Antibodies to Periodontal Pathogens and Stroke Risk

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Background and Purpose—The association between cerebrovascular events and periodontitis has been found in few studies based on clinical periodontal examinations. However, evidence on the association between periodontal pathogens and stroke is lacking. Therefore, the aim of the study was to investigate whether elevated levels of serum antibodies to major periodontal pathogens predict stroke in a case–control study.

Methods—The study population comprised 6950 subjects (aged 45 to 64 years) who participated in the Mobile Clinic Health Survey in 1973 to 1976 in Finland. During a follow-up of 13 years, a total of 173 subjects had a stroke. From these, 64 subjects had already experienced a stroke or had signs of coronary heart disease (CHD) at baseline, whereas 109 subjects were apparently healthy. Two controls per case were matched for age, gender, municipality, and disease status. Serum IgG and IgA class antibody levels to the periodontal pathogens, *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*, were determined by multiserotype enzyme-linked immunosorbent assay.

Results—The cases identified during the follow-up that were free of stroke or CHD at baseline were more often IgA-seropositive for *A. actinomycetemcomitans* than were their controls, 41.3% versus 29.3%. Compared with the seronegative, the seropositive subjects had a multivariate odds ratio of 1.6 (95% CI, 1.0 to 2.6) for stroke. The patients with a history of stroke or CHD at baseline were more often IgA-seropositive for *P. gingivalis* than were their controls, 79.7% versus 70.2%. When compared with the seronegative, the seropositive subjects had an odds ratio of 2.6 (1.0 to 7.0) for secondary stroke.

Conclusions—The present prospective study provides serological evidence that an infection caused by major periodontal pathogens is associated with future stroke. **(*Stroke*. 2004;35:2020-2023.)**

Key Words: cerebrovascular disorders ■ epidemiology ■ risk factors ■ stroke

Periodontitis is a persistent bacterial infection causing chronic inflammation in periodontal tissues. It is characterized by formation of pathological periodontal pockets concomitantly with destruction of periodontal ligament fibers attaching teeth to the alveolar bone and alveolar bone itself. The bacterial flora associated with periodontitis comprises a complex overgrowth of oral indigenous species. Species with elevated periodontopathogenic potential include mainly Gram-negative bacteria, particularly *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis*.[1]

Periodontitis has been linked to coronary heart disease (CHD) as a potential risk factor.[2–4] Its association with cerebrovascular events and stroke, however, is less studied. In 3 prospective studies,[3,5,6] 3 case–control studies,[7–9] and 1 cross-sectional study[10] based on clinical examinations, increased risk for stroke was associated with periodontitis and poor dental status. However, epidemiological evidence on the association between periodontal pathogens and stroke is lacking. Therefore, the aim of the present nested case–control study was to investigate whether elevated serum antibody levels to major periodontal pathogens are associated with stroke during a 13-year follow-up.

Materials and Methods

Serum samples were obtained from 3471 men and 3479 women aged 45 to 64 years during the Mobile Clinic Health Survey in 1973 to 1976 in Finland.[11,12] During a follow-up of 13 years, a total of 173 subjects (94 women, 79 men) experienced a fatal or nonfatal stroke (*International Classification of Diseases, Ninth Revision*, [ICD-9] code 430 to 438). From these, 109 subjects (55 women, 54 men) had no history and 64 subjects (39 women, 25 men) had a history of stroke or CHD at baseline. For the present nested case–control study, 2 controls per case were matched for age, municipality, gender, and history of stroke or CHD at baseline. A self-administered questionnaire provided information on sociodemographic background, diseases, medication, and smoking habits. Height and weight for the calculations of body mass index and blood pressure were measured in a physical examination. Data on deaths were obtained from the National Death Certificate Register, and underlying cause of death was taken as that assigned at the Central Statistical Office of Finland. Data on nonfatal strokes were derived from the National Hospital Discharge Data Register. The guidelines for human experimentation

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TABLE 1. Mean Baseline Levels (SD) of Potential Confounding Factors of Stroke Cases and Controls

	No History of Stroke or CHD at Baseline		History of Stroke or CHD at Baseline	
Baseline Variable	Cases (n=109)	Controls (n=206)	Cases (n=64)	Controls (n=121)
Age, y	55.7 (5.7)	55.7 (5.6)	57.7 (4.9)	57.1 (4.7)
Gender, % male	49.5	49.5	39.1	39.1
Current smoker, %	27.8	26.9	43.8	21.9
Diabetic subjects, %	8.3	3.7	18.8	9.4
Hypertensive subjects, %	42.2	24.8	59.4	33.6
Body mass index, kg/m^2	27.0 (4.7)	26.6 (2.8)	27.7 (4.8)	26.7 (3.1)
Alcohol consumption, g/wk	62.4	56.9	49.2	54
Cholesterol concentration, mmol/L	7.35 (1.3)	7.35 (1.0)	7.25 (1.2)	7.25 (1.0)

of the National Public Health Institute, Helsinki, Finland were followed in the conduct of this study.

Serum cholesterol concentration was determined by an auto-analyzer modification of the Liebermann–Burchard reaction at baseline. Serum IgG and IgA class antibodies to *A. actinomycetemcomitans* and *P. gingivalis* were determined by multiserotype enzyme-linked immunosorbent assay.[13] Two dilutions of each serum (stored at −20°C) in duplicate were used and the results (ELISA units [EU]) consisting of mean absorbances were calculated as continuous variables. We included on each plate a high and a low control serum in duplicates to monitor the interassay variations. The interassay coefficients for variation were 5.2% and 4.6% for *A. actinomycetemcomitans* and 4.0% and 3.7% for *P. gingivalis* IgA and IgG, respectively. The ELISA results of each plate were corrected according to the mean of the high control values after the whole material was analyzed. The subjects were considered seropositive for *A. actinomycetemcomitans* and *P. gingivalis*, when the corresponding IgG value was ≥5.0 EU or the IgA value ≥2.0 EU, which represent the mean antibody levels plus 1.5×SD of periodontally healthy subjects.[13]

The mean levels of antibodies and the proportions of seropositive subjects were compared and the significance of differences between cases and controls were tested using *t* test or χ^2 test. The odds ratios and their 95% CIs of stroke between subjects seropositive and seronegative for *A. actinomycetemcomitans* and *P. gingivalis* were estimated using the conditional logistic model.[14] Potential confounding factors were included in the model. The statistical analyses were performed using SAS program version 6.12.

Results

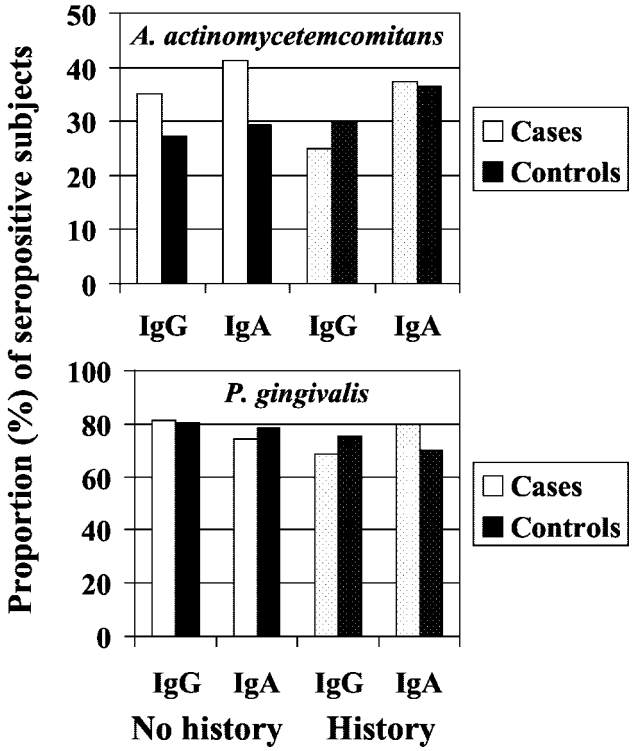
The characteristics of the cases and controls are summarized in Table 1. Among subjects without and with a history of stroke or CHD at baseline, the cases were more often hypertensive and were more likely to have diabetes than did the controls, and the cases with a history of stroke or CHD were more frequently current smokers than their controls. At baseline, there were no statistically significant differences in the mean antibody levels against *A. actinomycetemcomitans* or *P. gingivalis* between the groups of subjects with or without a history of stroke or CHD (data not shown). Furthermore, the proportion of seropositive subjects for either pathogen did not differ statistically significantly between these groups at baseline (data not shown).

In the study populations with or without a history of stroke or CHD at baseline, there were no statistically significant differences in the mean IgG or IgA class antibody levels to *A. actinomycetemcomitans* or *P. gingivalis* between the cases and the controls (Table 2). The cases identified during the follow-up but free of stroke and CHD at baseline were more often seropositive for *A. actinomycetemcomitans* in IgG and IgA classes than those remaining free of stroke, 35.2% versus 27.2%, and 41.3% versus 29.3%, respectively (Figure). Compared with the seronegative subjects, the univariate odds ratio for stroke among individuals IgA-seropositive for *A. actinomycetemcomitans* was 1.6 (95% CI, 1.0 to 2.6) (Table 3). The corresponding multivariate odds ratio adjusted for age, gender, place of residence, diabetes, smoking, alcohol consumption, body mass index, and serum cholesterol concentration was 1.7 (1.0 to 2.9). The proportion of *P. gingivalis*-

TABLE 2. Mean Serum Antibody Levels to Periodontal Pathogens

	No History of Stroke or CHD at Baseline			History of Stroke or CHD at Baseline		
	Cases (n=109) Mean (SD)	Controls (n=206) Mean (SD)	*P**	Cases (n=64) Mean (SD)	Controls (n=121) Mean (SD)	*P**
A. actinomycetemcomitans						
IgG (EU)	4.86 (2.79)	4.79 (2.65)	0.86	4.08 (2.51)	4.73 (2.29)	0.13
IgA (EU)	1.88 (1.14)	1.84 (0.96)	0.78	1.91 (1.09)	1.89 (1.03)	0.92
P. gingivalis						
IgG (EU)	8.05 (2.84)	8.21 (2.22)	0.62	7.58 (2.96)	7.73 (2.24)	0.71
IgA (EU)	3.43 (1.89)	3.22 (1.16)	0.32	3.34 (1.64)	3.22 (1.40)	0.57

**t* test.



Proportion of cases and controls seropositive for periodontal pathogens. Serum IgG and IgA class antibody levels to periodontal pathogens were determined by multiserotype ELISA. Samples were analyzed from subjects with and without a history of stroke at baseline, and from their matched controls. Subjects were considered seropositive when their serum IgG class antibody level was ≥5.0 EU and IgA class antibody level ≥2.0 EU to *A. actinomycetemcomitans* and *P. gingivalis*.

seropositive subjects did not differ significantly between these cases and controls: 81.5% versus 80.6% for IgG, and 74.1% versus 78.6% for IgA class antibodies, respectively (Figure).

The cases with a history of stroke or CHD at baseline were more frequently IgA-seropositve, but not IgG-seropositive for *P. gingivalis* than their controls: 79.7% versus 70.2%, and 68.8% versus 75.2%, respectively (Figure). The subjects IgA-seropositive for *P. gingivalis* had a univariate odds ratio of 1.7 (0.8 to 3.7) and a multivariate odds ratio of 2.6 (1.0 to 7.0) for stroke when compared with the seronegative subjects (Table 3). No statistically significant differences in the proportions of *A. actinomycetemcomitans*-seropositive subjects were found between these cases and controls (Figure).

Discussion

We found that an elevated serum IgA-class antibody level to *A. actinomycetemcomitans* predicted stroke in a prospective case–control study. In addition, an elevated IgA-antibody level to *P. gingivalis* predicted a recurrent stroke in subjects with a history of stroke or CHD at baseline. Elevated IgA class antibody levels to periodontal pathogens in saliva are believed to indicate persistent periodontitis with active tissue destruction.[15] The significance of elevated serum IgA levels against periodontal pathogens, however, is not fully understood. Nevertheless, when determined from dentate patients with periodontitis, serum and salivary IgA antibody levels to periodontal pathogens have a strong positive correlation with each other.[15] Therefore, our results suggest that aggressive forms of periodontitis addressed to *A. actinomycetemcomitans*, usually occurring particularly at young age (younger than 35 years), and to *P. gingivalis*, developing often at adult age, are associated with incidence of stroke.

Based on serum IgG antibody levels, 27.2% and 80.6% of the controls free of stroke at baseline were seropositive for *A. actinomycetemcomitans* and *P. gingivalis*, respectively. The proportion of *A. actinomycetemcomitans*-seropositive subjects was in the same range as in our previous study (32%) comprising middle-aged men in 1997.[4] In the present study, the proportion of *P. gingivalis*-seropositive subjects was much higher than in the earlier one (53%),[4] although here we also included women, who are known to suffer from periodontitis less frequently than men.[15] This suggests that the dental care services and/or dental hygiene have improved in Finland since the1970s.

In our previous study, 17% of the subjects free of CHD were edentulous, which proved to be the most important confounding factor for the serum IgG class antibody levels to

TABLE 3. Odds Ratios for Stroke in Subjects Seropositive Compared With Subjects Seronegative for Periodontal Pathogens

	No History of Stroke or CHD at Baseline		History of Stroke or CHD at Baseline	
	Univariate* OR (95% CI)	Multivariate† OR (95% CI)	Univariate* OR (95% CI)	Multivariate† OR (95% CI)
A. actinomycetemcomitans				
IgG‡	1.38 (0.86–2.23)	1.18 (0.70–1.98)	0.47 (0.24–0.92)	0.47 (0.21–1.07)
IgA§	1.61 (0.99–2.60)	1.68 (0.98–2.88)	1.03 (0.56–1.91)	1.10 (0.51–2.36)
P. gingivalis				
IgG‡	0.74 (0.35–1.56)	0.73 (0.34–1.57)	0.46 (0.17–1.21)	0.43 (0.13–1.46)
IgA§	0.77 (0.43–1.37)	0.65 (0.34–1.21)	1.72 (0.80–3.66)	2.62 (0.98–7.01)

*No adjustment; reference group=seronegative subjects.

†Adjusted for age, gender, place of residence, blood pressure, diabetes, smoking, alcohol consumption, BMI, and serum cholesterol.

‡Serum antibody level ≥5.0 EU vs <5.0 EU.

§Serum antibody level ≥2.0 EU vs <2.0 EU.

these periodontal pathogens.[4] Furthermore, the antibody levels correlated strongly, but not linearly, with the number of natural teeth. In the present study, we did not have any information on the dental status of the subjects. The present population is likely to comprise a significant proportion of edentulous subjects or subjects with only a few natural teeth, which may cause a bias toward low serum antibody levels and overemphasize their significance. The reliability of the results would have benefit if the edentulous and dentate subjects had been analyzed separately. However, the significant odds ratios in our study were of the same magnitude as reported in earlier prospective studies,[3,5] indicating a moderate association between periodontitis and stroke. Seropositivity for *P. gingivalis* IgA class antibodies predicted stroke only in subjects with evidence of known CVD at baseline, but not in subjects free from CVD. The results therefore do not suggest a causal role for *P. gingivalis* infection in the pathogenesis of stroke in a healthy population—contradictory to CHD.[16] However, the association of *A. actinomycetemcomitans* IgA seropositivity and stroke in initially healthy subjects is, to our knowledge, the first finding connecting infection by this pathogen to an increased risk for CVD in humans. There are several aspects that may contribute to the difference in the results between the subjects with and without a history of CVD at baseline. For example, efficacy of the immune response or epitope distribution of serum antibodies exhibits strain-to-strain variation depending on genotypes and serotypes of the pathogens.

The mechanisms by which chronic infections increase the likelihood of atherosclerosis or thrombosis are not clear, but the prerequisite is believed to be the long-term systemic exposure to the pathogens. In periodontitis, gingival inflammation accompanied by microulceration of the periodontal pocket epithelium and increasing subgingival space for bacterial deposits provide bacteria and their components access to the bloodstream. Local infection in the periodontal pockets triggers a systemic inflammatory response releasing inflammatory mediators, eg, C-reactive protein, whose elevation has been shown to be directly associated with atherogenesis.[17] Furthermore, periodontitis is accompanied by proatherogenic lipid profiles.[18,19] In an earlier study, the relative risk for cerebrovascular disease tended to be higher for periodontitis than for edentulousness, which supports the hypothesis of periodontal pathogens/periodontal inflammation being the cause for the association.[5] The direct role of periodontal pathogens in atherogenesis was recently supported by 2 studies using mouse models.[20,21] In these studies, intravenous and oral application of *P. gingivalis* exacerbated early atherosclerotic lesions, which were more advanced and occurred earlier in pathogen-challenged animals than in the vehicle control animals. One of the bacterial virulence factors behind the atherogenic properties of *A. actinomycetemcomitans* and *P. gingivalis* may be lipopolysaccharide, which activates macrophages and induces their conversion into foam cells.[22,23]

In conclusion, the present prospective study provides first serological evidence that a chronic infection caused by the periodontal pathogens *A. actinomycetemcomitans* and *P. gingivalis* is associated with incident stroke.

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