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RESEARCH ARTICLE

Serum IgG titers to periodontal pathogens predict 3-month outcome in ischemic stroke patients

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Abstract

Several cohort studies have shown that periodontal disease is associated with an increased risk for stroke. However, it remains unclear whether serum antibody titers for a specific periodontal pathogen are associated with outcome after ischemic stroke, and which kinds of pathogens are associated with ischemic stroke. We examined the relationship between serum IgG titers to periodontal pathogens and outcome in ischemic stroke patients. A total of 445 patients with acute ischemic stroke (194 female [44.0%], mean age 71.9±12.3 years) were registered in this study. Serum IgG titers to 9 periodontal pathogens (*Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Treponema denticola*, *Tannerella forsythensis*, *Campylobacter rectus*, *Eikenella corrodens*) were evaluated using the enzyme-linked immunosorbent assay (ELISA) method. An unfavorable outcome was defined as a 3 or higher on the modified Rankin Scale. The proportion of patients with unfavorable outcome was 25.4% (113 patients). Based on multivariate logistic regression analysis, numbers of IgG antibodies positive for periodontal pathogens (odds ratio 1.20, 95% CI 1.02–1.41, p = 0.03) were independent predictors of unfavorable outcome in ischemic stroke patients.

Introduction

Periodontal disease is a persistent bacterial infection causing chronic inflammation in periodontal tissues that is characterized by loss of connective tissue and alveolar bone support, leading to tooth loss [1]. The prevalence of periodontal disease is very high. Approximately >90% of the world population have mild to advanced periodontal disease.

Several cohort studies have shown that periodontal disease is associated with an increased risk of stroke [2–5]. A recent meta-analysis of 2 cohort studies found that periodontal disease increased the risk of ischemic stroke by 1.6-fold [6]. Furthermore, the Atherosclerosis Risk in Communities (ARIC) cohort study of 10362 stroke-free participants over a 15-year follow-up period demonstrated that periodontal disease was significantly associated with incidence of cardioembolic and thrombotic stroke, and regular dental care was related to lower adjusted stroke risk [7]. Periodontal disease has been established as one of the risk factors of ischemic stroke.

Measurement of serum antibody titers to a specific periodontal pathogen is considered to reflect its involvement in the disease process and has been used as one of the criteria required to identify causative organisms [8]. Recently, serum antibody titers related to a specific periodontal pathogen have been shown to indicate the risk factor of systemic diseases, such as coronary heart disease, non-alcoholic fatty liver disease and Alzheimer's disease [9–11]. We also previously reported that serum antibody titers related to a specific periodontal pathogen were associated with atrial fibrillation and carotid artery atherosclerosis [12].

However, it remains unclear whether serum antibody titers to periodontal pathogens can predict outcome after ischemic stroke. In this study, we examined the relationship between serum IgG titers to periodontal pathogens and outcome in ischemic stroke patients.

Materials and methods

Subjects

Consecutive acute ischemic stroke patients who were admitted to Hiroshima University Hospital and Suiseikai Kajikawa Hospital from January 2013 to April 2016 were enrolled in this prospective study. The study protocols were approved by the ethics committee of Hiroshima University Hospital and Suiseikai Kajikawa Hospital, and the study was performed according to the guidelines of the national government based on the Helsinki Declaration of 1964. Written informed consent was obtained from all patients or their relatives. All data analyses were blinded.

Data acquisition

Imaging analysis by computed tomography or magnetic resonance imaging was performed in all patients for diagnosis. Baseline clinical characteristic data, including age, sex, drinking and smoking habits, comorbidities (hypertension, diabetes mellitus, dyslipidemia, atrial fibrillation), past history of stroke, and C-reactive protein (CRP) levels were collected from all patients. Ischemic stroke subtypes were classified using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [13] by stroke specialists. Stroke severity on admission was evaluated using the National Institutes of Health Stroke Scale (NIHSS) scores. Hypertension was defined as use of anti-hypertensive medication before admission or confirmed blood pressure of $\geq 140/90$ mmHg at rest measured 2 weeks after onset. Diabetes mellitus was defined as glycated hemoglobin level of $\geq 6.5\%$, fasting blood glucose level of ≥ 126 mg/dL, or use of anti-diabetes medication. Dyslipidemia was defined as total cholesterol level of ≥ 220 mg/dL, low-density lipoprotein cholesterol level of ≥ 140 mg/dL, high-density lipoprotein cholesterol level of <40 mg/dL, triglyceride levels of ≥ 150 mg/dL, or use of anti-hyperlipidemia medication. Atrial fibrillation was defined as follows: (1) a history of sustained or paroxysmal atrial fibrillation or (2) atrial fibrillation detection on arrival or during admission. Renal function was calculated with the estimated glomerular filtration rate (eGFR) using a revised equation for the Japanese population as follows: eGFR (mL min−1 1.73 m−2) = 194 × (serum creatinine)−1.094 × (age)−0.287 × 0.739 (for women) [14]. Chronic kidney disease was defined as an eGFR<60

mL min−1 1.73 m−2. When we evaluated the 3-month outcome, patients who were disabled prior to stroke incidence (corresponding to premorbid modified Rankin scale [mRS] score ≥2) were excluded. An unfavorable 3-month outcome was defined as a 3 or higher on the mRS.

Measurement of serum antibody titers to periodontal pathogens

Serum IgG antibody titers to periodontal pathogens were determined using enzyme-linked immunosorbent assay (ELISA) as described previously [10]. Serum samples were collected from the patients within 3 days after stroke occurrence, and were stored at −80˚C. Sonicated preparations of the following periodontal pathogens were used as bacterial antigens *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *Treponema denticola*, *Tannerella forsythia*, *Campylobacter rectus*, and *Eikenella corrodens*. These pathogens were representative periodontal pathogens that were previously reported to be associated with serum antibody titers and stroke outcome [15]. Serum from 5 healthy subjects were pooled and used for calibration. Using serial dilutions of pooled control serum, the standard reaction was defined based on ELISA units (EU), such that 100 EU corresponded to 1:3200 dilution of the calibrator sample. For statistical analysis, we used the common logarithms of serum IgG antibody titers. Cut-off points for reactivity (positive decision point) to each antigen were defined as more than 1 standard deviation (SD) from mean ELISA units (EU).

Statistical analysis

Data are expressed as means ± SD or the median (minimum, maximum) for continuous variables and frequencies and percentages for discrete variables. Statistical analysis was performed using JMP 13 statistical software (SAS Institute Inc., Cary, NC, USA). The statistical significance of intergroup differences was assessed using unpaired t-test or Mann-Whitney's U-test (for continuous variables), or the Fisher exact test or χ^2 test (for discrete variables) as appropriate. Baseline data in ischemic stroke patients were analyzed, and two-step strategies were employed to assess the relative importance of variables in their association with poor outcome, respectively, using least square linear regression analysis. First, a univariate analysis was performed. Then, a multi-factorial analysis was performed with selected factors that had p<0.10 on univariate analysis. We considered p<0.05 to be statistically significant. Receiver operating characteristic (ROC) curves were configured to establish cut-off points for numbers of positive for periodontal pathogens that optimally predicted the unfavorable outcome.

Results

A total of 445 patients with acute ischemic stroke (194 female [44.0%], mean age 71.9±12.3 y) were registered in this study. Table 1 shows the baseline characteristics of all patients. The median NIHSS score was 3 (IQR 1–6), and the mean serum CRP was 0.57±1.60 mg/dL.

The proportion of patients with unfavorable outcome was 25.4% (113 patients). Table 2 shows the univariate analysis of baseline characteristics to determine associations with unfavorable outcome. Patients with unfavorable outcomes were significantly older and had a higher proportion of female sex, atrial fibrillation, and cardioembolism than those with favorable outcomes; patients with unfavorable outcomes also had a lower frequency of habitual drinking. The levels of serum CRP were significantly higher in patients with unfavorable outcomes than in those with favorable outcomes (p<0.001). The patients with unfavorable outcomes exhibited severe neurological deficits at admission.

Table 1. Baseline characteristics of study subjects.

	n = 445
Age	71.9±12.3
Sex (female), n (%)	194 (44.0)
Comorbidities	
Hypertension, n (%)	331 (74.4)
Diabetes mellitus, n (%)	119 (26.7)
Dyslipidemia, n (%)	201 (45.2)
Atrial fibrillation, n (%)	88 (19.8)
Chronic kidney disease, n (%)	148 (33.8)
Past history of stroke, n (%)	127 (28.5)
Current smoker, n (%)	184 (41.3)
Habitual drinker, n (%)	209 (47.0)
NIHSS score, median (IQR)	3 (1–6)
Serum CRP, mg/dL	0.57±1.60
Ischemic stroke subtypes	
Cardioembloism, n (%)	99 (22.2)
Large artery atherosclerosis, n (%)	103 (23.1)
Small artery occlusion, n (%)	91 (20.4)
Others, n (%)	152 (34.2)

NIHSS, National Institutes of Health Stroke scale; IQR, interquartile range; CRP, C-reactive protein.

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Table 3 shows the univariate analysis of antibody titers to periodontal pathogens (positive for periodontal pathogens) to determine associations with unfavorable outcome. The patients with unfavorable outcomes had a significantly higher proportion of positive for *F. nucleatum* and *T. denticola* than those patients with favorable outcomes. Variables with p<0.10 on univariate analysis were forced to enter into the multivariate logistic regression analysis procedure. From these results, age, NIHSS scores on admission, and serum CRP were independently associated with unfavorable outcome. Furthermore, we confirmed that IgG antibody titers to *F. nucleatum* (odds ratio 7.64, 95% CI 3.54–16.91, p<0.001) were independent predictors of unfavorable outcome in ischemic stroke patients (S1 Table). There were no significant correlations between serum CRP and serum IgG titers to each periodontal pathogen.

Patients with unfavorable outcomes had greater numbers of positive for periodontal pathogens than those with favorable outcomes (p = 0.001). The optimal cut-off point of the numbers of positive for periodontal pathogens for predicting unfavorable outcome was ≥3, with a sensitivity of 27.4%, specificity of 89.3%, and area under the ROC curve of 0.695. The mRS at 3-months was significantly higher in patients with ≥3 positive for periodontal pathogens than in those with <3 positive for periodontal pathogens (p<0.001, Fig 1). Variables with p<0.10 on univariate analysis were forced to enter into the multivariate logistic regression analysis procedure in Table 4. The numbers of IgG antibodies positive for periodontal pathogens (odds ratio 1.20, 95% CI 1.02–1.41, p = 0.03) were independent predictors of unfavorable outcome in ischemic stroke patients.

Discussion

We demonstrated that serum IgG titers to periodontal pathogens predict 3-month outcome in ischemic stroke patients. In particular, IgG antibody titers to *F. nucleatum* and numbers of

Table 2. Univariate analysis of baseline characteristics to determine associations with unfavorable outcome.

Factors	Favorable (n = 332)	Unfavorable (n = 113)	p-value
Age	70.0±12.2	77.2±11.1	<0.001
Sex (female), n (%)	135 (40.7)	61 (54.0)	0.012
Hypertension, n (%)	246 (74.1)	86 (76.1)	0.24
Diabetes mellitus, n (%)	89 (26.8)	30 (26.6)	0.96
Dyslipidemia, n (%)	156 (47.0)	45 (39.8)	0.18
Atrial fibrillation, n (%)	44 (13.3)	44 (38.9)	<0.001
Chronic kidney disease, n (%)	109 (32.8)	39 (34.5)	0.74
Past history of stroke, n (%)	98 (29.5)	29 (25.7)	0.43
Current smoker, n (%)	145 (43.7)	39 (34.5)	0.094
Habitual drinker, n (%)	167 (50.3)	42 (37.2)	0.023
NIHSS score, median (IQR)	2 (1–4)	10 (5–20)	<0.001
Serum CRP, mg/dL	0.38±1.26	1.12±2.24	<0.001
Ischemic stroke subtypes			<0.001
Cardioembloism, n (%)	48 (14.5)	51 (45.1)	
Large artery atherosclerosis, n (%)	82 (24.7)	21 (18.6)	
Small artery occlusion, n (%)	86 (25.9)	5 (4.4)	
Others, n (%)	116 (34.9)	36 (31.9)	

NIHSS, National Institutes of Health Stroke scale; IQR, interquartile range; CRP, C-reactive protein.

IgG antibodies positive for periodontal pathogens were independent predictors of unfavorable outcome in ischemic stroke patients.

The associations between periodontal disease and ischemic stroke have been reported in several studies. The first National Health and Nutrition Examination Survey showed that periodontal disease is one of the risk factors of ischemic stroke [16]. The ARIC study confirmed an independent association between periodontal disease and incident ischemic stroke risk, particularly cardioembolic and thrombotic stroke subtypes [7]. Furthermore, some cohort studies have reported that dental care or periodontal disease treatment could reduce the incidence of ischemic stroke [7, 17, 18]. On the other hand, the influence of periodontal disease on outcome of ischemic stroke patients has not been established. We revealed a significant association

Table 3. Univariate analysis of antibody titers to periodontal pathogens (positive for periodontal pathogens) to determine associations with unfavorable outcome.

| Factors	Favorable (n = 332)	Unfavorable (n = 113)	p-value
Periodontal pathogens			
Porphyromonas gingivalis, n (%)	39 (11.8)	15 (13.3)	0.67
Aggregatibacter actinomycetemcomitans, n (%)	35 (10.5)	16 (14.2)	0.30
Prevotella intermedia, n (%)	29 (8.7)	17 (15.0)	0.071
Prevotella nigrescens, n (%)	33 (9.9)	11 (9.7)	0.95
Fusobacterium nucleatum, n (%)	20 (6.0)	31 (27.4)	<0.001
Treponema denticola, n (%)	43 (13.0)	25 (22.1)	0.023
Tannerella forsythia, n (%)	42 (12.7)	18 (15.9)	0.39
Campylobacter rectus, n (%)	29 (8.7)	16 (14.2)	0.093
Eikenella corrodens, n (%)	36 (10.8)	16 (14.2)	0.35
Numbers of IgG antibodies positive for periodontal pathogens, median (IQR)	0 (0–1)	1 (0–3)	0.001

IQR, interquartile range.
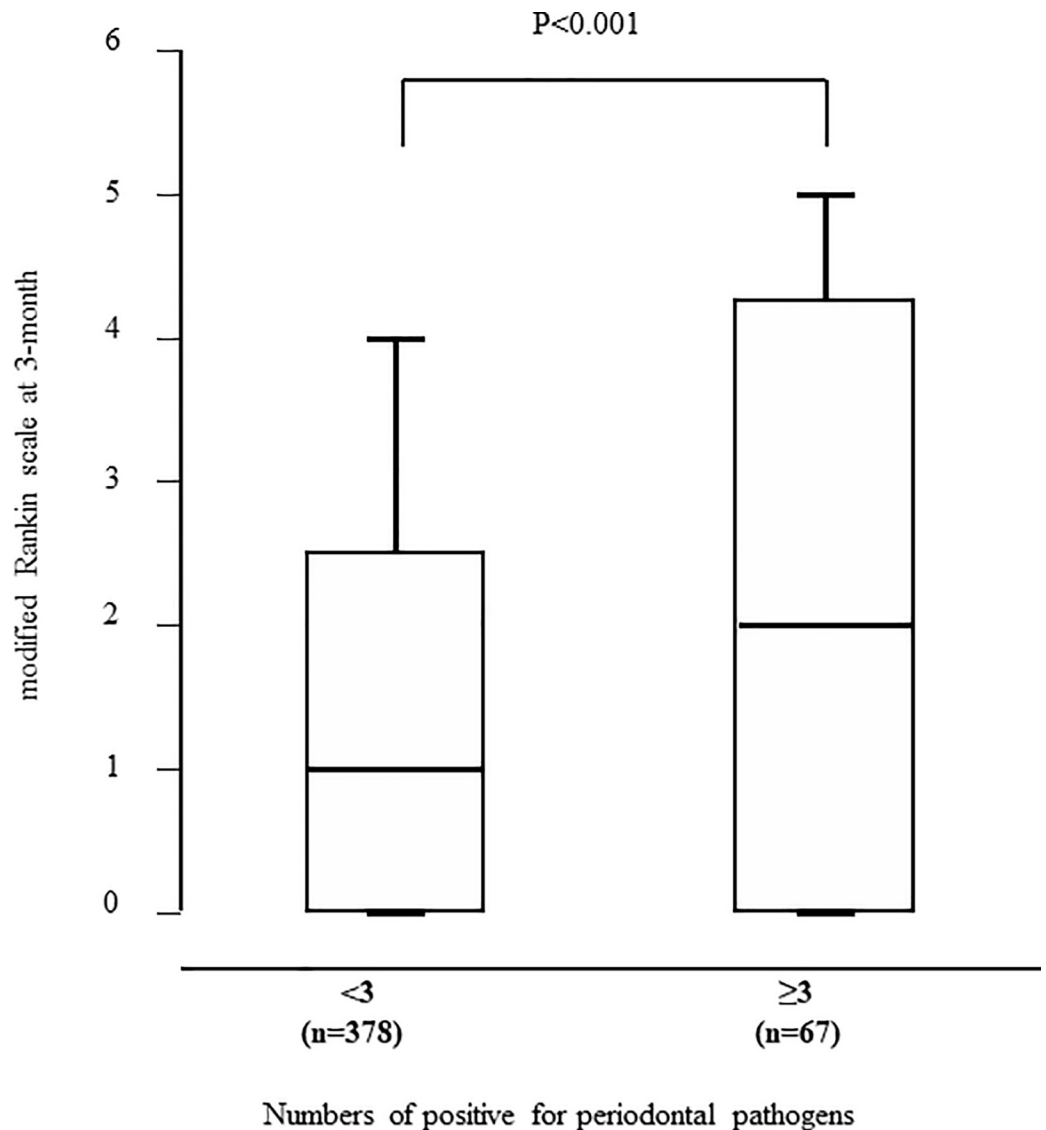
Fig 1. Relationship between the mRS at 3-months and numbers of IgG antibodies positive for periodontal pathogens. The mRS at 3-months was significantly higher in patients with ≥3 positive for periodontal pathogens than in those with <3 positive for periodontal pathogens.

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between serum IgG titers to periodontal pathogens and outcome of ischemic stroke patients. There is a possibility that regular dental care not only reduces the incidence of ischemic stroke, but also prevents severe neurological deficits in the acute phase of ischemic stroke.

We used serum IgG titers to periodontal pathogens to investigate the association between periodontal disease and outcome of ischemic stroke. Circulating IgG levels against periodontal pathogens may be more accurate measures of periodontal infection and its severity in previous studies [19, 20]. There are several reports demonstrating the association between antibodies to periodontal pathogens and coronary heart disease [21–23]. However, there have been few studies investigating the association between antibodies to periodontal pathogens and ischemic stroke. Pussinen *et al.* showed that antibodies to *A. actinomycetemcomitans* and *P. gingivalis* are associated with incident stroke [24]. Hosomi *et al.* reported that the levels of serum anti-

Table 4. Multivariate analyses to determine associations with unfavorable outcome.

Factors	Odds ratio	95% CI	p-value
Age	1.04	1.02–1.07	0.001
Sex (female)	1.31	0.67–2.58	0.44
Current smoker	1.21	0.62–2.37	0.58
Habitual drinking	0.43	0.22–0.81	0.008
NIHSS score on admission	1.22	1.15–1.29	<0.001
Cardioembolism	1.33	0.66–2.59	0.41
Serum CRP	1.19	1.03–1.40	0.023
Numbers of IgG antibodies positive for periodontal pathogens	1.20	1.02–1.41	0.031

CI, confidence interval; NIHSS, National Institutes of Health Stroke scale; IQR, interquartile range, CRP: C-reactive protein.

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P. intermedia antibody may be associated with atherothrombotic stroke [12]. We previously demonstrated that serum antibody titers to *F. nucleatum* were independent predictors of unfavorable outcome in all subtypes of stroke patients [15]. *F. nucleatum*, a Gram-negative anaerobe, is one of the most abundant species in the oral cavity, in both diseased and healthy individuals [25, 26]. It is considered to be a periodontal pathogen because it is frequently isolated from periodontitis lesions, produces a high number of tissue irritants, and often aggregates with other periodontal pathogens, as a bridge between early and late colonizers [27, 28]. It is frequently detected in atherosclerotic plaques, and is also one of the most common periodontal pathogens detected in ruptured cerebral aneurysm [29]. *F. nucleatum* elicits a variety of host responses, and is a potent stimulator of the inflammatory cytokines, IL-6, IL-8, and TNF α [30, 31].

In the present study, numbers of IgG antibodies positive for periodontal pathogens are independent predictors of unfavorable outcome in only ischemic stroke patients, not in hemorrhagic stroke patients. There is a possibility that an interaction between multiple periodontal pathogens may have negative effects on acute infarction. However, whether periodontal disease can modify acute ischemic brain damage is not fully understood. O'Boyle *et al.* showed that despite elevated systemic inflammation in periodontitis, oral inflammatory disease does not impact acute stroke pathology in terms of severity, determined primarily by infarct volume [32]. On the other hand, periodontal pathogens themselves are highly invasive; *P. gingivalis* can compromise and cross the blood brain barrier into the brain [33], and *Treponema* spp. have been speculated to enter the brain directly via peripheral trigeminal nerves [34]. *F.nucleatum* is also reported to be able to pass through the blood-brain barrier and has been found to be causative of brain abscesses in some case studies [35, 36]. Multiple periodontal pathogens infections are considered to cause higher-grade systemic inflammation. Several reports showed that high-grade systemic inflammation is deleterious in the context of ischemic stroke [37–39]. We speculate that this is a part of the reasons why numbers of IgG antibodies positive for periodontal pathogens are independent predictors of unfavorable outcome in ischemic stroke patients. Further studies are needed to clarify this point.

There are some limitations to our study. First, we did not evaluate the oral conditions such as tooth loss and grade of periodontal disease, socioeconomic status, and access to dental care in each patient. We cannot eliminate the possibility that these factors can influence the outcome in ischemic stroke patients. However, several reports have shown that IgG levels against periodontal pathogens are more accurate measures of periodontal infection and its severity. Takeuchi et al. showed that higher anti-*P. gingivalis* IgG levels were found in the periodontitis

group compared with the healthy control group [40]. Pussinen et al. found that ELISA is suitable for measuring antibodies to periodontal pathogens in large epidemiological studies in order to evaluate the role of periodontitis as a risk factor for other diseases [20]. Kudo et al. revealed that IgG titers of periodontitis patients were significantly higher than those of healthy controls, particularly in those with sites of probing depth over 4 mm [41]. In this way, IgG titers to periodontal pathogens are established as a surrogate marker of periodontal health. Moreover, we assessed the tooth status of 85 patients. The tooth status was evaluated by a dentist within 7 days after admission. The median numbers of remaining teeth were 23 (IQR 14–26), and treated teeth were 9 (IQR 4–14). Patients with unfavorable outcomes had significantly lower numbers of remaining teeth than patients with favorable outcomes. (20 [IQR 10–25] vs. 24 [IQR 16–27], p = 0.03). We found a positive correlation between the numbers of teeth lost and the numbers of positive for periodontal pathogens. Therefore, we believe that this limitation does not have a substantial influence on our results. Second, this is a cross-sectional observational study. It is difficult to elucidate the biological mechanisms responsible for the association between periodontal pathogens and ischemic stroke.

In conclusion, we demonstrated that numbers of IgG antibodies positive for periodontal pathogens were independent predictors of unfavorable outcome in ischemic stroke patients. It is possible that regular dental care not only reduces the incidence of ischemic stroke, but also prevents severe neurological deficits in the acute phase of ischemic stroke.

Supporting information

S1 Dataset.
(XLSX)

S1 Table. Multivariate analyses to determine associations with unfavorable outcome.
(DOCX)

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References

1. Pihlstrom BL, Michalowicz BS, Johnson NW. Periodontal diseases. Lancet. 2005; 366: 1809–1820. https://doi.org/10.1016/S0140-6736(05)67728-8 PMID: 16298220

2. Desvarieux M, Demmer RT, Rundek T, Boden-Albala B, Jacobs DR Jr, Sacco RL, et al. Periodontal microbiota and carotid intima-media thickness: the Oral Infections and Vascular Disease Epidemiology Study (INVEST). Circulation. 2005; 111: 576–582. https://doi.org/10.1161/01.CIR.0000154582.37101.15 PMID: 15699278

3. Scannapieco FA, Bush RB, Paju S. Associations between periodontal disease and risk for atherosclerosis, cardiovascular disease, and stroke. A systematic review. Ann Peridonol. 2003; 8: 38–53.

4. Syrjänen J, Peltola J, Valtonen V, Iivanainen M, Kaste M, Huttunen JK. Dental infections in association with cerebral infarction in young and middle-aged men. J Intern Med. 1989; 225: 179–184. https://doi.org/10.1111/j.1365-2796.1989.tb00060.x PMID: 2703800

5. Loesche WJ, Schork A, Terpenning MS, Chen YM, Kerr C, Dominguez BL. The relationship between dental disease and cerebral vascular accident in elderly United States veterans. Ann Periodontol. 1998; 3: 161–174. https://doi.org/10.1902/annals.1998.3.1.161 PMID: 9722700

6. Lafon A, Pereira B, Dufour T, Rigouby V, Giroud M, Béjot Y, et al. Periodontal disease and stroke: a meta-analysis of cohort studies. Eur J Neurol. 2014; 21: 1155–1161 https://doi.org/10.1111/ene.12415 PMID: 24712659

7. Sen S, Giamberardino LD, Moss K, Morelli T, Rosamond WD, Gottesman RF, et al. Periodontal Disease, Regular Dental Care Use, and Incident Ischemic Stroke. Stroke. 2018; 49: 353–362.

8. Dye BA, Herrera-Abreu M, Lerche-Sehm J, Vlachojannis C, Pikdoken L, Pretzl B, et al. Serum antibodies to periodontal bacteria as diagnostic markers of periodontitis. J periodontal. 2009; 80: 634–647.

9. Yamazaki K, Honda T, Domon H, Okui T, Kajita K, Amanuma R, et al. Relationship of periodontal infection to serum antibody levels to periodontopathic bacteria and inflammatory markers in periodontitis patients with coronary heart disease. Clin Exp Immunol. 2007; 149: 445–452 https://doi.org/10.1111/j.1365-2249.2007.03450.x PMID: 17645769

10. Nakahara T, Hyogo H, Ono A, Nagaoki Y, Kawaoka T, Miki D, et al. Involvement of Porphyromonas gingivalis in the progression of non-alcoholic fatty liver disease. J Gastroenterol. 2018; 53: 269–280. https://doi.org/10.1007/s00535-017-1368-4 PMID: 28741270

11. Sparks Stein P, Steffen MJ, Smith C, Jicha G, Ebersole JL, Abner E, et al. Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease. Alzheimers Dement. 2012; 8: 196–203. https://doi.org/10.1016/j.jalz.2011.04.006 PMID: 22546352

12. Hosomi N, Aoki S, Matsuo K, Deguchi K, Masugata H, Murao K, et al. Association of serum anti-periodontal pathogen antibody with ischemic stroke. Cerebrovasc Dis. 2012; 34: 385–392. https://doi.org/10.1159/000343659 PMID: 23207319

13. Adams HP Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, et al. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993; 24: 35–41. https://doi.org/10.1161/01.str.24.1.35 PMID: 7678184

14. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, et al. Revised equations for estimated gfr from serum creatinine in japan. Am J Kidney Dis. 2009; 53: 982–992. https://doi.org/10.1053/j.ajkd.2008.12.034 PMID: 19339088

15. Nishi H, Hosomi N, Ohta K, Aoki S, Nakamori M, Nezu T, et al. Serum IgG antibody titer to *Fusobacterium nucleatum* is associated with unfavorable outcome after stroke. Clin Exp Immunol, 2020; 200: 302–309. https://doi.org/10.1111/cei.13430 PMID: 32155293

16. Wu T, Trevisan M, Genco RJ, Dorn JP, Falkner KL, Sempos CT. Periodontal disease and risk of cerebrovascular disease: the first national health and nutrition examination survey and its follow-up study. Arch Intern Med. 2000; 160: 2749–2755. https://doi.org/10.1001/archinte.160.18.2749 PMID: 11025784

17. Chen ZY, Chiang CH, Huang CC, Chung CM, Chan WL, Huang PH, et al. The association of tooth scaling and decreased cardiovascular disease: a nationwide population-based study. Am J Med. 2012; 125: 568–575. https://doi.org/10.1016/j.amjmed.2011.10.034 PMID: 22483056

18. Lee YL, Hu HY, Huang N, Hwang DK, Chou P, Chu D. Dental prophylaxis and periodontal treatment are protective factors to ischemic stroke. Stroke. 2013; 44: 1026–1030. https://doi.org/10.1161/STROKEAHA.111.000076 PMID: 23422085

19. Rams TE, Listgarten MA, Slots J. Actinobacillus actinomycetemcomitans and Porphyromonas gingivalis subgingival presence, species-specific serum immunoglobulin G antibody levels, and periodontitis disease recurrence. J Periodontal Res. 2006; 41: 228–234. https://doi.org/10.1111/j.1600-0765.2005.00860.x PMID: 16677293

20. Pussinen PJ, Vilkuna-Rautiainen T, Alfthan G, Mattila K, Asikainen S. Multiserotype enzyme-linked immunosorbent assay as a diagnostic aid for periodontitis in large-scale studies. J Clin Microbiol. 2002; 40: 512–518. https://doi.org/10.1128/jcm.40.2.512-518.2002 PMID: 11825965

21. Beck JD, Eke P, Heiss G, Madianos P, Couper D, Lin D, et al. Periodontal disease and coronary heart disease: a reappraisal of the exposure. Circulation. 2005; 112: 19–24. https://doi.org/10.1161/CIRCULATIONAHA.104.511998 PMID: 15983248

22. Colhoun HM, Slaney JM, Rubens MB, Fuller JH, Sheiham A, Curtis MA. Antibodies to periodontal pathogens and coronary artery calcification in type 1 diabetic and nondiabetic subjects. J Periodontal Res. 2008; 43: 103–110. https://doi.org/10.1111/j.1600-0765.2007.01001.x PMID: 18230111

23. Lund Håheim L, Olsen I, Nafstad P, Schwarze P, Rønningen KS. Antibody levels to single bacteria or in combination evaluated against myocardial infarction. J Clin Periodontol. 2008; 35: 473–478. https://doi.org/10.1111/j.1600-051X.2008.01229.x PMID: 18410396

24. Pussinen PJ, Alfthan G, Rissanen H, Reunanen A, Asikainen S, Knekt P. Antibodies to periodontal pathogens and stroke risk. Stroke. 2004; 35: 2020–2023. https://doi.org/10.1161/01.STR.0000136148.29490.fe PMID: 15232116

25. Field CA, Gidley MD, Preshaw PM, Jakubovics N. Investigation and quantification of key periodontal pathogens in patients with type 2 diabetes. J Periodontal Res. 2012; 47: 470–478 https://doi.org/10.1111/j.1600-0765.2011.01455.x PMID: 22220967

26. Loozen G, Ozcelik O, Boon N, De Mol A, Schoen C, Quirynen M, et al. Inter-bacterial correlations in subgingival biofilms: a large-scale survey. J Clin Periodontol. 2014; 41: 1–10. https://doi.org/10.1111/jcpe.12167 PMID: 24102517

27. Han YW. Fusobacterium nucleatum: a commensal-turned pathogen. Curr Opin Microbiol. 2015; 23: 141–147. https://doi.org/10.1016/j.mib.2014.11.013 PMID: 25576662

28. Feuille F, Ebersole JL, Kesavalu L, Stepfen MJ, Holt SC. Mixed infection with Porphyromonas gingivalis and Fusobacterium nucleatum in a murine lesion model: potential synergistic effects on virulence. Infect Immun. 1996; 64: 2094–2100. https://doi.org/10.1128/IAI.64.6.2094-2100.1996 PMID: 8675312

29. Pyysalo MJ, Pyysalo LM, Pessi T, Karhunen PJ, Öhman JE. The connection between ruptured cerebral aneurysms and odontogenic bacteria. J Neurol Neurosurg Psychiatry. 2013; 84: 1214–1218. https://doi.org/10.1136/jnnp-2012-304635 PMID: 23761916

30. Han YW, Shi W, Huang GT, Kinder Haake S, Park NH, Kuramitsu H, et al. Interactions between periodontal bacteria and human oral epithelial cells: Fusobacterium nucleatum adheres to and invades epithelial cells. Infect Immun. 2000; 68: 3140–3146. https://doi.org/10.1128/iai.68.6.3140-3146.2000 PMID: 10816455

31. Park SR, Kim DJ, Han SH, Kang MJ, Lee JY, Jeong YJ, et al. Diverse Toll-like receptors mediate cytokine production by Fusobacterium nucleatum and Aggregatibacter actinomycetemcomitans in macrophages. Infect Immun. 2014; 82: 1914–1920. https://doi.org/10.1128/IAI.01226-13 PMID: 24566622

32. O'Boyle C, Haley MJ, Lemarchand E, Smith CJ, Allan SM, Konkel JE, et al. Ligature-induced periodontitis induces systemic inflammation but does not alter acute outcome after stroke in mice. Int J Stroke. 2020; 15:175-187.

33. Singhrao SK, Chukkapalli S, Poole S, Velsko I, Crean SJ, Kesavalu L. Chronic Porphyromonas gingivalis infection accelerates the occurrence of age-related granules in ApoE$^{-/-}$ mice brains. J Oral Microbiol. 2017; 9: 1270602. https://doi.org/10.1080/20002297.2016.1270602 PMID: 28326151

34. Riviere GR, Riviere KH, Smith KS. Molecular and immunological evidence of oral Treponema in the human brain and their association with Alzheimer's disease. Oral Microbiol Immunol. 2002; 17: 113–118. https://doi.org/10.1046/j.0902-0055.2001.00100.x PMID: 11929559

35. Heckmann JG, Lang CJ, Hartl H, Tomandl B. Multiple brain abscesses caused by Fusobacterium nucleatum treated conservatively. Can J Neurol Sci. 2003; 30: 266-268. https://doi.org/10.1017/s0317167100002717 PMID: 12945954

36. Kai A, Cooke F, Antoun N, Siddharthan C, Sule O. A rare presentation of ventriculitis and brain abscess caused by Fusobacterium nucleatum. J Med Microbiol. 2008; 57: 668-671. https://doi.org/10.1099/jmm.0.47710-0 PMID: 18436604

37. Dénes A, Ferenczi S, Kovács KJ. Systemic inflammatory challenges compromise survival after experimental stroke via augmenting brain inflammation, blood- brain barrier damage and brain oedema independently of infarct size. J Neuroinflammation. 2011; 8: 164. https://doi.org/10.1186/1742-2094-8-164 PMID: 22114895

38. McColl BW, Rothwell NJ, Allan SM. Systemic inflammatory stimulus potentiates the acute phase and CXC chemokine responses to experimental stroke and exacerbates brain damage via interleukin-1- and neutrophil-dependent mechanisms. J Neurosci. 2007; 27: 4403–4412. https://doi.org/10.1523/JNEUROSCI.5376-06.2007 PMID: 17442825

39. McColl BW, Rothwell NJ, Allan SM. Systemic inflammation alters the kinetics of cerebrovascular tight junction disruption after experimental stroke in mice. J Neurosci. 2008; 28: 9451–9462. https://doi.org/10.1523/JNEUROSCI.2674-08.2008 PMID: 18799677

40. Takeuchi Y, Aramaki M, Nagasawa T, Umeda M, Oda S, Ishikawa I. Immunoglobulin G subclass antibody profiles in Porphyromonas gingivalis-associated aggressive and chronic periodontitis patients. Oral Microbiol Immunol. 2006; 21: 314-318. https://doi.org/10.1111/j.1399-302X.2006.00296.x PMID: 16922931

41. Kudo C, Naruishi K, Maeda H, Abiko Y, Hino T, Iwata M, et al. Assessment of the plasma/serum IgG test to screen for periodontitis. J Dent Res. 2012 Dec; 91: 1190–1195. https://doi.org/10.1177/0022034512461796 PMID: 23018816