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Clinical and Bacterial Markers of Periodontitis and Their Association with Incident All-Cause and Alzheimer's Disease Dementia in a Large National Survey

May A. Beydoun[a,*,1], Hind A. Beydoun[b], Sharmin Hossain[a], Ziad W. El-Hajj[c], Jordan Weiss[d] and Alan B. Zonderman[a]

[a]*Laboratory of Epidemiology and Population Sciences, National Institutes on Aging, NIA/NIH/IRP, Baltimore, MD, USA*

[b]*Fort Belvoir Community Hospital, Fort Belvoir, VA, USA*

[c]*McGill University, Montreal, QC, Canada*

[d]*Population Studies Center and the Leonard Davis Institute of Health Economics, University of Pennsylvania, Philadelphia, PA, USA*

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Abstract. Microbial agents including periodontal pathogens have recently appeared as important actors in Alzheimer's disease (AD) pathology. We examined associations of clinical periodontal and bacterial parameters with incident all-cause and AD dementia as well as AD mortality among US middle-aged and older adults. Clinical [Attachment Loss (AL); probing pocket depth (PPD)] and bacterial [pathogen immunoglobulin G (IgG)] periodontal markers were investigated in relation to AD and all-cause dementia incidence and to AD mortality, using data from the third National Health and Nutrition Examination Surveys (NHANES III, 1988–1994) linked longitudinally with National Death Index and Medicare data through January 1, 2014, with up to 26 years of follow-up. Sex- and age-specific multivariable-adjusted Cox proportional hazards models were conducted. Among those ≥65 years, AD incidence and mortality were consistently associated with PPD, two factors and one cluster comprised of *IgG* titers against Porphyromonas gingivalis (*P. gingivalis*), Prevotella melaninogenica (*P. melaninogenica*) and Campylobacter rectus (*C. rectus*) among others. Specifically, AD incidence was linked to a composite of *C. rectus* and *P. gingivalis* titers (per SD, aHR = 1.22; 95% CI, 1.04–1.43, $p = 0.012$), while AD mortality risk was increased with another composite (per SD, aHR = 1.46; 95% CI, 1.09–1.96, $p = 0.017$) loading highly on *IgG* for *P. gingivalis*, *Prevotella intermedia*, *Prevotella nigrescens*, *Fusobacterium nucleatum*, *C. rectus*, *Streptococcus intermedius*, *Capnocylophaga Ochracea*, and *P. melaninogenica*. This study provides evidence for an association between periodontal pathogens and AD, which was stronger for older adults. Effectiveness of periodontal pathogen treatment on reducing sequelae of neurodegeneration should be tested in randomized controlled trials.

Keywords: Aging, Alzheimer's disease, dementia, periodontal pathogens, periodontitis

INTRODUCTION

Dementia, a common disorder affecting older adults, has an estimated prevalence of 4.7% (≥60 years) [1], with 4.6–7.7 million additional annual cases occurring worldwide [3.5–10.5 per 1,000 in various world regions] [1–3]. Generally, ∼60–80%

*Correspondence to: May A. Beydoun, PhD, NIH Biomedical Research Center, National Institute on Aging, IRP, 251 Bayview Blvd., Suite 100, Room #: 04B118, Baltimore, MD 21224, USA. Fax: +1 410 558 8236; E-mail: baydounm@mail.nih.gov.

[1]MAB had full access to the data used in this manuscript and completed all the statistical analyses.

of dementia is ascribed to Alzheimer's disease (AD) [1], a progressive neurodegenerative disorder with a multi-factorial etiology. AD triggers progressive episodic memory deterioration followed by impairment in other domains of cognition [4]. AD is likely caused by age-dependent and progressive Aβ-amyloid brain deposition [5], with a second pathological hallmark being the neurofibrillary tangles arising from hyperphosphorylated tau proteins [6]. It constitutes the primary cause of disability among older adults [7], the leading health care burden in developed countries [8], and the sixth leading cause of death in the US [9]. The number of AD-affected Americans is expected to rise from currently 5.4 million to 13.8 million by 2050 [9]. In 2016, US long-term and hospice care cost for all-cause dementia (including AD, vascular dementia and other rare forms) was estimated at $236 billion [9].

Despite no effective treatment, epidemiologic research has uncovered genetic markers for late-onset AD (e.g., ApoE ε4) and several modifiable risk factors. The combined effects of low education, smoking, physical inactivity, depression, mid-life obesity, hypertension, and type 2 diabetes explains ∼54% of AD risk [10], leaving much variation unaccounted for. Identifying novel mid-life modifiable risk factors is essential for planning cost-effective interventions. Microbial agents have recently appeared as important actors of AD's etiology [8], notably periodontal pathogens [11–15], many of which can cause periodontitis (Pd), a condition shown to increase risk of diabetes, atherosclerosis, cardiovascular events [16], and adverse cognitive outcomes [11–15].

Pd affects 20–50% of older adults and is initiated by periodontal bacteria, the most well-known being *Porphyromonas gingivalis (P. gingivais), Tannerella forsythia (T. forsythia)*, *Aggregatibacter actinomycetemcomitans (A. actinomycetemcomitans),* and *Treponema denticola (T. denticola)*, triggering gingival inflammation, connective tissue destruction, periodontal pocket formation, alveolar bone loss, and edentulation [17]. Given its increased prevalence with age, Pd may be highly predictive of AD. In fact, several hypothesized pathways link Pd to AD, including brain tissue invasion by periodontal gram-negative bacteria found in the dental biofilm, release of bacterial byproducts into the brain via bloodstream invasion and direct impacts of peripheral nerves [18]. Periodontal pathogens can affect brain cytokines through systemic or neural pathways [19]. A recent comprehensive study suggests that *P. gingivalis* and its associated gingipains in the brain play a central role in AD pathogenesis and suggests that $A\beta_{1\text{-}42}$ is produced in the brain partly as a response to this infection [20]. However, the epidemiological evidence as to the relationship between various Pd-related pathogens, including *P. gingivalis*, and AD remains scarce.

We examined age and sex-specific associations of serum immunoglobulin G (IgG) humoral immune response against periodontal pathogens and Pd markers with incident all-cause and AD dementia as well as AD mortality among U.S. middle-aged and older adults (45+years at baseline) using the National Health and Nutrition Examination Survey (NHANES) III linked with Center for Medicare & Medicaid Services (CMS) data.

MATERIALS AND METHODS

Database: NHANES-CMS

The NHANES, sponsored by the National Center for Health Statistics (NCHS), consists of cross-sectional surveys providing nationally representative data on U.S. population health and nutritional status. Sampling follows a stratified, multistage probability cluster design. It includes in-home interviews for basic health and demographic information [21]. This was a retrospective cohort study whereby publicly available data was linked to restricted medical and death records and analyzed at the Research Data Center (RDC). CMS-Medicare and NDI linkage methodology are provided in Supplementary Material 1.

The present study was approved for ethical treatment of participants by the Institutional Review Board of the National Institute on Aging, Intramural Research Program.

Study sample

A participant flowchart is presented in Fig. 1, including the sample at risk and number of events. First, we included NHANES III participants aged 45+years with complete data on at least one of 19 periodontal pathogens Immunoglobulin G (*IgG)* humoral immune response (1988–1994, surplus serum, SPSDEPPX), mortality status and CMS-linkage data. Among 33,199 participants (aged 1–90 years) recruited in NHANES III (1988–1994) with complete socio-demographics (i.e., age and

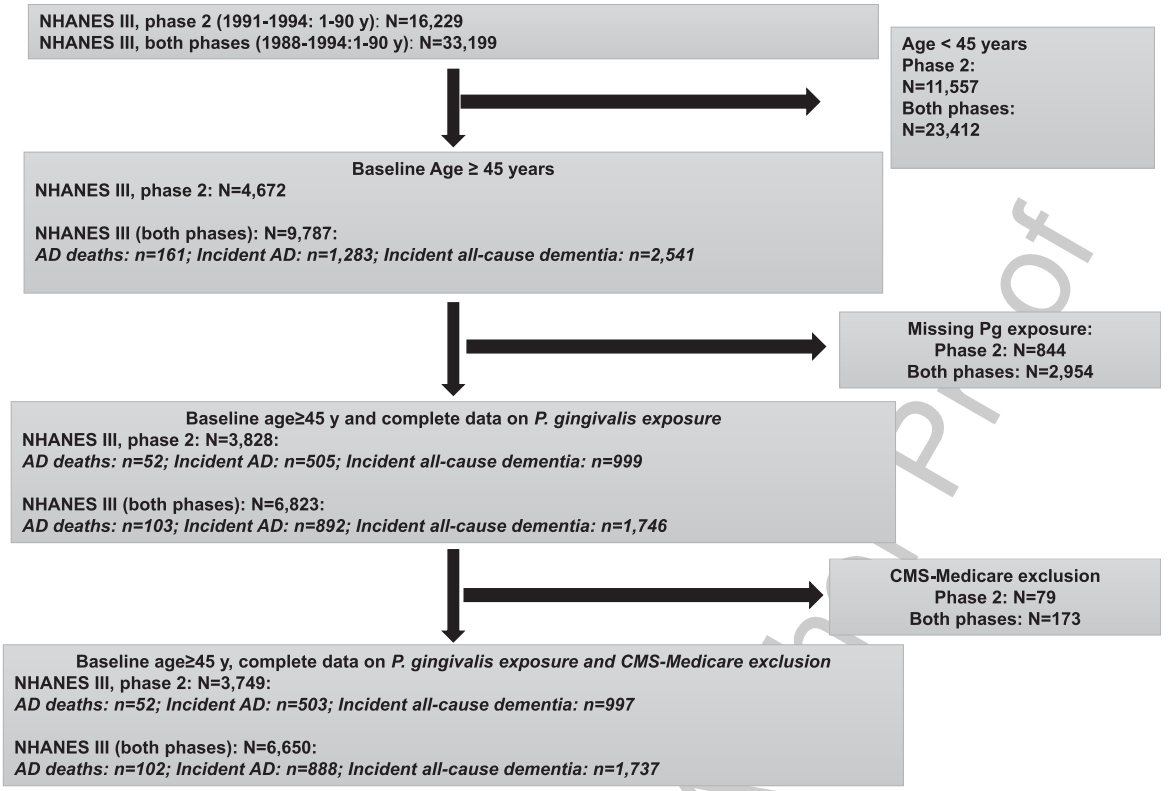

Fig. 1. Participant Flowchart. Both phases: 1988–1994; Phase 2:1991–1994. AD, Alzheimer's disease; CMS, Centers for Medicare and Medicaid Services; NHANES III, Third National Health and Nutrition Examination Surveys.

sex), 9,787 were aged 45+years, of whom 6,823 had complete surplus serum periodontal pathogen data.

Second, 4,672 participants aged 45+years had data on humoral immune response (*IgG*) against two periodontal pathogens only for phase 2 (1991–1994) of NHANES III (DEPP file), namely *Pg* and *Aa*. Of this sub-sample, 3,828 had complete data in the DEPP file (specifically on *Pg*). Sample sizes varied for both samples, depending on exposure of interest.

Participants without CMS-Medicare data were assumed to have no event of interest until end of 2013 or censored upon death. Thus, unweighted samples consisted of 6,823 participants with *Pg* measured at both phases and 3,828 with *P. gingivalis* measured at phase 2. After exclusion due to Health Maintenance Organization (HMO) utilization, those samples were reduced to 6,650 and 3,749, respectively. Third, for PPD/AL exposures, the sample size was reduced to 5,088 (45+, both phases combined, CMS-Medicare exclusion) and PPD/AL exposures with complete both phase *P. gingivalis* and CMS-Medicare exclusion amounted to N = 4,465.

Dementia and AD onset

The CMS Chronic Condition Data Warehouse Categories included a summary file with 21 chronic conditions and varying reference time periods, numbers and types of claims to qualify, exclusions and a set of International Classification of Diseases, version 9 (ICD-9)/CPT4/HCPCS codes. AD was diagnosed using ICD-9 code 331.0 (any diagnosis on the claim) from inpatient, Skilled Nursing Patient [SNP], Home Health Agency [HHA], Health Options Program [HOP] or Carrier claims during a 3-year period. All-cause dementia was assessed using similar criteria with the following diagnostic codes: 331.0, 331.1, 331.11, 331.19, 331.2, 331.7, 290.0, 290.10, 290.11, 290.12, 290.13, 290.20, 290.21, 290.3, 290.40, 290.41, 290.42, 290.43, 294.0, 294.1, 294.10, 294.11, 294.8, and 797. We computed time-to-event starting from Medical Examination Center (MEC) examination date, using the earliest occurrence date. The summary file was available for 1999–2013 follow-up period. Using the same algorithm, we estimated AD/dementia's earliest diagnosis

date during 1991–1998 [22]. Follow-up time was truncated to January 1, 2014 and was expressed in months.

Mortality from AD

AD mortality was a primary outcome and was determined using underlying cause of death ICD-10 code G30 [23]. Additional AD cases were included when earliest AD diagnosis date was unavailable but AD-related death was assigned. Follow-up was censored at death or, if participants were alive by end of follow-up, January 1, 2014.

Dental examination and clinical periodontal markers

Attachment loss (AL) and probing pocket depth (PPD) defined Pd in our study [24]. Briefly, dental examiners assessed oral health during both phases of MEC examinations in NHANES III. AL and PPD were measured at mid-buccal and mesio-buccal sites on every tooth in two randomly selected quadrants-the maxilla and the mandible (Range: 14–28 teeth) [25]. AL was defined as distance between cemento-enamel junction and base of periodontal pocket, while PPD was distance from base of periodontal pocket to free gingival margin [26]. Mean AL and PPD were calculated for 28 sites. Two non-missing sites per tooth were required for AL/PPD measure and at least one tooth measurement was required to estimate the mean [26].

Periodontal pathogens

Serum immunoglobulin G (IgG) titers were measured for humoral immune response against 19 periodontal bacteria using a series of 1:1,000 serum dilutions and checkerboard immunoblotting as previously described [27]. Those pathogens are: 1) *A. Actinomycetemcomitans* (American Type Culture Collection [ATCC] strains 43718, 29523, and 33384); 2) *P. gingivalis* (ATCC strains 3327 and 53978); 3) *T. forsythia* (ATCC strain 43037); 4) *T. denticola* (Oral Microbiology, Gothenburg, Sweden [OMGS] strain 3271); 5) *Campylobacter rectus* (*C. rectus*, ATCC strain 33238); 6) *Eubacterium nodatum* (*E. nodatum*, ATCC strain 33099); 7) *Prevotella intermedia* (*P. intermedia*, ATCC strain 25611); 8) *Prevotella nigrescens* (*P. nigrescens*, ATCC strain 33563); 9) *Prevotella melaninogenica* (*P. melaninogenica*, ATCC strain 25845); 10) *Fusobacterium nucleatum* (*F. nucleatum*, ATCC strain 33563); 11) *Parvimonas micra* aka *Micromonas micros* (*M. micros*, ATCC strain 10953); 12) *Selenomonas noxia* (*S. noxia*, ATCC strain 43541); 13) *Eikenella corrodens* (*E. corrodens*, ATCC strain 23834); 14) *Capnocylophaga ochracea* (*C. ochracea*, ATCC strain 33624); 15) *Streptococcus intermedius* (*S. intermedius*, ATCC strain 35037); 16) *Streptococcus oralis* (*S. oralis*, ATCC strain 35037); 17) *Streptococcus mutans* (*S. mutans*, ATCC strain 25175); 18) *Vellonella Parvula* (*V. parvula*, ATCC strain 10790); 19) *Actinomyces naeslundii* (*A. naeslundii*, ATCC strain 49340) [28]. IgG titers were quantified using chemiluminescent signal-measuring instrument and compared to human IgG standard curve [27]. Specifically, 8,153 stored serums among NHANES III participants aged 40 years or older were analyzed at Columbia University College of Dental Medicine, New York, NY between 2003 and 2006 (Phase 2 then Phase 1 samples). A factor analysis was conducted among individuals >45 years of age, to extract independent common factors, using eigenvalue and scree plot criteria and varimax rotation (Supplementary Material 2). These factors were entered simultaneously into models examining associations between periodontal pathogens and AD mortality, AD incidence and all-cause dementia incidence. Finally, we created modified mutually-exclusive color-coded clusters that were determined using cluster analysis in a previous analysis of all available periodontal pathogen data (k = 19, 40+years, NHANES III, both phases) [28], and analysis also used by others [29]. Those clusters were defined based on correlated Log_e transformed pathogen IgG titers as shown in Supplementary Figure 1 [28]. In our present study, we first summed Log_e transformed IgG to form the clusters of correlated pathogen titers. Those summation clusters were then z-scored to allow for better interpretation in our main models.

In phase 2 of NHANES III, 9,371 surplus sera on two periodontal pathogens were analyzed (*P. gingivalis* and *A. actinomycetemcomitans*) among participants aged 12 years or older of whom 3,828 were 45+years. Antibody concentrations were measured in ELISA units of IgG (EU) and were examined in both untransformed and Log_e transformed metrics, for comparative purposes.

Covariates

Models were stratified by baseline age group (45+, 55+, and 65+) and sex. Demographic,

socio-economic, social support, lifestyle and health-related factors, dietary quality and nutritional biomarkers were potential confounders included in all models (See Supplementary Material 2 for details).

Statistical analysis

We performed analyses using Stata 15.0 (StataCorp, College Station, TX) [30]. We accounted for survey design complexity by incorporating 6-year (NHANES III, 1988–1994) and 3-year (NHANES III, 1991–1994) primary sampling units and strata. Standard errors were estimated using Taylor series linearization (i.e., *svy*: commands) [30]. Multivariate imputed data (m = 5 imputations, 10 iterations) with chained equations [31] estimated means and proportions across age groups and measures of association, after adjusting for sampling design complexity.

The main exposures of interest were 19 periodontal pathogens added simultaneously (Model 1) and separately (Model 2) for the entire NHANES III sample (1988–1994). Factors extracted from those 19 periodontal pathogens, as well as pre-determined color-coded clusters, were also considered as predictors of interest. For phase 2 NHANES III, *A. Actinomycetemcomitans* and *P. gingivalis* were main predictors, analyzed as standardized z-scores (as is and Log_e transformed). These periodontal pathogens were then entered as predictors (both phases and phase 2, separately) into linear regression models with periodontitis measured with AL and PPD as outcomes while adjusting for all covariates. Finally, means of AL/PPD were considered as separate predictors in main causal models, specifically incident AD and all-cause dementia due to smaller sample size for AD mortality outcome. In those models, defining time-to-event from any age ≥45 years since baseline visit (i.e., delayed entry) until death or censoring or outcome of interest (AD death, AD incidence, all-cause dementia incidence), we conducted Cox proportional hazards models for three outcomes stratifying separately by sex and baseline age to ≥45, ≥55, and ≥65 years. We present fully adjusted models accounting for demographic, socio-economic, lifestyle/social support factors, nutritional biomarkers, and health-related factors (Supplementary Material 2). Weighted mean times of follow-up are estimated from weighted person-months of follow-up and the weighted sample in each model ([Person-months$_{(\text{weighted})}$]/[Persons$_{(\text{weighted})}$]). A type I error of 0.05 was considered for statistical significance and 0.10 for borderline (or marginal) significance. Multiple testing adjustment was done using a familywise Bonferroni approach while accounting for outcome multiplicity (e.g., AL/PPD: 2 outcomes; incident AD/all-cause dementia/AD mortality: 3 outcomes) and assuming that each exposure, model and strata was a distinctive hypothesis [32].

RESULTS

Participant characteristics by age group and sex

Cumulative incidence (weighted) of all three outcomes increased linearly with baseline age, with AD dementia, all-cause dementia and AD mortality reaching 18%, 38%, and 3% in the 65+ baseline age group, respectively. Women in this sample were more likely to be older, and baseline age was also directly related to Non-Hispanic white race, smaller household size, higher proportion widowed, larger means of AL and PPD, and higher proportions completely or partially edentulous (Table 1 and Supplementary Table 1). However, most periodontal pathogen titers were either unrelated or inversely linked to age, with the clearest inverse relationship shown for Orange Blue and Yellow Orange clusters, and for *Factor 3* comprised of *E. nodatum* and *A. naeslundii IgG* titers. Moreover, socio-economic status was associated with younger age, while age was directly related to better dietary quality as measured by the 1995 Healthy Eating Index, co-morbidity and AL, reduced physical activity, smoking and drug use, lower prevalence of obesity, reduced mean of 25(OH)D coupled with increased levels of folate, vitamin A, vitamin E, total carotenoids, and ferritin.

Periodontal pathogens' association with AD mortality, AD and all-cause dementia incidence

After correction for multiple testing (Table 2), Phase 2 *P. gingivalis IgG* titers (un-transformed, z-scores) were associated with increased risk for incident AD dementia, particularly among women (1 SD = 212, HR = 1.14, 95% CI: 1.05–1.23, $p = 0.004$) and individuals above 55 (HR = 1.06) or 65 years (HR = 1.12) at baseline. Log_e transformed *P. gingivalis IgG* titers were marginally associated with increased risk for AD mortality in 65+ age group, while the reverse was true for *A. Actinomycetemcomi-*

Table 1
Baseline characteristics of selected participants by age group, NHANES III, 1988–1994 [N = 3,749 (phase 2:1991–1994); N = 6,650 (both phases)][a]

Selected participant characteristics	Age group (y)			*p*-value* (Design-based F-test)[b]	
	45–55	55–65	65+		
Unweighted N (both phases)	(N = 1,701)	(N = 1,698)	(N = 3,251)	55–65 versus	65+ versus
	25.6%	25.5%	48.9%	45–55	45–55
Cumulative incidence of AD and all-cause dementia and of AD mortality, weighted %					
AD dementia	1.7 ± 0.3	11.2 ± 1.1	18.3 ± 0.9	<0.001	<0.001
All-cause dementia	4.3 ± 0.5	18.9 ± 1.3	37.5 ± 1.1	<0.001	<0.001
AD mortality	0.1 ± 0.1	1.1 ± 0.4	2.9 ± 0.4	0.028	0.001
Dental measures					
Periodontitis, mean ± SE	(N = 1,435)	(N = 1,225)	(N = 1,805)		
Attachment Loss	1.45 ± 0.05	1.76 ± 0.07	1.98 ± 0.07	0.03	<0.001
Probing Pocket Depth	1.50 ± 0.03	1.53 ± 0.04	1.45 ± 0.03	0.29	0.28
Factors (z-scores), mean ± SE	(N = 1,597)	(N = 1,604)	(N = 3,077)		
Factor 1	–0.05 ± 0.09	+0.00 ± 0.09	0.02 ± 0.08	0.35	0.24
Factor 2	–0.05 ± 0.04	–0.09 ± 0.04	–0.15 ± 0.04	0.46	0.063
Factor 3	+0.21 ± 0.04	+0.13 ± 0.04	–0.04 ± 0.03	0.10	<0.001
Factor 4	–0.07 ± 0.06	–0.15 ± 0.04	–0.09 ± 0.04	0.09	0.62
Factor 5	+0.05 ± 0.07	–0.04 ± 0.05	+0.03 ± 0.04	0.12	0.84
Clusters (z-scores), mean ± SE	(N = 1,655)	(N = 1,655)	(N = 3,169)		
Orange Red	–0.097 ± 0.04	–0.150 ± 0.040	–0.183 ± 0.036	0.35	0.078
Red Green	–0.031 ± 0.010	–0.071 ± 0.085	–0.046 ± 0.071	0.55	0.83
Yellow Orange	+0.02 ± 0.06	–0.02 ± 0.06	–0.10 ± 0.05	0.51	0.020
Orange Blue	+0.19 ± 0.04	+0.10 ± 0.05	–0.10 ± 0.04	0.08	<0.001
Dentate status	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Completely edentulous	9.2 ± 1.11	19.0 ± 1.24	32.2 ± 1.87	--	--
Edentulous in one arch	10.5 ± 1.22	14.5 ± 1.07	14.8 ± 0.95	<0.001	<0.001
Teeth complete	80.3 ± 1.48	66.5 ± 1.54	52.9 ± 2.04	0.001	<0.001
Periodontal pathogen IgG					
Phase 2	(N = 895)	(N = 914)	N = 1,826)		
P. gingivalis					
Continuous	109.2 ± 6.34	105.2 ± 4.2	122.3 ± 7.55	0.49	0.16
Log_e transformed	4.49 ± 0.03	4.47 ± 0.02	4.51 ± 0.02	0.30	0.64
A. Actinomycetemcomitans (Aa)					
Continuous	99.3 ± 5.53	102.8 ± 5.15	94.5 ± 2.87	0.66	0.39
Log_e transformed	4.45 ± 0.03	4.46 ± 0.02	4.44 ± 0.02	0.79	0.61
Both Phases, Log_e transformed[c]	(N = 1.622–1,692)	(N = 1,617–1685)	(N = 3,116–3,227)		
P. Gingivalis (Pg) mix	5.7 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	0.44	0.34
P. Intermedia (Pi)	5.6 ± 0.1	5.5 ± 0.1	5.3 ± 0.0	0.57	0.002
P. Nigrescens (Pn)	5.3 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0.20	0.06
T. Forsythia (Tf)	4.8 ± 0.1	4.7 ± 0.1	4.7 ± 0.1	0.37	0.76
A. Actinomycetemcomitans (Aa) mix	6.7 ± 0.1	6.7 ± 0.1	6.6 ± 0.1	0.65	0.41
F. Nucleatum (Fn)	4.4 ± 0.1	4.4 ± 0.1	4.4 ± 0.1	0.78	0.15
S. Oralis (So)	4.3 ± 0.1	4.3 ± 0.1	4.2 ± 0.1	0.64	0.54
M. Micros (Mm)	5.2 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	0.52	0.13
C. Rectus (Cr)	4.4 ± 0.1	4.3 ± 0.1	4.4 ± 0.1	0.11	0.73
E. Corrodens (Ec)	5.2 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	0.63	0.15
E. Nodatum (En)	7.3 ± 0.1	7.1 ± 0.1	6.8 ± 0.1	0.09	<0.001
S. Intermedius (Si)	5.2 ± 0.1	5.1 ± 0.1	5.0 ± 0.1	0.68	0.002
C. Ochracea (Co)	5.0 ± 0.1	4.8 ± 0.1	4.7 ± 0.0	0.052	0.002
V. Parvula (Vp)	3.6 ± 0.1	3.7 ± 0.1	3.8 ± 0.1	0.97	0.021
A. Naeslundii (An)	6.1 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	0.10	<0.001
P. Melaninogenica (Pm)	5.3 ± 0.1	5.4 ± 0.1	5.4 ± 0.1	0.99	0.35
S. Noxia (Sn)	3.7 ± 0.2	3.6 ± 0.1	3.5 ± 0.1	0.21	0.21
T. Denticola (Td)	4.9 ± 0.1	4.8 ± 0.1	4.8 ± 0.1	0.35	0.43
S. Mutans (Sm)	4.5 ± 0.1	4.5 ± 0.1	4.4 ± 0.1	0.82	0.40
Socio-demographic characteristics	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Age (y)	49.1 ± 0.11	59.3 ± 0.09	73.6 ± 0.24	<0.001	<0.001
Sex, % male	48.5 ± 1.75	44.8 ± 1.12	41.4 ± 1.12	0.07	0.002

Race/ethnicity	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Non-Hispanic White	79.7 ± 1.80	79.0 ± 2.06	86.6 ± 1.30	--	--
Non-Hispanic Black	8.8 ± 0.74	9.6 ± 0.90	7.3 ± 0.76	0.39	0.04
Mexican-American	4.3 ± 0.43	2.9 ± 0.31	1.9 ± 0.17	0.003	<0.001
Other	7.3 ± 1.46	8.5 ± 1.97	4.3 ± 0.85	0.54	0.02
Urban/rural area of residence	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Urban	50.1 ± 4.69	46.1 ± 4.53	43.2 ± 5.11	0.18	0.06
Rural	49.1 ± 4.69	53.9 ± 4.53	56.8 ± 5.11	--	--
	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Household size	2.9 ± 0.06	2.5 ± 0.04	1.9 ± 0.03	<0.001	<0.001
Marital status	(N = 1,701)	(N = 1,698)	(N = 3,251)		
Never married	5.4 ± 0.97	3.1 ± 0.43	4.0 ± 0.41	0.02	0.93
Married	73.7 ± 2.12	71.8 ± 1.5	54.9 ± 1.45	--	--
Divorced	13.7 ± 1.72	10.4 ± 1.06	5.4 ± 0.57	0.14	0.003
Widowed	2.3 ± 0.39	9.7 ± 0.9	33.3 ± 1.17	<0.001	<0.001
Other	4.8 ± 0.61	4.9 ± 0.85	2.5 ± 0.39	0.82	0.08

25(OH)D, 25-hydroxyvitamin D; AD, Alzheimer's disease; EU, ELISA units; HEI, Healthy Eating Index; HS, high school; IgG, Immunoglobulin G; MAR, Mean Adequacy Ratio; NHANES, National Health and Nutrition Examination Surveys. [a] Values are weighted means ± SEM or percent ± SEP, taking into account sampling design complexity (PSU and strata), across 5 imputations with 10 iterations. [b]Design-based F-test accounting for design complexity in terms of sampling weights, PSU and stratum. for categorical variables, this was the equivalent of a χ^2 test of independence restricting the sample first to 55–64/45–54, then to 65 + /45–54. For continuous variables, it was the equivalent of a Wald test in a linear regression model with the variable being the outcome predicted by age group and in which 45–54 years was the referent category to which "55–64" and "65+" were compared. [c]SD of Log_e transformed periodontal pathogens across groups ranged between 1.2–1.3 (*Co, Vp*) and 1.8–2.0 (*Pg, En, An*), with the remaining ranging between 1.4 and 1.6.

tans IgG (both transformed and untransformed, 65+). When examining all 19 periodontal pathogens (Log_e transformed, z-scored, 1988–1994) in relation to the three dementia outcomes of interest (Supplementary Tables 2 and 3), and upon multiple testing adjustment, we found that *S. oralis IgG* was linked with increased risk for all-cause dementia among men, a pattern observed among women for *E. corrodens IgG* (*Model 1*: all pathogens entered). Similarly, *C. rectus IgG* was associated with increased risk for all-cause dementia in all age groups, a pattern that was consistent between models 1 and 2 among the older group (65+). *C. rectus IgG* was also marginally and directly associated with incident AD risk in the 55+ age group (Model 1), while *S. intermedius* was marginally and inversely associated with incident AD risk among women (Model 1). For AD mortality (Table 3), most of our findings emerged when each periodontal pathogen was entered separately into the model (*Model 2*). Most notably, *P. gingivalis IgG* (Log_e transformed, z-score) was associated with increased AD mortality risk among those aged 65+ at baseline (1 SD = 2.03, HR = 1.36, 95% CI: 1.10–1.69, $p = 0.010$), as was the case for *P. melaninogenica IgG* (1 SD = 1.28, HR = 1.43, 95% CI: 1.11–1.85, $p = 0.009$). The latter finding was further strengthened by adding the remaining 18 titers into the model (HR = 1.73, $p = 0.005$). Consistent with all-cause dementia (Supplementary Table 2), Table 3 also indicates that *So* was directly related to AD mortality risk among men (Model 2). Additionally, AD mortality risk was increased with higher *S. intermedius IgG*.

Periodontal pathogen factor and clusters and their association with AD mortality, AD and all-cause dementia incidence

Using factor analysis and pre-defined clusters (Table 4), our results indicated that AD incidence was associated with *Factor 4* in the 65+ age group, which loaded highly on *C. rectus* and *P. gingivalis* titers (aHR = 1.22; 95% CI, 1.04–1.43, $p = 0.012$). In this model, the effect of 1 SD increase in *Factor 4* on AD incidence was equivalent to two years of aging on the Log_e(HR) scale. Moreover, AD mortality risk was increased with higher baseline Factor 2 in that age group (per SD, aHR = 1.46; 95% CI, 1.09–1.96, $p = 0.017$) which loaded highly on *P. gingivalis*, *P. intermedia*, *P. nigrescens*, *F. nucleatum*, *C. rectus*, *S. intermedius*, *C. ochracea* and *P. melaninogenica* titers. In both 55+ and 65+ age group, Orange-Red cluster (*P. melaninogenica*, *P. intermedia*, *P. nigrescens*, *P. gingivalis*) was associated with increased AD mortality risk, while Red-Green cluster (*T. forsythia*, *T. denticola*, *A. actinomycetemcomitans*, *E. corrodens*, *S. noxia*, *V. parvula*, *C. rectus*) was only marginally associated with AD and all-cause dementia among women ($p < 0.033$), after correction for multiple testing.

Table 2
P. gingivalis and *A. actinomycetemcomitans* serum IgG's association with incident all-cause and Alzheimer's disease (AD) dementia and with AD mortality in multiple Cox proportional hazards model, overall and stratified by sex and race: NHANES III, 1991–1994[a]

	z-scored periodontal pathogen IgG (Phase II)[b]			z-scored, Log_e transformed periodontal pathogen IgG (Phase II)[b]		
	Log_e (HR)	(SE)	p	Log_e(HR)	(SE)	p
All-cause dementia[c]						
Men (N = 1,646)						
P. gingivalis	–0.19	(0.17)	0.31	–0.12	(0.10)	0.26
A. actinomycetemcomitans	+0.03	(0.06)	0.62	+0.03	(0.06)	0.66
Women (N = 1,979)						
P. gingivalis	+0.07	(0.07)	0.34	–0.04	(0.10)	0.69
A. actinomycetemcomitans	+0.05	(0.05)	0.36	+0.03	(0.07)	0.62
45+ at baseline (N = 3,625)						
P. gingivalis	–0.01	0.11	0.92	–0.08	(0.06)	0.19
A. actinomycetemcomitans	+0.04	(0.04)	0.34	+0.03	(0.06)	0.60
55+ at baseline (N = 2,731)						
P. gingivalis	+0.01	(0.09)	0.88	–0.08	(0.06)	0.21
A. actinomycetemcomitans	–0.01	(0.05)	0.91	–0.01	(0.05)	0.81
65+ at baseline (N = 1,817)						
P. gingivalis	+0.04	(0.07)	0.59	–0.06	(0.06)	0.33
A. actinomycetemcomitans	**+0.12**	**(0.06)**	**0.051**	+0.08	(0.06)	0.19
AD dementia[d]						
Men (N = 1,646)						
P. gingivalis	–0.05	(0.21)	0.81	–0.11	(0.12)	0.37
A. actinomycetemcomitans	+0.10	(0.11)	0.34	+0.11	(0.11)	0.34
Women (N = 1,979)						
P. gingivalis	+0.13	(0.04)	**0.004****	+0.04	(0.06)	0.58
A. actinomycetemcomitans	–0.03	(0.11)	0.81	–0.09	(0.11)	0.40
45+ at baseline (N = 3,625)						
P. gingivalis	**+0.06**	**(0.03)**	**0.034**	–0.04	(0.06)	0.50
A. actinomycetemcomitans	+0.01	(0.06)	0.84	–0.00	(0.07)	0.95
55+ at baseline (N = 2,731)						
P. gingivalis	**+0.06**	**(0.02)**	**0.015****	–0.03	(0.06)	0.64
A. actinomycetemcomitans	–0.04	(0.08)	0.62	–0.07	(0.07)	0.28
65+ at baseline (N = 1,817)						
P. gingivalis	**+0.11**	**(0.03)**	**0.003****	+0.03	(0.07)	0.72
A. actinomycetemcomitans	+0.07	(0.07)	0.35	+0.02	(0.07)	0.73
AD mortality[e]						
45+ at baseline (N = 3,625)						
P. gingivalis	+0.11	(0.07)	0.13	+0.14	(0.14)	0.31
A. actinomycetemcomitans	–0.03	(0.34)	0.94	–0.27	(0.30)	0.31
55+ at baseline (N = 2,731)						
P. gingivalis	+0.09	(0.06)	0.13	+0.16	(0.14)	0.27
A. actinomycetemcomitans	–0.02	(0.35)	0.95	–0.33	(0.36)	0.38
65+ at baseline (N = 1,817)						
P. gingivalis	**+0.19**	**(0.15)**	**0.21**	**+0.35**	**(0.15)**	**0.033***
A. actinomycetemcomitans	**–2.61**	**(0.73)**	**0.002****	**–1.23**	**(0.28)**	**<0.001****

25(OH)D, 25-hydroxyvitamin D; AD, Alzheimer's disease; EU, ELISA units; HEI, Healthy Eating Index; HR, hazard ratio; HS, high school; IgG, Immunoglobulin G; MAR = Mean Adequacy Ratio; NHANES = National Health and Nutrition Examination Surveys. [a]Models were adjusted for age, sex, race/ethnicity, poverty income ratio, education (years), urban-rural area of residence, household size, marital status, nutritional factors (HEI, MAR), nutritional biomarkers (25(OH)D, folate, vitamin C, vitamin A, total carotenoids, vitamin E, ferritin, selenium and normalized calcium), lifestyle (smoking, drug use, alcohol, physical activity), health-related factors (self-rated health, co-morbidity index, allostatic load, weight status), dentate status and social support variables. Covariates (other than exposures) were imputed and analysis is across 5 imputations with 10 iterations. [b]Standardized into z-scores. 1 SD of untransformed Pg is 434 (45+), 148 (55+), 276 (65+), 605 (Men), 212 (Women); 1 SD of untransformed Aa is 87 (45+), 82 (55+), 72 (65+),85 (Men), 88 (Women). 1 SD of Log_e transformed Pg is ~0.85–0.90 for all groups; 1 SD of Log_e transformed Aa is ~0.85–0.90 for all groups. [c]997 unweighted incident dementia cases for 45+, weighted mean follow-up time: 185 months. [d]503 unweighted incident AD cases for 45+, weighted mean follow-up time: 189 months. [e]52 unweighted AD deaths for 45+, weighted mean follow-up time: 192 months. *$p < 0.033$, marginally significant after correction for multiple testing; **$p < 0.016$, significant after correction for multiple testing.

Table 3
Periodontal pathogens' serum IgG association with AD mortality in multiple Cox proportional hazards model, overall and restricted by baseline age group and sex: NHANES III, 1988–1994[a,b]

	≥45 y[c]			≥55 y[d]			≥65 y[e]			Men[f]			Women[g]		
	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p
AD mortality															
P. Gingivalis mix															
Model 1	+0.14	(0.13)	0.88	+0.15	(0.14)	0.28	**+0.35**	**(0.17)**	**0.046**	+0.25	(0.30)	0.40	+0.02	(0.25)	0.93
Model 2	+0.15	(0.10)	0.16	+0.17	(0.11)	0.13	**+0.31**	**(0.11)**	**0.010****	+0.33	(0.20)	0.11	–0.02	(0.13)	0.87
P. Intermedia															
Model 1	–0.08	(0.25)	0.74	–0.14	(0.27)	0.61	–0.19	(0.32)	0.57	+0.11	(0.52)	0.84	–0.04	(0.38)	0.93
Model 2	+0.20	(0.14)	0.15	+0.19	(0.13)	0.16	**+0.29**	**(0.13)**	**0.026***	+0.02	(0.14)	0.86	+0.11	(0.19)	0.58
P. Nigrescens															
Model 1	+0.18	(0.20)	0.36	+0.28	(0.21)	0.20	+0.18	(0.29)	0.54	–0.10	(0.66)	0.87	+0.10	(0.26)	0.69
Model 2	**+0.22**	**(0.13)**	**0.085**	**+0.25**	**0.13**	**0.063**	**+0.33**	**(0.14)**	**0.023***	**+0.43**	**(0.21)**	**0.049**	+0.11	(0.11)	0.46
T. Forsythia															
Model 1	**+0.43**	**(0.22)**	**0.058**	**+0.41**	**(0.21)**	**0.060**	+0.39	(0.25)	0.13	+0.86	(0.57)	0.14	**+0.64**	**(0.26)**	**0.019***
Model 2	+0.23	(0.14)	0.10	+0.22	(0.14)	0.12	+0.34	(0.16)	0.036	+0.49	(0.22)	**0.031***	+0.13	(0.17)	0.43
A. Actinomycetemcomitans (Aa) mix															
Model 1	**–0.38**	**(0.26)**	**0.15**	**–0.48**	**(0.27)**	**0.078**	–0.71	(0.45)	0.12	–0.05	(0.35)	0.88	**–0.80**	**(0.38)**	**0.039**
Model 2	–0.03	(0.15)	0.82	–0.10	(0.15)	0.52	–0.02	(0.19)	0.92	+0.59	(0.28)	0.042	–0.31	(0.20)	0.12
F. Nucleatum															
Model 1	–0.08	(0.28)	0.77	–0.04	(0.28)	0.89	+0.26	(0.35)	0.46	–0.77	(0.48)	0.12	+0.17	(0.40)	0.68
Model 2	+0.03	(0.13)	0.84	+0.02	(0.13)	0.86	+0.18	(0.14)	0.18	**+0.38**	**(0.19)**	**0.053**	–0.04	(0.16)	0.78
S. Oralis															
Model 1	+0.10	(0.26)	0.69	+0.03	(0.26)	0.90	+0.14	(0.31)	0.66	**+1.14**	**(0.46)**	**0.019***	–0.29	(0.29)	0.32
Model 2	+0.05	(0.13)	0.72	+0.02	(0.13)	0.87	+0.16	(0.13)	0.22	**+0.64**	**(0.25)**	**0.014****	–0.16	(0.16)	0.31
M. Micros															
Model 1	+0.21	(0.19)	0.29	+0.17	(0.20)	0.41	–0.20	(0.24)	0.39	–0.34	(0.32)	0.29	+0.42	(0.28)	0.15
Model 2	+0.15	(0.10)	0.16	+0.10	(0.12)	0.42	+0.07	(0.14)	0.64	+0.19	(0.21)	0.38	+0.13	(0.14)	0.35
C. Rectus															
Model 1	–0.13	(0.18)	0.49	–0.03	(0.19)	0.89	–0.11	(0.26)	0.69	–0.80	(0.52)	0.13	+0.02	(0.22)	0.91
Model 2	+0.07	(0.12)	0.57	+0.01	(0.16)	0.96	+0.23	(0.14)	0.10	+0.18	(0.22)	0.42	–0.04	(0.14)	0.76
E. Corrodens															
Model 1	+0.12	(0.30)	0.68	–0.01	(0.28)	0.98	+0.27	(0.35)	0.44	–0.13	(0.39)	0.74	+0.13	(0.38)	0.74
Model 2	+0.08	(0.18)	0.67	+0.02	(0.17)	0.90	+0.19	(0.15)	0.23	+0.29	(0.24)	0.23	–0.02	(0.24)	0.92
E. Nodatum															
Model 1	–0.01	(0.19)	0.96	–0.01	(0.21)	0.98	+0.15	(0.22)	0.50	–0.51	(0.40)	0.21	–0.12	(0.31)	0.71
Model 2	+0.06	(0.17)	0.73	+0.02	(0.17)	0.90	+0.20	(0.19)	0.31	**+0.32**	**(0.19)**	**0.094**	–0.12	(0.25)	0.64

(*Continued*)

Table 3
(*Continued*)

	≥45 y[c]			≥55 y[d]			≥65 y[e]			Men[f]			Women[g]		
	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p
S. Intermedius															
Model 1	–0.09	(0.24)	0.71	–0.23	(0.23)	0.33	–0.11	(0.31)	0.72	+0.93	(0.37)	0.78	–0.35	(0.32)	0.28
Model 2	+0.04	(0.12)	0.72	–0.02	(0.12)	0.88	+0.15	(0.15)	0.33	**+0.72**	**(0.21)**	**0.001****	–0.19	(0.16)	0.26
C. Ochracea															
Model 1	+0.08	(0.17)	0.64	+0.10	(0.18)	0.56	+0.07	(0.23)	0.76	+0.10	(0.34)	0.78	–0.04	(0.22)	0.86
Model 2	+0.05	(0.13)	0.72	+0.06	(0.14)	0.68	+0.09	(0.17)	0.58	+0.21	(0.16)	0.19	–0.08	(0.17)	0.61
V. Parvula															
Model 1	–0.13	(0.28)	0.65	+0.02	(0.25)	0.93	–0.10	(0.29)	0.73	+0.08	(0.41)	0.85	–0.06	(0.32)	0.85
Model 2	+0.06	(0.14)	0.68	+0.02	(0.16)	0.91	+0.17	(0.15)	0.26	**+0.46**	**(0.23)**	**0.051**	–0.10	(0.18)	0.58
A. Naeslundii															
Model 1	+0.03	(0.18)	0.89	–0.02	(0.19)	0.92	–0.04	(0.23)	0.86	+0.57	(0.51)	0.27	–0.06	(0.21)	0.77
Model 2	+0.04	(0.16)	0.80	+0.22	(0.14)	0.12	+0.11	(0.19)	0.57	**+0.48**	**(0.20)**	**0.020***	–0.18	(0.21)	0.39
P. Melaninogenica															
Model 1	+0.30	(0.21)	0.17	+0.36	(0.22)	0.11	**+0.55**	**(0.19)**	**0.005****	+0.20	(0.38)	0.59	+0.42	(0.27)	0.12
Model 2	+0.20	(0.13)	0.14	+0.22	(0.14)	0.12	**+0.36**	**(0.13)**	**0.009****	**+0.41**	(0.20)	**0.047**	+0.15	(0.18)	0.40
S. Noxia															
Model 1	–0.15	(0.22)	0.50	–0.14	(0.22)	0.53	–0.14	(0.25)	0.57	–0.04	(0.32)	0.91	–0.34	(0.31)	0.28
Model 2	+0.02	(0.12)	0.87	+0.04	(0.13)	0.77	+0.13	(0.13)	0.31	**+0.37**	**(0.21)**	**0.092**	–0.16	(0.17)	0.36
T. Denticola															
Model 1	–0.10	(0.25)	0.68	–0.06	(0.24)	0.79	–0.18	(0.25)	0.48	–0.15	(0.32)	0.64	–0.03	(0.32)	0.92
Model 2	–0.09	(0.13)	0.49	–0.09	(0.13)	0.49	–0.05	(0.13)	0.70	+0.17	(0.25)	0.50	–0.13	(0.18)	0.48
S. Mutans															
Model 1	–0.26	(0.23)	0.27	–0.26	(0.24)	0.30	–0.19	(0.27)	0.49	–0.40	(0.29)	0.18	+0.03	(0.27)	0.90
Model 2	–0.07	(0.13)	0.59	–0.08	(0.13)	0.52	+0.02	(0.14)	0.86	**+0.35**	**(0.19)**	**0.074**	–0.22	(0.16)	0.17

AD, Alzheimer's disease; BMI, body mass index; HR, hazard ratio; HS, high school; IgG, Immunoglobulin G; NHANES, National Health and Nutrition Examination Surveys. [a]Models were adjusted for age, sex, race/ethnicity, poverty income ratio, education (years), urban-rural area of residence, household size, marital status, nutritional factors (HEI, MAR), nutritional biomarkers (25(OH)D, folate, vitamin C, vitamin A, total carotenoids, vitamin E, ferritin, selenium and normalized calcium), lifestyle (smoking, drug use, alcohol, physical activity), health-related factors (self-rated health, co-morbidity index, allostatic load, weight status), dentate status and social support variables, as well as Phase of NHANES III. Covariates (other than exposures) were imputed and analysis is across 5 imputations with 10 iterations. Model 1: adjusted for all other periodontal pathogens; Model 2: one periodontal pathogen at a time. [b]Periodontal pathogen exposures were Log_e transformed and then standardized into z-scores. [c]Unweighted N = 6,277–6,581, weighted mean follow-up time: 200 months; [d]Unweighted N = 4,681–4,912, weighted mean follow-up time = 177 months; [e] Unweighted N = 3,077–3,229, weighted mean follow-up time: 148 months; [f]Unweighted N = 2,924–3,088, weighted mean follow-up time: 196 months [g]Unweighted N = 3,353–3,517, weighted mean follow-up time: 205 months. *$p < 0.033$, marginally significant after correction for multiple testing; **$p < 0.016$, significant after correction for multiple testing.

Table 4
Periodontal pathogens' serum IgG (Factor scores and pre-defined clusters) association with AD mortality, AD incidence and all-cause dementia incidence in multiple Cox proportional hazards model, overall and restricted by baseline age group and sex: NHANES III, 1988–1994[a,b]

	≥45 y[c]			≥55 y[d]			≥65 y[e]			Men[f]			Women[g]		
	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p
Factor scores															
AD mortality															
Factor 1: So/Sm/Ec	–0.12	(0.16)	0.48	–0.23	(0.15)	0.14	–0.08	(0.17)	0.61	+0.56	(0.40)	0.17	–0.30	(0.24)	0.24
Factor 2:Pi/Pn/Pm	**+0.26**	**(0.14)**	**0.073**	**+0.31**	**(0.15)**	**0.043**	**+0.38**	**(0.15)**	**0.017****	+0.43	(0.29)	0.15	+0.19	(0.20)	0.35
Factor 3:An/En	+0.06	(0.19)	0.75	+0.02	(0.19)	0.90	+0.13	(0.23)	0.58	**+0.44**	**(0.24)**	**0.081**	–0.14	(0.27)	0.35
Factor 4:Pg/Cr	+0.12	(0.14)	0.40	+0.16	(0.15)	0.31	+0.19	(0.16)	0.24	–0.29	(0.28)	0.30	+0.16	(0.16)	0.32
Factor 5:Co/Sn	+0.04	(0.18)	0.82	**+0.23**	**(0.13)**	**0.081**	+0.18	(0.19)	0.34	–0.44	(0.39)	0.27	+0.17	(0.24)	0.48
AD incidence															
Factor 1: So/Sm/Ec	+0.06	(0.06)	0.34	+0.00	(0.07)	0.97	+0.06	(0.08)	0.48	+0.08	(0.14)	0.57	+0.08	(0.07)	0.25
Factor 2:Pi/Pn/Pm	+0.01	(0.05)	0.92	+0.01	(0.05)	0.86	+0.02	(0.06)	0.80	–0.00	(0.13)	1.00	–0.03	(0.07)	0.25
Factor 3:An/En	–0.08	(0.06)	0.20	–0.10	(0.06)	0.14	–0.11	(0.07)	0.13	–0.06	(0.11)	0.59	–0.10	(0.08)	0.25
Factor 4:Pg/Cr	+0.10	(0.06)	0.14	+0.11	(0.07)	0.11	**+0.20**	**(0.08)**	**0.012****	+0.12	(0.15)	0.44	**+0.17**	**(0.08)**	**0.038**
Factor 5:Co/Sn	–0.06	(0.09)	0.49	–0.02	(0.10)	0.83	–0.02	(0.12)	0.89	–0.05	(0.14)	0.72	–0.06	(0.09)	0.52
All-cause dementia incidence															
Factor 1: So/Sm/Ec	**+0.10**	**(0.05)**	**0.062**	+0.06	(0.05)	0.28	**+0.09**	**(0.05)**	**0.073**	+0.11	(0.09)	0.22	**+0.10**	**(0.06)**	**0.073**
Factor 2:Pi/Pn/Pm	–0.05	(0.04)	0.23	–0.03	(0.04)	0.55	–0.02	(0.04)	0.70	+0.02	(0.08)	0.83	**–0.10**	**(0.06)**	**0.070**
Factor 3:An/En	–0.07	(0.04)	0.15	–0.06	(0.04)	0.19	–0.07	(0.05)	0.16	–0.04	(0.08)	0.66	–0.08	(0.06)	0.17
Factor 4:Pg/Cr	+0.02	(0.05)	0.67	+0.04	(0.05)	0.39	+0.09	(0.06)	0.12	–0.10	(0.11)	0.33	+0.06	(0.06)	0.32
Factor 5:Co/Sn	–0.09	(0.06)	0.13	–0.06	(0.06)	0.39	–0.05	(0.07)	0.48	–0.15	(0.09)	0.10	–0.02	(0.07)	0.72
Pre-defined clusters															
AD mortality															
Cluster 1: Orange-Red	**+0.36**	**(0.16)**	**0.031***	**+0.44**	**(0.18)**	**0.016****	**+0.56**	**(0.17)**	**0.002****	+0.46	(0.47)	0.34	+0.29	(0.27)	0.28
Cluster 2: Red-Green	–0.22	(0.30)	0.47	–0.20	(0.31)	0.54	–0.16	(0.32)	0.61	–0.41	(0.75)	0.59	–0.18	(0.35)	0.61
Cluster 3: Yellow-Orange	+0.01	(0.20)	0.97	–0.07	(0.21)	0.73	–0.09	(0.25)	0.72	+0.38	(0.42)	0.37	–0.06	(0.23)	0.81
Cluster 4: Orange-Blue	+0.05	(0.18)	0.79	+0.01	(0.18)	0.94	+0.11	(0.22)	0.63	+0.34	(0.24)	0.16	–0.15	(0.26)	0.55

(*Continued*)

Table 4
(*Continued*)

	≥45 y[c]			≥55 y[d]			≥65 y[e]			Men[f]			Women[g]		
	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p	Log_e(HR)	(SE)	p
AD incidence															
Cluster 1: Orange-Red	+0.02	(0.07)	0.75	+0.05	(0.08)	0.52	+0.05	(0.09)	0.57	–0.04	(0.14)	0.79	–0.00	(0.09)	0.99
Cluster 2: Red-Green	+0.16	(0.10)	0.12	+0.14	(0.09)	0.12	**+0.22**	**(0.11)**	**0.052**	–0.17	(0.21)	0.40	**+0.30**	**(0.13)**	**0.028***
Cluster 3: Yellow-Orange	–0.12	(0.09)	0.21	**–0.16**	**(0.09)**	**0.093**	–0.16	(0.12)	0.16	+0.22	(0.16)	0.19	**–0.22**	**(0.13)**	**0.092**
Cluster 4: Orange-Blue	–0.08	(0.06)	0.19	–0.08	(0.06)	0.17	–0.10	(0.06)	0.11	–0.05	(0.10)	0.64	–0.10	(0.08)	0.21
All-cause dementia incidence															
Cluster 1: Orange-Red	–0.05	(0.06)	0.37	–0.03	(0.06)	0.64	–0.04	(0.06)	0.53	+0.02	(0.10)	0.86	–0.11	(0.08)	0.15
Cluster 2: Red-Green	+0.12	(0.07)	0.11	**+0.12**	**(0.07)**	**0.083**	**+0.15**	**(0.09)**	**0.092**	–0.15	(0.15)	0.33	**+0.21**	**(0.09)**	**0.018***
Cluster 3: Yellow-Orange	–0.05	(0.06)	0.44	–0.07	(0.06)	0.22	–0.04	(0.09)	0.64	+0.14	(0.13)	0.25	–0.08	(0.10)	0.43
Cluster 4: Orange-Blue	–0.06	(0.04)	0.18	–0.05	(0.04)	0.21	–0.07	(0.04)	0.64	–0.02	(0.07)	0.79	–0.08	(0.05)	0.16

See Table 1 for periodontal pathogen abbreviations. AD, Alzheimer's disease. [a]Models were adjusted for age, sex, race/ethnicity, poverty income ratio, education (years), urban-rural area of residence, household size, marital status, nutritional factors (HEI, MAR), nutritional biomarkers (25(OH)D, folate, vitamin C, vitamin A, total carotenoids, vitamin E, ferritin, selenium and normalized calcium), lifestyle (smoking, drug use, alcohol, physical activity), health-related factors (self-rated health, co-morbidity index, allostatic load, weight status), dentate status and social support variables, as well as Phase of NHANES III. Covariates (other than exposures) were imputed and analysis is across 5 imputations with 10 iterations. [b]19 Periodontal pathogen exposures (both phases) were Log_e transformed and then standardized into z-scores. Factor analysis was conducted from which 5 factors were extracted each explaining >4% of total variance. After varimax rotation, factor 1 loaded highest ($\lambda > 0.40$) on *T. forsythia* (0.68), *A. actinomycetemcomitans* (0.65), *F. nucleatum* (0.53), *S. oralis* (0.81), *M. micros* (0.51), *C. rectus* (0.54), *E. corrodens* (0.68), *S. intermedius* (0.63), *V. parvula* (0.64), *S. noxia* (0.62), *T. denticola* (0.63) and *S. mutans* (0.73); factor 2 on *P. gingivalis* (0.42), *P. itermedia* (0.85), *P. nigrescens* (0.84), *F. nucleatum* (0.44), *C. rectus* (0.40), *S. intermedius* (0.42), C. *ochracea*(0.44), *P. melaninogenica* (0.70); factor 3 on *E. nodatum* (0.75), *A. naselundii* (0.76); factor 4 on *P. gingivalis* (0.44) and *C. rectus* (0.42); and factor 5 on C. *ochracea* (0.45) and *S. noxia* (0.41). Factors were labelled based on up to 3 highest loadings, using a shortcut name for each. See Supplementary Figure 1 and methods section for definition of each cluster. [c]Unweighted N = 6,277–6,278, weighted mean follow-up time: 200 months (AD mortality), 197 months (AD incidence), 192 months (all-cause dementia); [d] Unweighted N = 4,681–4,682, weighted mean follow-up time: 177 months (AD mortality), 171 months (AD incidence), 165 months (all-cause dementia); [e] Unweighted N = 3,077, weighted mean follow-up time: 148 months (AD mortality), 141 months (AD incidence), 133 months (all-cause dementia); [f]Unweighted N = 2,924, weighted mean follow-up time: 196 months (AD mortality), 193 months (AD incidence), 189 months (all-cause dementia) [g]Unweighted N = 3,353–3,354, weighted mean follow-up time: 205 months (AD mortality), 200 months (AD incidence), 194 months (all-cause dementia). *$p < 0.033$, marginally significant after correction for multiple testing; **$p < 0.016$, significant after correction for multiple testing.

Clinical Pd markers and their association with periodontal pathogens and AD/all-cause dementia outcomes

Moreover, P. gingivalis IgG, the Orange-Red, Red-Green and Yellow-Orange clusters, Factors 2 and 4 were independently associated with clinical Pd markers (AL/PPD) (Supplementary Table 3). Nevertheless, only a marginal association between PPD and incident AD risk was detected among men and older individuals upon multiple testing adjustment (Supplementary Table 4).

DISCUSSION

To our knowledge, this is the first large retrospective cohort study to examine the association between periodontal pathogens (and measures of Pd: AL/PPD) with AD incidence and mortality and incident all-cause dementia. Our findings indicated that IgG against P. gingivalis, P. melaninogenica, and C. rectus, two empirical periodontal pathogen factors, and two empirical periodontal pathogen clusters as well as PPD were consistently linked with at least one of the 3 outcomes among older adults. Moreover, findings with all-cause dementia and not AD pertained mostly to the outcome of vascular dementia, given that it is the second most common cause of dementia.

Although there are no other studies examining the association between periodontal pathogens and incidence of dementia per se, several studies have examined the relations between periodontal pathogens and cognitive impairments that could yield dementia outcomes. Our findings of positive associations between Pd and periodontal pathogens with various dementia outcomes mirror previous findings with cognitive outcomes. Specifically, using NHANES III, a study found that the highest Pg IgG (119 ELISA Units [EU]) were more likely to exhibit poor delayed verbal recall (OR 2.89, 95% CI 1.14 to 7.29) and impaired subtraction (OR 1.95, 95% CI 1.22 to 3.11) [13]. Two other nested case-control studies of periodontal pathogens found that participants with elevated A. naeslundii IgG (0.640 ng/ml) level exhibited higher risk of AD [14], as did titers for F. nucleatum and P. intermedia [15]. Similarly, the risk of developing dementia was higher among Pd patients compared to controls (HR = 1.16, 95% CI = 1.01–1.32, $p = 0.03$) [33]. Nevertheless, recent reviews and meta-analyses examining pooled evidence on Pd and dementia came to different conclusions [12]. This discrepancy highlights the need to examine associations between periodontal pathogens with AD and other types of dementia within different sub-groups (preferably at different baseline ages), as was done in our present study. Furthermore, our study indicated that PPD, a measure of current periodontitis was associated with incident AD among older adults, though that was not the case of AL, a measure of cumulative exposure. This finding needs to be replicated in other comparable cohorts.

Suggested mechanisms linking Pd or periodontal pathogens with cognitive impairment and dementia are still speculative. First, bacterial pathogens can spread from periodontal regions to blood stream into other bodily organs. Second, toxins produced by pathogens can damage the vascular system via oxidative stress leading to atherosclerosis which may trigger dementia or stroke [12]. Third, inflammatory mediators of Pd including cytokines, chemokines, and prostaglandins can contribute to AD by triggering brain inflammation [19]. P. gingivalis and P. melaninogenica are related rod-shaped, black-pigmented, strictly anaerobic gram-negative bacteria [18]. Perhaps the most characteristic feature of P. gingivalis induced periodontitis is the production of gingipains, enzymes that can cleave proteins specifically after arginine or lysine amino acids [34], and which are secreted through a complex known as Type IX Secretion System (T9SS) protein secretion system [34]. Gingipains target host peptides with antimicrobial or anti-inflammatory activities, and by inactivating them induce edema and bleeding, in addition to allowing bacterial cells to infiltrate neutrophils [35]. Together with other virulence factors, this allows P. gingivalis to induce inflammation while evading host immune response [36], and to make use of inflammatory fluids as a source of essential nutrients (e.g., iron) [37] required for bacterial growth [38]. P. gingivalis produces proteolytic enzymes that target immunoglobulins and cell surface adhesion proteins, which could facilitate invasion and weaken host immune resp Mouse models show that the lipopolysaccharides (LPS) and the gingipains produced by Pg respectively increase accumulation of amyloid-β (Aβ) [20, 39] and enhance migration and inflammation of microglia [40], which are two hallmark pathologies of AD. Recent mouse studies have demonstrated that repeated exposure to P. gingivalis, resulting in gingipain accumulation in and around brain cells, was responsible for neurodegeneration and strongly correlated with hippocampal Aβ accumulation [20, 41] onset [42]. Importantly, Poole et al.

confirmed in an *in vitro* study of AD brain tissue and controls that that LPS from periodontal bacteria can access the AD brain during life given that labeling in the matched controls was absent. This demonstration of a known chronic oral-pathogen-related virulence factor reaching the human brains suggests an inflammatory role in the existing AD pathology [43]. Moreover, Dominy et. al have found that small-molecule inhibitors of gingipains may be an effective treatment against P. gingivalis-induced brain inflammation and bacterial colonization and thus may slow neurodegeneration [20]. The present study adds to the epidemiological evidence suggesting that P. gingivalis eradication among others may be an effective means to delay onset of AD, pending randomized clinical trials.

Just like P. gingivalis, P. melaninogenica expresses a complete T9SS secretion system. Although P. melaninogenica does not use gingipains, T9SS is important in biofilm formation and is involved in Pd, possibly by secreting proteases [44]. Our results indicate that Pg and Pm may independently or interactively induce cognitive impairment leading to AD as an underlying cause of death among older adults.

Moreover, another study showed that P. gingivalis LPS alone was sufficient to antagonize IL-6 and IL-8, but not IL-1β stimulation by another pathogen, namely C. rectus, suggesting that mixed infections, particularly interactions between P. gingivalis and C. rectus may impair host immune responses through cytokine level reduction of direct relevance to both periodontitis and AD [45].

Our study has several notable strengths including the use of a large, nationally representative sample, inclusion of middle-aged adults (≥45 years), assessment of AD incidence and mortality and incident all-cause dementia over a long follow-up period of up to 26 years, measurement of serum antibody levels for periodontal pathogens combined with dental examination and adjustment for key potential confounders.

Limitations include observational study design, even though temporality of associations were ascertained. Underdiagnosis of AD and other dementias is a possibility despite the fact that over 90% of the US population is eligible for and uses Medicare after the age of 65 years and that the linkage was comprehensive, including all aspects of health care utilization (e.g., inpatient and outpatient) with continuous follow-up between 1991 and 2014. Nevertheless, a few cases missed by Medicare were added using NDI to assess incident AD and all-cause dementia. Moreover, the data lacked some key biochemical biomarkers of AD (such as blood or CSF markers of Aβ and tau) and neuroimaging of patients. Additionally, clinical periodontal measures were only estimated based on partial-mouth examination. This could underestimate Pd severity, thus attenuating observed associations. An in-depth study examining other alternative measures of clinically defined categories for periodontitis may be warranted. Furthermore, serum IgG humoral immune response exposures, though normalized through Log_e transformation, exhibited moderate collinearity. Finally, residual confounding bias particularly by genetic risk factors (e.g., ApoE4 status) cannot be discounted.

This study provides evidence for an association between periodontal pathogens and AD, which was stronger for older adults and calls for a line of inquiry, including randomized controlled trials, on the effectiveness of periodontal treatment against onset and progression of neurodegenerative disorders such as AD.

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SUPPLEMENTARY MATERIAL

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