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Serum antibodies to periodontal pathogens are a risk factor for Alzheimer's disease

Pamela Sparks Stein^{a,*}, Michelle J. Steffen^c, Charles Smith^c, Gregory Jicha^c, Jeffrey L. Ebersole^b, Erin Abner^c, and Dolph Dawson III^b

^aDepartment of Oral Health Science, College of Dentistry, University of Kentucky, Lexington, KY 40536 USA

^bCenter for Oral Health Research, College of Dentistry, University of Kentucky, Lexington, KY 40536 USA

^cAlzheimer's Disease Research Center, University of Kentucky, Lexington, KY 40536 USA

Abstract

Background—Chronic inflammation in periodontal disease has been suggested as a potential risk factor in Alzheimer's disease. The purpose of this study was to examine serum antibody levels to bacteria of periodontal disease in participants who eventually converted to Alzheimer's disease (AD) compared to the antibody levels in control subjects.

Methods—Serum from 158 participants in the BRAINS (Biologically Resilient Adults in Neurological Studies) research program at the University of Kentucky were analyzed for IgG antibody levels to 7 oral bacteria associated with periodontitis including: *Aggregati-bacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Campylobacter rectus*, *Tre-ponema denticola*, *Fusobacterium nucleatum*, *Tannerella forsythia*, and *Prevotella intermedia*. All 158 participants were cognitively intact at baseline venous blood draw. Eighty one of the participants developed either mild-cognitive impairment (MCI) or Alzheimer's disease (AD) or both, and 77 controls remained cognitively intact in the years of follow up. Antibody levels were compared between controls and AD subjects at baseline draw and after conversion and controls and MCI subjects at baseline draw and after conversion using the Wilcoxon rank-sum test. AD and MCI participants were not directly compared. Linear regression models were used to adjust for potential confounding.

Results—Antibody levels to *F. nucleatum* and *P. intermedia*, were significantly increased ($\alpha = 0.05$) at baseline serum draw in the AD patients compared to controls. These results remained significant when controlling for baseline age, Mini-Mental State Exam (MMSE) score and apolipoprotein epsilon 4 (*APOE* $\epsilon 4$) status.

Conclusions—This study provides initial data that demonstrate elevated antibodies to periodontal disease bacteria in subjects years prior cognitive impairment and suggests that periodontal disease could potentially contribute to the risk of AD onset/progression. Additional cohort studies profiling oral clinical presentation with systemic response and AD and prospective studies to evaluate any cause-and-effect association are warranted.

Corresponding Author: Pam Stein, D.M.D., M.P.H., Department of Oral Health Science, Division of Public Health Dentistry, University of Kentucky College of Dentistry, 333 Waller Avenue, Suite 180, Lexington, KY 40504, Phone: 859-323-5591, Fax: 859-257-9634, pam.stein@uky.edu.

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Keywords

Alzheimer's disease; periodontitis; antibody; periodontal bacteria; periodontal disease; mild cognitive impairment

1. Background

While researchers have continued to investigate potential treatments for Alzheimer's disease (AD), it remains a devastating, fatal condition resulting in a significant burden both socially and economically [1]. As the world's elderly population increases, the prevalence of AD will also increase [2]. Identification of modifiable risk factors and preventive strategies are important for preventing and managing this chronic disease in the future. Previously identified risk factors such as age, the presence of the APOE $\epsilon 4$ (apolipoprotein epsilon 4) allele, and a family history are not modifiable [3]. In the present study we investigated periodontal disease as a potential modifiable risk factor for AD.

Periodontal disease is a peripheral, chronic infection, which elicits a systemic inflammatory response [4]. The chronic trickling of Gram negative, anaerobic periodontal bacteria into the systemic bloodstream result in elevated levels of various inflammatory mediators in the serum of periodontitis patients [5–6]. Chronic systemic inflammation induced by periodontal disease has been suggested as a risk factor for several conditions including stroke [7], cardiovascular disease [8], diabetes complications [9], and preterm birth [10].

While the role of inflammation in Alzheimer's disease is debated, studies suggest systemic inflammation may increase the risk or progression of AD [11–14]. Some inflammatory mediators associated with periodontal disease, e.g. C Reactive Protein (CRP), Interleukin 6 (IL 6), Interleukin 1 (IL 1 β), and TNF- α have been suggested to increase the risk of cognitive decline and/or Alzheimer's disease [11–14]. Elevated levels of CRP and IL6 in serum have been associated with an increased risk of Alzheimer's disease [12]. Another study found subjects with higher levels of serum TNF- α at baseline had a 4 fold increase in the rate of cognitive decline compared to controls during 6 months of observation [13]. Further, serum levels of IL 1 β and incidence of systemic infection increased the rate of cognitive decline in Alzheimer's patients [14].

An "inflammatory model" has been proposed by Kamer et al., [15] whereby periodontal disease induces systemic inflammatory products which stimulate the production of beta amyloid and tau protein in brain tissue leading to Alzheimer's neuropathology. Watts et al. present a similar model in which periodontal infection may serve to contribute to or exacerbate Alzheimer's disease through inflammatory mechanisms [16].

Limited studies have investigated periodontal disease as a potential risk factor for dementia. A few studies have used tooth loss as a potential marker of history of periodontal disease. One of the earliest investigations, a case control study by Kondo et al. [17], found a loss of more than half of the teeth to be associated with Alzheimer's disease. However, it is not possible to determine if the low number of teeth was a result of oral disease earlier in life or the result of dementia related poor oral care and the subsequent removal of teeth.

A twin study conducted by Gatz et al. [18] found that those who had lost more than half their teeth at age 35 had a greater risk of developing Alzheimer's disease later in life. The accuracy of the tooth loss data may be questionable as tooth loss was measured by self-report only. Subjects were asked to recall how many teeth they had at age 35 choosing from all/most, half or few/none. The average age of the subjects when asked the question about tooth loss was 44 [18]. Stein et al. [19] also found a low number of teeth (9 or fewer) to be

associated with increased risk of dementia in a subset of Nun Study participants. Results from these studies should be cautiously interpreted as tooth loss may mark conditions other than periodontal disease i.e. dental caries and trauma.

Recent studies have moved beyond using tooth loss as a proxy for periodontal disease to looking at markers of periodontal disease in the serum. Investigators have demonstrated profiles of serum antibodies to selected periodontal pathogens as indicative of periodontitis [20–21]. Using data from NHANES III, Nobel et al.[22] found those participants with the highest levels of serum antibodies to periodontal bacteria to have significantly lower scores on delayed word recall and calculation tasks. A case control study [23] found elevated levels of TNF- α and an increased number of serum antibodies to periodontal bacteria in subjects diagnosed with Alzheimer's disease compared to cognitively normal subjects. While findings from both studies are intriguing, neither addresses the temporal nature of the association. Perhaps, cognitive impairments in the subjects resulted in a lack of oral hygiene and an increase in periodontal disease which could explain the findings of increased levels of periodontal antibodies and TNF- α in the AD subjects.

Questions remain about which came first, the periodontal disease or the cognitive impairment. The present study investigates serum antibodies to periodontal pathogens but does so in a manner that addresses temporal concerns that the subject's cognitive impairment could explain the periodontal findings. All subjects were cognitively intact at baseline when the serum samples were drawn. Based on the inflammatory models proposed by Kamer [15] and Watts [16] and the findings from the aforementioned studies, we hypothesized that the levels of antibodies to periodontal pathogens would be elevated in the serum of cognitively normal participants who would later convert to mild cognitive impairment (MCI) or AD, compared to the levels in cognitively normal control subjects who remained normal over the same observation interval. We included MCI in our hypothesis because systemic inflammation may also play a role in MCI. A recent study shows subjects with MCI have elevated levels of several inflammatory mediators including TNF- α and IL 1 β compared to cognitively normal subjects [24].

We further hypothesized that in those subjects who did not remain cognitively intact during follow up, levels of antibodies would remain elevated or increase further in the serum drawn after MCI or AD conversion due to lack of oral care.

2. Methods

2.1 Study population

The serum samples for this study were obtained from research subjects participating in the BRAINS (Biologically Resilient Adults in Neurological Studies) program at the Sanders Brown Center on Aging at the University of Kentucky. The BRAINS research program, which started in 1989, is a longitudinal study in which participants are followed annually with medical, neurologic, and neuropsychological evaluations and also agree to brain donation upon death. Details of BRAINS participant recruitment have been previously described [25]. This unique cohort allows researchers to observe the transition from normal cognition to mild cognitive impairment and dementia over time. Over 1,000 subjects have participated in the BRAINS program since its inception, with approximately 500 followed currently.

The current study is a retrospective study which uses longitudinal data collected in the BRAINS study over time. The study design is a case-control study nested within a cohort study. Our study population included a subset of the BRAINS research subjects who were cognitively intact at their baseline serum draw and had available serial blood draws (n=158).

BRAINS research subjects who converted to MCI or AD but did not have samples available both at baseline serum draw and after the date of diagnosis were excluded. MCI and AD subjects were identified first, and then suitable controls were identified using frequency matching on age and sex.

Of the 158 participants included in our study who were normal at baseline serum draw, a subset of 81 subsequently developed either mild-cognitive impairment (MCI) or Alzheimer's disease (AD) or both over time in the longitudinal follow up of the BRAINS study, and a subset of 77 remained cognitively intact over the same observation interval. Diagnosis was determined annually by the consensus of a neuropsychologist and the participant's physician using the criteria outlined by McKhann et al. [26] for AD and Petersen et al. [27–28] for MCI.

2.2 Serum samples

Baseline venous blood samples were drawn from each participant in the BRAINS program and drawn annually thereafter during the years of follow up. Serum samples for our study had been stored at -80°C . Baseline sera were available for all 158 cognitively intact participants. Forty-six of these participants eventually converted to MCI, and 35 additional participants converted to AD during the years of follow up. The median time from baseline assessment to diagnosis for the MCI and AD subjects was 9.8 years and 9.6 years, respectively. The total mean length of follow-up for the controls was 12.5 years (Table 1). Five groups of serum samples were analyzed in this study. This included: (i) baseline serum from control subjects who remained cognitively intact over the years of follow up; (ii) baseline serum from cognitively intact participants who converted to MCI over the years of follow up; (iii) baseline serum from cognitively intact participants who converted to AD over the years of follow up; and serum collected from participants after conversion at the time of (iv) MCI or (v) AD diagnosis.

Serum from a different study population was evaluated for levels of Immunoglobulin G (IgG) antibody to the same oral bacteria for comparison with the serum from the subjects participating in BRAINS program (the five groups described above).

2.3 Analyte measures

IgG antibody levels to 7 oral bacteria associated with periodontopathic biofilms were analyzed blindly as to group or sampling time using an ELISA as we have described previously [29]. The bacterial antigens included: *Aggregatibacter actinomycetemcomitans* (Aa) JP2, *Porphyromonas gingivalis* (Pg) ATCC 33277, *Campylobacter rectus* (Cr) ATCC 33238, *Treponema denticola* (Td) ATCC 35405, *Fusobacterium nucleatum* (Fn) ATCC 25586, *Tannerella forsythia* (Tf) ATCC 43037, and *Prevotella intermedia* (Pi) ATCC 25611. The ELISA results were determined spectrophotometrically using a Spectromax M2 (Molecular Devices) at 405 nm. The IgG antibody was expressed as $\mu\text{g/mL}$ by comparison to a standard IgG curve included in each assay.

Since the antibody levels in the MCI and AD patients were examined within the context of surrogate biomarkers of periodontal infections reflecting periodontal disease, we included the serum antibody results to these oral bacteria derived from normal adult subjects 70 years of age with or without periodontitis. Included in this population were 67 patients (age range: 31–70) from previous studies [29–33]. Chronic periodontitis was classified based upon clinical criteria provided by the American Academy of Periodontology [34–35]. Additionally, 42 subjects (age range: 23–67) were included as periodontally health controls demonstrated no sites with pocket depths >4 mm, attachment loss >3 , and $<10\%$ sites with bleeding on probing [36].

2.4 Statistical analysis

Statistical methods used include the Wilcoxon Rank Sum test and general linear regression. Wilcoxon Rank Sum tests were used to test the hypothesis that antibody levels are elevated prior to conversion to MCI or AD and remain elevated thereafter. Antibody levels were compared between controls and AD-converting subjects before (at baseline) and after conversion, and between controls and MCI-converting subjects before (at baseline) and after conversion. AD and MCI subjects were not directly compared. To assess the effects of potential confounders, general linear regression models were fit using log-transformed antibody levels as the dependent variables and group (e.g., control or AD before conversion), baseline age (which could modify antigen exposure), baseline MMSE (which could modify dental hygiene), diabetes and smoking status (which increase the risk of periodontal disease), and *APOE* $\epsilon 4$ status (which could modify immune response to antigens) as the independent variables. The normality of the log-transformed variables was confirmed via the Kolmogorov-Smirnov test. Statistical significance was set at $\alpha = 0.05$. All analyses were performed using PC-SAS9.2©.

3. Results

Characteristics of the Population

Controls, MCI converters, and AD converters were similar with regard to gender, years of education, baseline MMSE (Mini-Mental State Exam) scores, proportion of current or former smokers, proportion of persons with diabetes, and occupation types (Table 1). Thirty two of the control participants were male and 45 were female, 24 of the MCI converters were male and 22 were female and nine of the AD converters were male and 26 female. AD converters were older than controls at the baseline assessment. All participants were Caucasian with the exception of one participant in the MCI converters group.

Figures 1A–B illustrate the levels of antibody to the oral bacteria in the various subject groups. Antibody levels to *F. nucleatum* ($p < 0.0001$), *P. intermedia* ($p < 0.0001$), and *T. denticola* ($p = 0.027$) were significantly increased at baseline draw in the AD patients compared to controls. After adjustment for baseline age, baseline MMSE, years of education, sex, *APOE*- $\epsilon 4$, diabetes, and smoking status, *F. nucleatum* ($p = 0.0003$), *P. intermedia* ($p = 0.0001$), and *T. denticola* ($p = 0.0299$) remained significant at $\alpha = 0.05$, although applying a Bonferroni corrected $\alpha = 0.007$ negates the *T. denticola* result. Antibody levels to *F. nucleatum* ($p = 0.0003$), *P. gingivalis* ($p = 0.0077$), *P. intermedia* ($p < 0.0001$), and *T. denticola* ($p = 0.050$) were elevated in the AD patients after conversion. Adjusted results for *F. nucleatum* ($p = 0.0055$), *P. gingivalis* ($p = 0.013$), *P. intermedia* ($p < 0.0001$), and *T. denticola* ($p = 0.044$) all remain significant at $\alpha = 0.05$, but only the results for *F. nucleatum* and *P. intermedia* survive the Bonferroni correction for multiple comparisons.

Elevated antibodies were observed in the MCI group at baseline compared to controls for *F. nucleatum* ($p = 0.0001$), *C. rectus* ($p < 0.0001$), and *P. intermedia* ($p = 0.0001$), both in the unadjusted and adjusted (*F. nucleatum* [$p = 0.0002$], *C. rectus* [$p = 0.014$], and *P. intermedia* [$p = 0.0001$]) analyses; however, these antibody levels dropped in the MCI group following conversion. Unadjusted comparisons based on Wilcoxon's Signed Rank test on antibody levels before and after MCI conversion show significant ($\alpha = 0.05$) decreases in all antibodies with the exception of *P. gingivalis*. Antibody levels to *T. denticola* ($p = 0.0095$) and *T. forsythia* ($p = 0.0082$) were depressed in the MCI patients after conversion compared to control. In the adjusted analyses, *C. rectus* ($p = 0.04$), *P. gingivalis* ($p = 0.043$), *T. denticola* ($p = 0.03$), and *T. forsythia* ($p = 0.0054$) levels are significantly lower than control at $\alpha = 0.05$.

As has been reported previously [20–21], chronic periodontitis patients demonstrate significantly elevated serum antibody to putative oral pathogens, including *A. actinomycetemcomitans*, *C. Rectus*, *F. nucleatum*, *P. intermedia*, *P. gingivalis*, *T. denticola*, and *T. forsythia*. In the current study, antibody levels to *A. actinomycetemcomitans*, *C. Rectus*, *F. nucleatum*, and *P. intermedia* in the MCI patients at baseline and the AD patients at baseline were consistent with the levels in chronic periodontitis patients (Table 2 and Figure 1A–B). In addition, similar levels of antibodies to *P. gingivalis* were found between patients diagnosed with chronic periodontitis and AD patients at baseline (Table 2 and Figure 1A).

4. Discussion

Alzheimer's disease has a significant inflammatory component that results in the neurological damage [37]. While increased β -amyloid plaques in brain tissue and modification of the amyloid precursor protein have been linked to AD, the factors that initiate these changes and individual variation in the progression of neurodegeneration is not clear [37]. It is possible that other chronic diseases, such as periodontitis, that increase the overall "set point" of systemic inflammation in individual patients, would increase the risk for development/progression of AD [15–16].

Substantial epidemiologic evidence supports a relationship between periodontal disease and complications associated with chronic diabetes [9], cerebrovascular and cardiovascular events [7–8] and adverse pregnancy outcomes [10]. It has recently been shown that metabolic syndrome and Type II diabetes in midlife are associated with increased likelihood of AD in later years [38]. Furthermore, inflammatory markers have been linked with metabolic syndrome [39–41]. Periodontal disease has also been associated with increases in systemic inflammatory markers [4–6].

Our previous research found an association between a low number of teeth (0–9) and increased risk in developing dementia in Nun Study participants [19]. Alveolar bone loss, a marker of history of periodontal disease, was evaluated by radiographic interpretation but no association was found with risk of dementia. However radiographs were only available for a subset of the participants. Further, variability in angulation, density, processing, and patient positioning may have affected the findings of radiographic interpretation. Another of our previous studies found participants with a low number of teeth (0–9) declined more rapidly over time in their ability to recall words. Those who had both a low number of teeth and the *APOE* $\epsilon 4$ allele were the worst performers with lower delayed word recall scores at baseline and more rapid decline over time [42]. The current study adds to the evidence of our previous studies by utilizing a stronger marker of periodontal disease, serum antibodies to periodontal pathogens. In addition, because the current study is longitudinal, it addresses the temporality of the association of periodontal disease and AD. Previous serum studies [22–23] that have suggested an association between elevated periodontal antibodies and cognitive impairments or AD have not addressed the temporal nature of the association.

In the current study, both the AD and MCI subjects demonstrated significant elevations in antibody to *P. intermedia* and *F. nucleatum* at baseline, prior to diagnosis of the neurological changes. Additionally, the AD subjects expressed significantly elevated antibody to *T. denticola*, and *P. gingivalis* at baseline. These sera were obtained years prior to the clinical diagnosis of AD or MCI, while subjects were still cognitively normal. Therefore these elevations cannot be attributed to secondary effects of the AD disease process, such as poor nutrition or other dementia-related neglect. While it could be suggested that the antibody to these oral pathogens may have been cross-reactive with antigens from other sources, the literature is replete with studies supporting the specificity of these antibodies for oral

infections [20–21, 43–46], and that successful treatment and maintenance of periodontitis significantly lowers these antibody levels [47]. Comparison of these antibody levels to those described in numerous populations show levels in the AD and MCI subjects in the current study to be similar to chronic periodontitis patients [45–49]. Interestingly, the control group also showed antibody levels higher than healthy values for four of the seven bacteria (*A. actinomycetemcomitans*, *C. rectus*, *T. forsythia* and *P. gingivalis*) with three of the four at levels consistent with chronic periodontal disease. This may be because the study population was older, with a mean age at baseline of 70–74 years of age and periodontal disease occurs more frequently in elder adults. Regardless, the levels of antibodies in the control group were significantly less than the levels of those who converted to AD at baseline for five of the seven bacteria studied.

It should be noted that periodontal disease could be marking other risk factors for MCI and AD that we were unable to control. However, the college-equivalent education should be a reasonable indicator of similar socioeconomic status, i.e., that periodontal disease is not simply a proxy for low socioeconomic status and poor nutrition in the MCI and AD groups. Moreover, occupations in the control, MCI, and AD groups were rated per Barona et al. [50] and compared using Wilcoxon rank-sum statistics and chi-square tests. No significant differences were found.

It is also possible that the expression and severity of periodontitis reflects a fundamental alteration in the manner in which certain individuals respond to a chronic inflammatory challenge. Irrespective of the mechanism through which periodontitis-triggered systemic inflammation could potentially influence chronic neurological diseases such as AD, the data presented clearly document a strong association that deserves further investigation.

Substantial data have been developed supporting that the detection of serum IgG antibody reactive with a range of oral bacteria is specific for these microorganisms [31–33]. Thus, the findings from a range of clinical studies comparing periodontitis to oral health, documenting changes in the levels of specific serum antibody to selected oral bacteria with the progression and severity of periodontitis, and decreases in antibody levels to these bacteria that accompany successful treatment of the oral disease combine to emphasize the likelihood that elevated levels of antibody to these putative etio-logic agents of periodontitis are explicitly reflecting periodontal infections and disease [51–54]. Considering periodontal antibody reactivity as a surrogate biomarker for periodontal disease, these results suggest that the AD patients, in particular, likely expressed periodontitis prior to the neurological changes and continued to express the antibodies and oral disease after conversion. In contrast, the MCI group was much more limited in the repertoire of these serum responses, and the antibody levels decreased for 6 of the 7 oral bacterial species following diagnosis. The basis for these findings is not clear. Altered approaches to the medical management of the MCI patients may have contributed to improving the oral health of this group after diagnosis.

Interleukin-11 (IL-11) has been implicated in the pathogenesis of periodontitis [55–56]. Evidence from animal models of AD suggests that IL-11 might be neuroprotective in a beta mediated neurotoxicity [57]. The observed increase in IL-11 levels in cerebrospinal fluid of patients with AD could represent an ineffective compensatory response within the central nervous system (CNS) compartment [58]. Recent studies have demonstrated a selective decrease in stimulated IL-11 production from peripheral blood monocytes in MCI but not in AD [59]. Since IL-11 can mediate T helper cell 2 (TH2) polarization [60], decreased stimulated IL-11 levels at the time of MCI diagnosis could result in a decreased TH2 polarization and decreased antibody response to periodontal organisms, potentially accounting for our observations. To explore this hypothesized link, we plan future studies of

stimulated IL-11 in blood of MCI patients for correlation with serum antibodies to periodontal pathogens.

This study provides data that demonstrate elevated host responses to oral pathogens associated with periodontitis in patients with AD, and to a lesser extent MCI. The overall thrust of these investigations was to understand if the chronic oral infection of periodontitis was associated with AD, and if the chronic oral infection of periodontitis could potentially contribute to the risk of AD expression. The current findings support this association; however, additional cohort studies profiling oral clinical presentation with systemic response and AD and prospective studies to evaluate any cause-and-effect association will have to be developed to more fully determine the importance of maintaining oral health as a fundamental part of healthy aging and lowering the risk of these types of neurological changes.

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ABBREVIATIONS USED IN MANUSCRIPT

AD	Alzheimer's disease
MCI	Mild cognitive impairment
APOE ε4	Apolipoprotein epsilon 4
MMSE	Mini-Mental Status Exam
BRAINS	Biologically Resilient Adults in Neurological Studies
IgG	Immunoglobulin G
Aa	Aggregatibacter actinomycetemcomitans
Pg	Porphyromonas gingivalis
Cr	Campylobacter rectus
Td	Treponema denticola
Fn	Fusobacterium nucleatum
Tf	Tannerella forsythia
Pi	<i>Prevotella intermedia</i>
IL-11	Interleukin 11
CNS	Central nervous system
TH2	T helper cell 2

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Figure 1A

Figure 1B

Figure 1.

Figure 1A and 1B. Levels of IgG antibodies to the common periodontal pathogens *Aggregatibacter actinomycetemcomitans* (Aa), *Campylobacter rectus* (Cr), *Fusobacterium nucleatum* (Fn), *Prevotella intermedia* (Pi), *Porphyromonas gingivalis* (Pg), *Treponema denticola* (Td), and *Tannerella forsythia* (Tf), are compared in five groups of subjects: controls from the BRAINS group who remained cognitively intact ($n=77$ dark blue bar); subjects who eventually converted to AD at baseline serum draw ($n=35$, red bar, AD before); subjects who converted to AD after conversion, at AD diagnosis ($n=35$ green bar, AD after); subjects who eventually converted to MCI at baseline serum draw ($n=46$, purple bar, MCI before); subjects who converted to MCI after conversion, at MCI diagnosis ($n=46$, light blue bar, MCI after). The mean raw data values are presented in the figures, although the p-values for the adjusted means were based on models where the outcome was the log-transformed values.

Table 1

Baseline characteristics of normal participants from the BRAINS group who subsequently developed either MCI or AD and controls from the BRAINS group who remained normal over the same observation interval. Means were compared with t-tests, and proportions were compared with the chi-square test.

	Control (n = 77)	MCI (n = 46)	p-value (MCI vs. control)	AD (n = 35)	p-value (AD vs. control)
Baseline Age, mean yrs (SD)	70.0 (6.5)	72.1 (6.1)	NS	74.1 (7.5)	0.004
Sex (% male)	41.6	52.2	NS	25.7	NS
Education, mean yrs (SD)	15.9 (2.6)	15.0 (3.1)	NS	16.0 (2.5)	NS
Baseline MMSE, mean (SD)	29.4 (0.8)	28.8 (1.3)	0.01	28.8 (1.2)	0.006
Follow up, mean years (SD)	12.5 (4.8)	11.7 (5.1)	NS	10.8 (5.1)	NS
ApoE-4 positive (%)	15.6	37.0	0.007	37.1	0.01
Current or former smoker (%)	47.8	43.5	NS	38.9	NS
Diabetes (%)	11.6	13.0	NS	8.3	NS
Profession/technical/ office occupation (%)	81.6	77.8	NS	77.8	NS

Table 2

Serum IgG antibody levels in chronic adult periodontitis patients (n=67) and healthy controls (n=42) 70 years of age.

Serum Analyte	Healthy Controls	Chronic Periodontitis
<i>A. actinomycetemcomitans</i>	12.1 ± 1.4	30.4 ± 4.3 *
<i>C. rectus</i>	31.7 ± 4.2	38.8 ± 4.6
<i>F. nucleatum</i>	6.1 ± 0.4	10.0 ± 1.5 *
<i>P. intermedia</i>	6.2 ± 0.4	15.9 ± 2.2 *
<i>P. gingivalis</i>	8.2 ± 0.9	43.1 ± 6.4 *

* Mean ± SD; Significantly different than healthy controls at least at p<0.001 using Wilcoxon Mann Whitney U test.