periodontal pocket deeper than 5 mm and clinical attachment loss (see Table 1).

Periodontal status and possible infectious foci of periodontium and/or alveolar bone were detected from analog panoramic tomographs. The number of periapical lesions, *angular bony defects* (they were at least to the middle third of the root length), furcation lesions in molars, and pericoronitis in third molars were recorded.

Plasma and salivary samples

Plasma samples were collected once a year for 15 years from the 21 participants and stored at -20°C. The following samples were collected at the end of the 15-year period.

Paraffin-stimulated salivary samples were collected for five minutes. The salivary sample was divided into two aliquots, of which 2 ml was centrifuged immediately at 12,000 rpm for five minutes and frozen on dry ice. The frozen samples were brought to the research laboratory at the Institute of Dentistry, University of Helsinki, Helsinki, within 48 hours and stored at -70°C.

Bacterial samples and antibody measurements

From the 21 participants in the study, pooled bacterial samples from the 14 most inflamed periodontal pockets in each subject were collected with sterile curettes at the end of the study. The samples were transported to the laboratory in vials containing 2 ml of viability preserving medium no. III (VMGA III) medium, and cultured. *A. actinomycetemcomitans* and *P. gingivalis* were identified as previously described (18, 19). Briefly, for *A. actinomycetemcomitans*, 100-µl aliquots of undiluted and 10^{-2} dilutions of samples were inoculated on Tryptic soy bacitracin vancomycin (TSBV)-agar plates and incubated in 5% CO_2 in air at 36°C for three days. *A. actinomycetemcomitans* grew as typical adherent colonies, which were catalase-positive. For *P. gingivalis*, 100-µl aliquots of 10^{-4} and 10^{-5} were inoculated on Brucella agar plates and incubated in anaerobic jars at 37°C for 10 days. *P. gingivalis* grew as dark-pigmented colonies, which had

Table 1. Clinical characteristics in periodontitis ($n=6$) and periodontally healthy ($n=15$) subjects in the end of the study

Variable	Periodontitis *n* (%)	Periodontally healthy *n* (%)	*p*-Value[a]
Gender			
Men	1 (17)	8 (53)	ns
Women	5 (83)	7 (47)	ns
General health			
Elevated blood pressure	3 (50)	2 (13)	ns
Cardiovascular diseases	2 (33)	0	ns
Diabetes (type II)	0	1 (7)	ns
Osteoporosis	0	1 (7)	ns
Current smoking	0	3 (20)	ns
	Mean ± SD	Mean ± SD	
Age	66 ± 5	59 ± 13	0.302
Periodontal examination			
Total number of teeth	19.5 ± 4.1	15.7 ± 10.8	0.569
Visible plaque (% of sites)	13.33 ± 9.2	11.2 ± 9.6	0.558
Bleeding on probing (% of sites)	36.8 ± 9.5	24.6 ± 17.2	0.112
Number of teeth with periodontal pockets (≥5 mm)	2.0 ± 2.5	0	0.000
Periodontal pockets (% of sites)	1.8 ± 2.3	0	0.000
Clinical attachment loss (mm)	4.17 ± 4.54	0	0.000
Highest CPITN value[b]	3.8 ± 0.4	2.4 ± 0.5	0.000
Total number of angular bony defects[c]	1.5 ± 2.0	0.3 ± 0.5	0.213

[a]Mann–Whitney test or Chi-square test.
[b]CPITN = community periodontal index of treatment needs. Tracking of plasma antibodies against *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* during 15 years.
[c]Radiological analyses.
Note: ns = non-significant.

positive trypsin-like enzyme activity as detected with carbo-benzoxy-L-arginine-7-amino-4-methylcoumarin amide HCl (CAAM)-reagent.

Bacterial DNA was isolated from the VMGA III medium according to the manufacturer's instructions using Chelex 100 resin, and *A. actinomycetemcomitans* and *P. gingivalis* were detected by PCR as reported earlier (20, 21). In every series of PCR, chromosomal DNA extracted from *A. actinomycetemcomitans* (ATCC43718) and *P. gingivalis* (W50) strains served as positive controls, and water served as a negative control.

Plasma IgG and salivary IgG and IgA antibodies against *A. actinomycetemcomitans* and *P. gingivalis* were detected by multiserotype-ELISA as previously described (6). The strains used as formalin-killed whole cell antigens were ATCC29523, ATCC43781, ATCC33384, IDH781, IDH1708, and C59 representing *A. actinomycetemcomitans* serotypes a, b, c, d, e, and a non-serotypeable strain, respectively, and ATCC33277, W50, and OMGS434 representing *P. gingivalis* serotypes a, b, and c, respectively. Plasma or saliva diluted in antibody buffer (PBS containing 0.05% Tween 20 and 0.5% bovine plasma albumin) was incubated at room temperature for two hours. For the determinations, four dilutions of each sample were used in duplicate. The dilutions were as follows: plasma samples for the detection of *A. actinomycetemcomitans* IgG 1:500, 1:1,500, 1:4,500, and 1:13,500 and for the detection of *P. gingivalis* IgG 1:100, 1:400, 1:1,600, and 1:6,400, and salivary samples for the detection of either *A. actinomycetemcomitans* or *P. gingivalis* IgG and IgA were 1:3.3, 1:10, 1:30, and 1:90. The results were calculated as area under the plasma concentration time curve (AUC) from the dilution curves. The inter-assay coefficients for variation as calculated from values of the reference serum applied on each plate were 6.3 and 5.5% for *A. actinomycetemcomitans* and 5.1 and 4.7% for *P. gingivalis* IgA and IgG, respectively.

Statistical analysis

Data analysis was performed using the SPSS for Windows, version 13.0 (SPSS Inc, Chicago, IL, USA). Differences in the clinical characteristics and antibody levels between the two study groups were analyzed by the Mann–Whitney *U* test or Chi-square test. Interactions between parameters were examined using Pearson

Table 2. Antibody levels in periodontitis and periodontally healthy subjects in the end of the study

Variable	Periodontitis Mean ± SD	Periodontally healthy Mean ± SD	*p*-Value[a]
Plasma antibody levels (EU)[b]			
A. actinomycetemcomitans IgG	20.51 ± 12.76	11.06 ± 5.70	0.066
P. gingivalis IgG	20.35 ± 6.76	11.94 ± 4.26	0.008
Salivary antibody levels (EU)			
A. actinomycetemcomitans IgG	0.63 ± 0.58	0.14 ± 0.16	0.045
A. actinomycetemcomitans IgA	1.11 ± 0.88	0.59 ± 0.50	0.205
P. gingivalis IgG	0.44 ± 0.39	0.03 ± 0.02	0.001
P. gingivalis IgA	0.85 ± 0.76	0.46 ± 0.37	0.235

[a]Mann–Whitney test.
[b]EU = ELISA units.

Correlation. The statistical significance of the intra-individual differences in annual plasma IgG levels was analyzed by the Kruskall–Wallis test. *P*-values ≤ 0.05 were considered as statistically significant.

Results

Demographic and clinical data

Table 1 provides a summary of the demographic and clinical data of the subject sample. According to our classification criteria, the subjects with periodontitis at the time of the clinical examination had higher number of deep (≥ 5 mm) periodontal pockets, attachment loss levels, and community periodontal index of treatment needs (CPITN) value compared to those without periodontitis. No differences were found in the bleeding on probing and visible plaque or in the number of *angular bony defects* detected from the radiographic pictures between the two groups. There were no statistical differences in the general health and current smoking between the subjects with ($n = 6$) and without ($n = 15$) periodontitis.

Antibody levels at the end of the study

Mean plasma and salivary IgG antibody and salivary IgA antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* in the periodontitis and non-periodontitis groups at the end of the study are shown in Table 2.

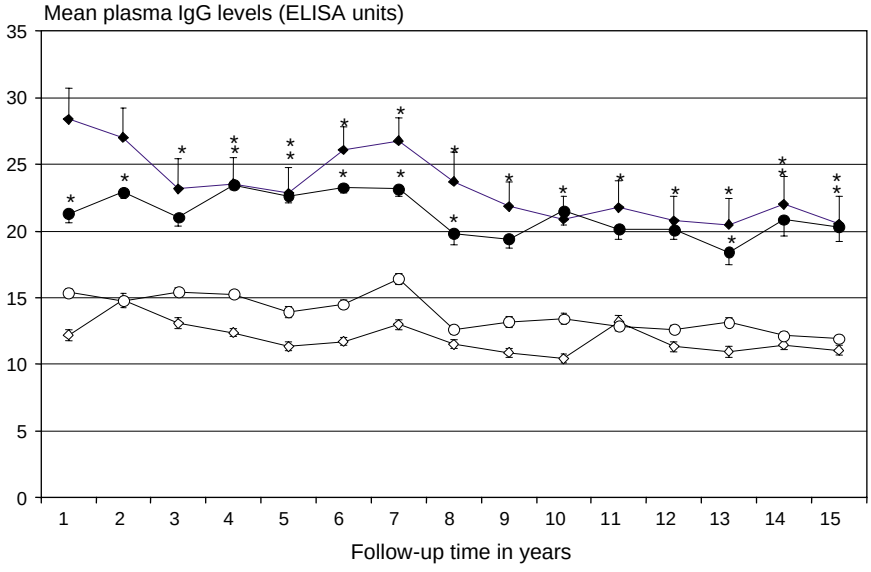

Fig. 1. Plasma immunoglobulin G (IgG) levels against *A. actinomycetemcomitans* and *P. gingivalis* levels during 15 years. The classification into those with and without periodontitis was done in the connection of the last sampling. The *A. actinomycetemcomitans* levels (ELISA units, mean ± SEM) of periodontitis subjects ($n = 6$) (◆) and periodontally healthy subjects ($n = 15$) (◇), *P. gingivalis* levels (ELISA units, mean ± SEM) in periodontitis subjects (●) and periodontally healthy subjects (○). Statistically significant differences are marked with asterisk ($p < 0.05$).

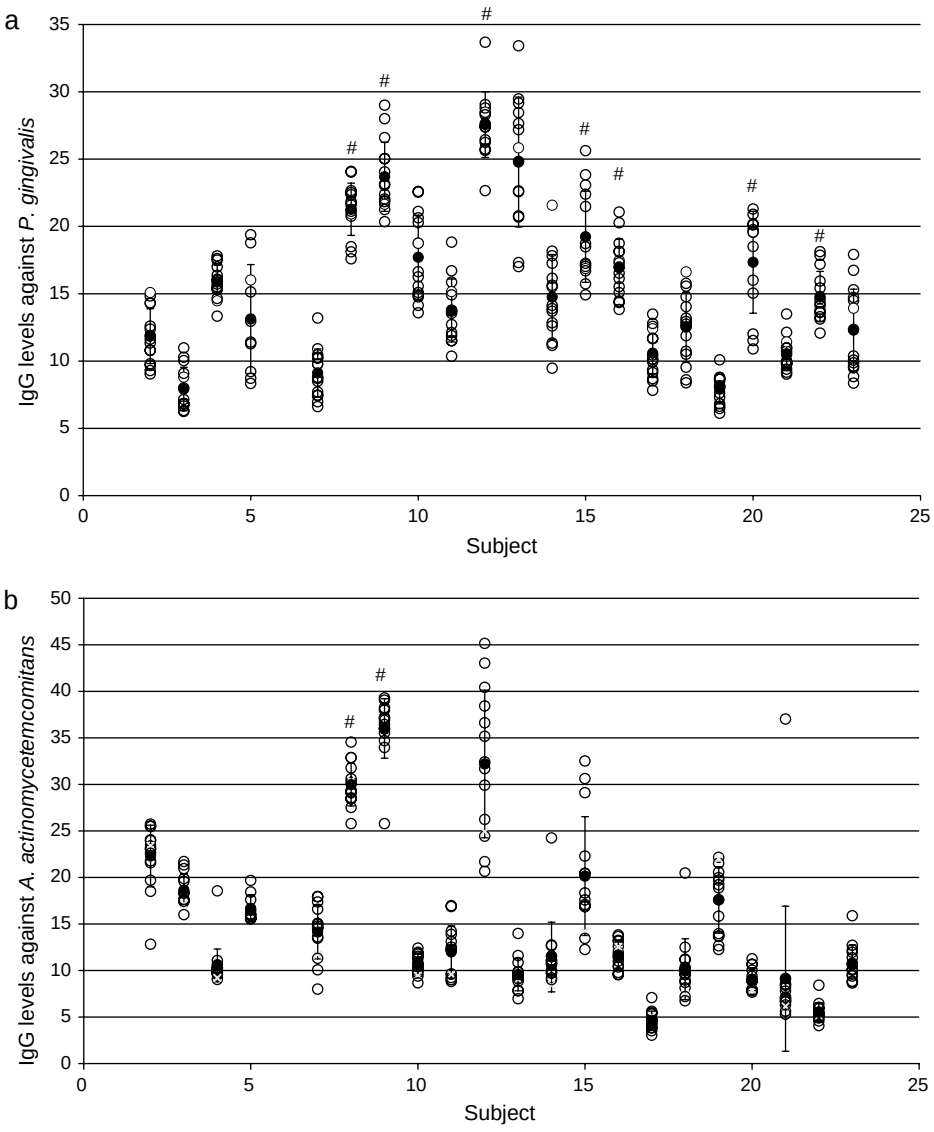

Fig. 2. Individual variation in plasma immunoglobulin G (IgG) levels (ELISA units) against *P. gingivalis* (a) and *A. actinomycetemcomitans* (b) during 15 years. The box plot columns show antibody levels in each year (○), mean antibody levels (●), and standard deviation (vertical lines). One box plot column represents one subject. Subjects marked with # are those who were PCR-positive for *P. gingivalis* (a) and *A. actinomycetemcomitans* (b) at the end of the study.

Mean salivary IgG antibody levels against *A. actinomycetemcomitans* and both salivary and plasma IgG levels against *P. gingivalis* were significantly higher in subjects with periodontitis compared to the levels in the periodontally healthy subjects. There were no differences in the salivary IgA antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* between the groups.

Long-term inter and intra-individual antibody variations

The mean annual plasma antibody IgG levels against *A. actinomycetemcomitans* and *P. gingivalis* during the 15-year period in subjects with and without periodontitis are shown in Fig. 1. Plasma IgG levels against *A. actinomycetemcomitans* were 1.6–2.3 fold higher in subjects with periodontitis than in those without ($p < 0.05$). Mean IgG levels against *P. gingivalis* were 1.4–1.7 fold higher in subjects with periodontitis than in periodontally healthy subjects ($p < 0.05$). The intra-individual variation in plasma IgG antibodies against *P. gingivalis* (Fig. 2a) and *A. actinomycetemcomitans* (Fig. 2b) during the 15 years are shown for all subjects. The individual variation during the *study* was not statistically significant in any of the subjects.

Table 3. Antibody levels in subjects positive and negative for PCR-detected *A. actinomycetemcomitans* and *P. gingivalis* in the end of the study

	PCR negative Mean ± SD	PCR-positive Mean ± SD	*p*-Value[a]
Antibodies against *A. actinomycetemcomitans*	Negative for *A. actinomycetemcomitans* $n = 19$[b]	Positive for *A. actinomycetemcomitans* $n = 2$[c]	
IgG in plasma	11.45 ± 5.70	35.72 ± 1.65	0.023
IgG in saliva	5.05 ± 5.59	21.29 ± 1.84	0.023
IgA in saliva	16.72 ± 10.05	27.73 ± 6.47	0.093
Antibodies against *P. gingivalis*	Negative for *P. gingivalis* $n = 14$[d]	Positive for *P. gingivalis* $n = 7$[e]	
IgG in plasma	11.89 ± 4.24	19.24 ± 7.06	0.014
IgG in saliva	0.03 ± 0.02	0.38 ± 0.39	0.007
IgA in saliva	0.48 ± 0.38	0.76 ± 0.72	0.456

[a]Mann–Whitney *U* test.
[b]Subjects negative for PCR detection of. *A. actinomycetemcomitans*.
[c]Subjects positive for PCR detection of *A. actinomycetemcomitans*.
[d]Subjects negative for PCR detection of *P. gingivalis*.
[e]Subjects positive for PCR detection of *P. gingivalis*.

Detection of bacteria

Mean plasma and salivary IgG levels were associated with the bacteria detected by PCR (Table 3). IgG levels against *A. actinomycetemcomitans* and *P. gingivalis* in both plasma and saliva were significantly higher in subjects positive for *A. actinomycetemcomitans* and *P. gingivalis* compared to the subjects negative for the bacteria, respectively. Salivary IgA levels against *A. actinomycetemcomitans* and *P. gingivalis* were not significantly elevated in PCR-positive subjects for either of the bacteria. Of the two *A. actinomycetemcomitans* PCR-positive subjects one was culture-positive. Seven subjects were PCR-positive for *P. gingivalis*, and five of them were culture-positive.

Correlation analyses

Correlation coefficients between the periodontal status and plasma, and salivary IgG and IgA antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* at the end of the *study* are shown in Table 4. The number of *angular bony defects* correlated with IgG antibody levels against *P. gingivalis* in plasma and saliva and IgA antibody levels against *A. actinomycetemcomitans* in saliva. The number of deepened periodontal pockets (≥ 5 mm) correlated with IgG antibody levels against *P. gingivalis* in both plasma and saliva, IgA antibody levels against *P. gingivalis* in saliva, and IgG and IgA antibody levels against *A. actinomycetemcomitans* in saliva. Mean clinical attachment loss correlated with IgG and IgA antibody levels against *P. gingivalis* and *A. actinomycetemcomitans* in both plasma and saliva. The highest CPITN value correlated with IgG levels against *P. gingivalis* and *A. actinomycetemcomitans* in both plasma and saliva.

Discussion

Tracking of plasma antibodies

The present study is a retrospective 15-year tracking of plasma antibody levels against *A. actinomycetemcomitans* and *P. gingivalis*. Throughout the whole time frame, the mean plasma antibody levels remained extremely stable, and only minor, non-significant individual variation was seen. Furthermore, our data showed elevated mean antibody levels against both *A. actinomycetemcomitans* and *P. gingivalis* in subjects, who were diagnosed as having periodontitis at the end of the 15-year time period, compared to the periodontally healthy.

The main result in the present study was the remarkable stability of plasma IgG levels in both the periodontitis and periodontally healthy subjects, even though the periodontal classification was done at the end of the study. The limitation of the study is that we did not have the dental history of the subjects who participated in the study, and it is possible that they had had active periodontitis at some point during the 15 years. This is, however, not likely since at the time of the dental examination, none of them were currently in periodontal treatment or in the maintenance care. The subjects had got occasional periodontal treatment by their general practitioners, but none of them had sought for treatment by a specialized periodontologist.

Table 4. Correlation coefficients between plasma IgG levels to *A. actinomycetemcomitans* and *P. gingivalis* and periodontal status at the time of periodontal examination

	Pg IgG in plasma	Aa IgG in plasma	Pg IgA in saliva	Pg IgG in saliva	Aa IgA in saliva	Aa IgG in saliva	Highest CPI value	Mean attachment loss (mm)	Periodontal pockets[a]	Vertical bone loss (number of pockets)	Bleeding on probing (%)
Bleeding on probing (%)	0.164	0.225	0.064	0.262	0.125	0.210	0.233	0.258	0.170	0.050	1
Vertical bone loss (number of pockets)	0.589*	0.264	0.660*	0.623*	0.633*	0.432	0.381	0.451	0.901	1	
Periodontal pockets[a]	0.564*	0.315	0.515*	0.642*	0.548*	0.438*	0.535*	0.380	1		
Mean attachment loss (mm)	0.637*	0.637*	0.660*	0.895*	0.581*	0.798*	0.598*	1			
Highest CPI[b] value	0.628*	0.526*	0.224	0.663*	0.236	0.498*	1				
Aa IgG[c] in saliva	0.664*	0.881*	0.719*	0.895*	0.713*	1					
Aa IgA[c] in saliva	0.513*	0.497*	0.938*	0.706*	1						
Pg IgG[c] in saliva	0.783*	0.729*	0.732*	1							
Pg IgA[c] in saliva	0.452*	0.504*	1								
Aa IgG[c] in plasma	0.493*	1									
Pg IgG[c] in plasma	1										

[a]Number of sites with deepened (≥5 mm) periodontal pockets. [b]CPI = clinical plaque index. [c]ELISA units per stimulated salivary flow rate.
*Statistically significant ($p < 0.05$).

The antibody detection method we use in our laboratory has been published earlier (22). Antigens in the assay represent *A. actinomycetemcomitans* serotypes a–e and a non-serotypeable strain, and *P. gingivalis* serotypes a–c (6). When the IgG-class antibody levels to pathogens are summed up, the assay has a specificity and sensitivity of 90 and 71%, respectively, for finding periodontitis by measuring antibody levels from plasma samples (6). Since the aim of the present study, however, was not to validate further the multiserotype-ELISA, the antibody levels were not summed up.

Our findings are consistent with previous studies. Papapanou and co-workers demonstrated an overall stability of plasma IgG-class antibody titers to 19 periodontal bacteria over a 30-month period for both periodontitis patients and periodontally intact control subjects (14). Periodontal treatment among those with the disease did not affect the plasma antibody levels. Furthermore, Dye and co-workers (2009) reported that serum IgG titers to selected periodontal species are feasible in epidemiologic studies using a combined serologic/demographic approach (23). Several other studies have shown higher plasma IgG levels against specific periodontal pathogens in patients with periodontitis than in periodontally healthy individuals (5, 9, 24, 25). It has been suggested that elevated antibody levels against *A. actinomycetemcomitans* and *P. gingivalis* exert a protective role in the progression of periodontitis (11). In a 36-month follow-up study, the patients who had low homologous plasma antibody levels together with cultivable *A. actinomycetemcomitans* and *P. gingivalis*, showed a positive predictive value for periodontitis disease recurrence (11). Our retrospective study, however, does not suggest a protective role for the plasma IgG-class antibody levels.

Cultivable *A. actinomycetemcomitans* and *P. gingivalis* can serve as markers for destructive periodontal disease in adult subjects (26). In the present study, we were able to detect two *A. actinomycetemcomitans*-positive subjects and seven *P. gingivalis*-positive subjects by PCR. From those subjects, bacteria could be cultivated in one and five cases, respectively. Plasma and salivary IgG levels were elevated in the *P. gingivalis*-positive subjects as detected with both PCR method and cultivation. In the *A. actinomycetemcomitans*-positive subjects, the plasma and salivary IgG levels were elevated in the PCR-positive group. This can be explained by the small number of *A. actinomycetemcomitans*-positive subjects in the population.

Plasma and salivary IgG levels correlated with each other. At the year of periodontal examination, both plasma and salivary antibody levels against *P. gingivalis* were significantly higher in the periodontitis group than in the periodontally healthy group. Concerning *A. actinomycetemcomitans* only the salivary IgG levels were elevated in the periodontitis group. A trend was seen also for plasma IgG against *A. actinomycetemcomitans* to be elevated in

the periodontitis group even though not statistically significant. The small study population may explain this; from 28 subjects participating originally in the study annually during the 15 years, only 21 attended the clinical examination. In addition, all affected subjects had chronic periodontitis, that is more commonly associated with the presence of *P. gingivalis* than *A. actinomycetemcomitans* (27). Although IgA is the predominant immunoglobulin in saliva, our study showed elevated salivary IgG levels against both *A. actinomycetemcomitans* and *P. gingivalis* in periodontitis. In the whole saliva samples, the total immunoglobulin originates both from crevicular fluid and exocrine secretion. Therefore, there is some discrepancy compared to previous studies, in which salivary IgA have been measured in subjects with periodontitis.

Hägewald and co-workers reported that a group of patients with aggressive periodontitis had lower total IgA levels than periodontally healthy subjects (28), whereas Henskens and associates found no difference in saliva IgA concentrations between patients with chronic periodontitis and periodontally healthy subjects (29). The present study supports the latter observation showing no differences in the IgA levels of periodontitis and periodontally healthy groups.

Overall, plasma and salivary IgG and IgA levels against *A. actinomycetemcomitans* and *P. gingivalis* correlated significantly with periodontal status. There was a positive correlation with the number of sites with deepened periodontal pockets, mean attachment loss, and *angular bony defects* detected from the radiographic pictures. Only three of the 21 participants in the present study were current smokers, and all of them belonged to the periodontally healthy group. Smoking is a strong risk factor for periodontal diseases and may have an impact also on immunoglobulin levels (8, 12, 30), but in this study the number of current smokers was too small to study the effect of smoking on the antibody production.

In conclusion, the mean plasma IgG levels against *A. actinomycetemcomitans* and *P. gingivalis* were stable during the entire 15-year period. The mean plasma antibody levels were also correlated with the periodontal status, the levels were significantly higher in subjects with periodontitis compared to the periodontally healthy. In this study population, periodontitis was moderate and the number of subjects with periodontitis was small. Therefore, further studies are needed to confirm the observations among patients with severe periodontitis.

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Conflict of interest and funding

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