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Prospective association of periodontal disease with cardiovascular and all-cause mortality: NHANES III follow-up study

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ABSTRACT

Background: It has been suggested that periodontal disease (PD) was associated with an increased risk for cardiovascular diseases (CVD), although evidence is inconclusive.
Purpose: We first sought to prospectively evaluate the relationship of PD to CVD and all-cause mortality using a national representative sample in the United States.
Methods: The study population consisted of 10,849 participants who were 30 years or older and received a periodontal examination from NHANES III mortality follow-up sample (1988–2006). CVD and all-cause deaths were ascertained from the National Death Index records. The causes of death were defined using the International Classification of Disease coding (ICD-10). The severity of PD was categorized as non-PD, modest and severe PD based on clinical attachment loss and pocket depth.
Results: Of the study sample, 3105 and 561 participants were identified as modest and severe PD cases, respectively. After up to 18 years of follow-up, there were total 2894 deaths, of which 1225 were from CVD. The levels of inflammation markers (high sensitivity C-reactive protein, white cell count and fibrinogen) were significantly higher in men with severe PD compared to men without PD ($p < 0.05$). The prospective associations were evaluated using multivariable Cox proportional-hazards models. After adjusting for age, gender, race, household income and traditional risk factors of CVD, severe PD was associated with an increase risk of CVD mortality and all-cause mortality in men aged 30–64 years (HR = 2.13 with 95% confidence interval of 1.37–3.31 for CVD mortality; HR = 1.64 with 95% confidence interval of 1.25–2.15 for all-cause mortality). In addition, significant linear trends were found in CVD and all-cause mortality across the severity of PD ($p < 0.001$). However, no significant associations were found in men aged ≥65 and in women.
Conclusions: There appears to be prospective associations between PD and CVD and all-cause mortality in men aged 30–64 years. Inflammation may be one possible pathway to link PD with CVD.

1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in the United States [1]. Traditional risk factors such as cigarette smoking, hyperlipidemia, hypertension, and diabetes only explain part of the variation in the risk of CVD [2]. In the last decade, low-grade systemic inflammation has been advocated as one of the important novel risk factors for CVD [3]. Periodontal disease (PD), which is a chronic infection in the supportive tissue of teeth and caused by complex dental biofilms composed of microorganisms found in the oral microbiota, is common among U.S. adults [4]. The association between PD and CVD has been reviewed extensively in the literature [5–7]. The majority of epidemiological studies have shown the presence of a significant positive association between PD and CVD [8–10], although reports from the Health Professionals Study [11] and the Physicians' Health Study [12] observed no association between PD and either coronary heart disease or stroke among more than 66,000 male health professionals. The large sample sizes of these two studies provide a good reason for caution with regard to the overall hypothesis. To date, few studies have investigated the prospective associations between PD and CVD and all-cause mortality, especially in a national representative sample. We therefore sought to assess the associations of PD with CVD and all-cause mortality based on National Health and Nutrition Examination Survey III (NHANES III) mortality follow-up data (1988–2006).

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2. Methods

2.1. Study population

The data for this study were taken from the National Health and Nutrition Examination Survey III (NHANES III), a nationally representative health survey conducted between 1988 and 1994 by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC) [13]. NHANES III used a multistage cluster design with oversampling of racial/ethnic minorities, children, and the elderly. Sample weights are provided for each individual that correct for non-response and unequal probability of selection. Participants completed a household interview and a physical examination in the Mobile Examination Center (MEC). We limited analyses to the 10,849 adults aged more than 30 years (male 5156, female 5693) who received a periodontal examination and were eligible for mortality follow-up.

2.2. Mortality follow-up

Participants were linked to death records from the National Death Index for the years 1988–2006. Vital status was determined by NCHS through a rigorous process of probabilistic matching and death certificate review based on participant social security number, full name, date and state of birth, gender, race/ethnicity, state of residence, and marital status [14]. The primary underlying cause of death was used for case definition. These causes were defined using the International Classification of Disease coding (ICD-10). CVD mortality was defined as ICD-10 codes I00-I99 including all deaths from coronary heart disease, cerebrovascular diseases and other disorders of circulatory system.

2.3. Assessment of periodontal disease

The periodontal examination for NHANES III was conducted in the mobile examination centers. Briefly, the periodontal examination was conducted at 2 sites, mid-buccal and mesiobuccal for each tooth, in 2 randomly chosen quadrants, 1 maxillary and 1 mandibular, on the assumption that conditions in these 2 quadrants would represent the mouth. Third molars were excluded because of their frequent extraction in young adulthood, so a maximum 14 teeth and 28 sites per individual were examined. Detailed examination procedures can be obtained from the CDC website [13]. Currently uniform criteria for an accurate definition of PD have not been established in epidemiological studies [15]. For this study, we defined modest PD as at least one site with >4 mm clinical attachment loss or at least one site with probing depths >5 mm; severe PD as at least one site with CAL $\geq$6 mm and one or more sites with PD $\geq$5 mm in 2 quadrants (half mouth).

2.4. Assays of markers of inflammation

Laboratory methods and quality control procedures are described in detail elsewhere [13,16]. Serum C-reactive protein was quantified using latex-enhanced nephelometry. The assay was standardized using the World Health Organization's international reference preparation of C-reactive protein. Both within- and between-assay quality control procedures were used, and the coefficient of variation of the method was 3.2–16.1% (median, 6.3%) through the study period. The white blood cell count was obtained using a Coulter Counter. The coefficient of variation for this assay was no more than 3.0%. Fibrinogen was measured in citrated plasma using an automated coagulation analyzer. The total coefficient of variation across normal pooled plasma was 3.9%.

2.5. Potential confounders

We evaluated several variables for potential confounding. Demographic variables included age (in years), race (non-Hispanic white, non-Hispanic black and all others), education (high school education or less versus more than high school) and household income level. Household income level was measured based on poverty income ratio (PIR) which is the ratio of family income to the appropriate poverty threshold: low (PIR < 1.35), medium ($1.35 \leq$ PIR < 3.0) and high (PIR $\geq$ 3.0). The potential risk factors for CVD included smoking status, alcohol use, total to HDL cholesterol ratio (TC/HDL ratio), hypertension, diabetes, and a history of coronary heart disease (CHD) or stroke. Smoking was categorized as never, current and former smoking. Alcohol use was dichotomized as at least 12 drinks in the last 12 months or not. TC/HDL ratio was calculated as total cholesterol divided by HDL cholesterol (<5 and $\geq$5) [17]. Obesity was defined as body mass index $\geq$30 kg/m^2. Hypertension was defined as mean SBP $\geq$140 mm Hg, mean DBP $\geq$90 mm Hg, or current treatment for hypertension with medication. Diabetes was defined from self-report of diagnosis or taking diabetes medications. A history of coronary heart disease (CHD) or stroke was determined by self-reported.

2.6. Statistical analysis

Analyses were performed using SAS-callable SUDAAN version 10 (Research Triangle Institute) to account for the complex sampling scheme used in NHANES III. Descriptive statistics were used to summarize variables as well as to detect outliers and missing values. We compared characteristics of the sample according to periodontal status. Person-years were calculated from the date of NHANES III survey participation (1988–1994) through the date of death or December 31, 2006, whichever came first. All the analyses were stratified by gender and two age groups ($30 \leq$ age < 65 and age $\geq$ 65). We evaluated the associations between PD and inflammation markers by fitting 3 multiple linear regression models with hsCRP, white blood cell count and fibrinogen as continuous outcomes respectively. hsCRP and white blood cell count were natural logarithm transformed in the analyses due to the skewed distributions. Then we compared Kaplan–Meier curves to graphically display the survival functions across participants with and without PD, and log-rank tests were used to test the difference. The unadjusted and multivariable-adjusted associations were evaluated using Cox proportional-hazards model, stratified by gender and age groups. The independent association of CVD and all-cause mortality with PD was assessed in multivariable models after adjusting for age, race, household income level, education, smoking status, alcohol use, TC/HDL ratio, hypertension, obesity, diabetes, and a history of coronary heart disease (CHD) or stroke. The effects of PD and other covariates were evaluated using adjusted hazard ratios with 95% confidence intervals. The proportional hazard assumption was tested based on the smoothed plots of the scaled Schoenfeld residuals [18].

3. Results

Demographic characteristics and potential risk factors of CVD in the study population with and without PD are presented in Table 1. Of 10,849 participants who were 30 years or older, 3105 and 561 participants were identified as modest and severe PD cases, respectively. The median follow up time was 14 years. Individuals with modest or severe PD were more likely to be younger, non-Hispanic black, current smoker and have lower levels of education and family income, and higher levels of TC/HDL ratio, and more likely to have hypertension, diabetes and a history of CHD or stroke compared

Table 1
Baseline characteristics of study participants by periodontal disease (PD), NHANES III mortality follow-up study, 1988–2006.[a]

	Men				Women				Total			
	Non (n = 3046)	Modest (n = 1729)	Severe (n = 381)	P	Non (n = 4137)	Modest (n = 1376)	Severe (n = 180)	P	Non (n = 7183)	Modest (n = 3105)	Severe (n = 561)	P
Age (year) %												
30–39	41.58	21.18	25.88		36.92	19.25	19.06		38.98	20.32	23.73	
40–49	27.12	25.57	26.10		26.54	19.88	22.89		26.80	23.07	25.09	
50–59	12.88	23.02	17.84		14.07	19.65	16.59		13.54	21.54	17.45	
60–69	10.58	16.76	18.64		11.52	20.30	21.94		11.11	18.32	19.68	
70–79	5.95	10.04	10.10		7.64	13.61	15.72		6.89	11.61	11.87	
80+	1.89	3.44	1.44	<0.001	3.31	7.30	3.80	<0.001	2.68	5.14	2.18	<0.001
Race %												
Non-Hispanic white	81.62	73.22	64.07		77.43	73.76	57.96		79.29	73.46	62.15	
Non-Hispanic black	8.14	11.86	21.36		10.55	12.92	28.49		9.48	12.33	23.61	
Mexican-American	4.55	5.15	5.27		4.21	4.45	5.92		4.36	4.84	5.48	
All others	5.70	9.77	9.29	<0.001	7.81	8.87	7.62	<0.001	6.87	9.38	8.77	<0.001
Poverty ratio %												
Low (<1.35)	16.00	21.63	32.20		22.82	28.60	43.67		19.79	24.70	35.81	
Medium (1.35–2.99)	30.52	30.75	36.30		30.92	36.09	41.70		30.74	33.10	38.00	
High (≥3.00)	53.48	47.62	31.50	<0.001	46.26	35.31	14.63	<0.001	49.47	42.21	26.19	<0.001
Education (<12 yrs) %	21.17	27.79	44.89	<0.001	21.25	32.11	36.59	<0.001	21.21	29.69	42.28	<0.001
Smoking %												
Never	38.23	23.70	20.10		58.27	45.82	40.59		49.36	33.43	26.56	
Current	25.32	37.53	48.01		20.24	30.44	34.04		22.50	34.41	43.61	
Former	36.45	38.77	31.89	<0.001	21.49	23.74	25.36	<0.001	28.14	32.16	29.83	<0.001
Alcohol use %	66.75	59.89	67.43	0.012	43.15	35.30	29.61	<0.001	53.63	49.07	55.52	0.016
TC/HDL ≥ 5.0[b]	41.42	44.92	39.29	0.188	19.29	23.31	33.57	0.002	29.12	35.42	37.49	0.001
Hypertension %	24.36	27.13	30.63	0.001	24.83	31.97	40.34	<0.001	24.62	29.26	33.69	0.001
Obesity %	21.81	21.09	26.73	0.391	26.36	28.04	42.21	0.024	24.34	24.15	31.61	0.078
Diabetes %	4.27	7.54	12.76	<0.001	5.70	7.70	13.82	0.002	5.06	7.61	13.09	<0.001
CHD/stroke history %	3.62	6.48	6.09	0.005	1.77	2.75	0.61	0.036	2.60	4.84	4.36	0.001

[a] All proportions are weighted to account for the sample design.
[b] TC/HDL, total cholesterol/HDL cholesterol ratio.

Table 2
Periodontal disease (PD) and levels of high-sensitivity C-reactive protein (hsCRP), white blood cell count and fibrinogen (regression parameter estimates β and standard errors, SE).[a]

	hsCRP[b]		White cell count[b]		Fibrinogen, mg/dL	
	β (SE)	P	β (SE)	P	β (SE)	P
Men						
Non-PD	Referent		Referent		Referent	
Modest	−0.01 (0.03)	0.774	0.02 (0.01)	0.270	4.34 (5.98)	0.471
Severe	0.15 (0.06)	0.010	0.07 (0.02)	0.001	20.34 (8.63)	0.022
Women						
Non-PD	Referent		Referent		Referent	
Modest	−0.02 (0.03)	0.553	0.01 (0.02)	0.417	−6.01 (3.38)	0.073
Severe	0.14 (0.09)	0.096	0.04 (0.03)	0.239	34.21 (10.00)	0.001

with non-PD participants. We explored the associations between PD and systemic inflammation markers including hsCRP, white cell count and fibrinogen. The separate multiple linear regression models illustrated that there were consistent relationships between PD and systemic inflammation markers after adjusting for other covariates (Table 2). The levels of hsCRP, white cell count and fibrinogen were significantly higher in men with severe PD compared to men without PD ($p < 0.05$). In women severe PD cases have higher levels of fibrinogen than non-PD participants ($p = 0.001$).

After up to 18 years of follow-up, there were total 2894 deaths, of which 1225 were from CVD (233 from stroke, 685 from coronary heart disease). In comparisons of Kaplan–Meier estimators of cumulative CVD mortality (Fig. 1), we found that cumulative CVD mortality rates were increased across the severity of PD in both men and women aged 30–64 years ($p < 0.001$). In contrast, no significant differences in cumulative CVD mortality were found in men and women aged more than 65 years ($p > 0.250$). Similar results were found in Kaplan–Meier estimators of cumulative all-cause mortality (results are not shown).

Table 3 presents unadjusted and multivariable adjusted hazard ratios of CVD and all-cause mortality separately in different age and gender groups. In unadjusted analysis, subjects with PD were at higher risk for CVD mortality and all-cause mortality compared to subjects without PD in both men and women aged 30–64 years, but not in older age group (age ≥ 65 years). The relationships also demonstrated a significant linear trend across the severity of PD ($p < 0.01$). After controlling for age, race, education, household income, smoking status, alcohol use, TC/HDL ratio, hypertension, obesity, diabetes and a history of CHD or stroke in Cox proportional hazard models, the risk was reduced significantly for both CVD and all-cause mortality. Men with severe PD still have an increased risk for CVD and all-cause mortality compared to men without PD in the younger group (30–64 years) (HR = 2.13 for CVD mortality with 95% confidence interval of 1.37–3.31, $p < 0.001$; HR = 1.64 for all-cause mortality with 95% confidence interval of 1.25–2.15, $p < 0.001$). In addition, significant linear trends were found in CVD and all-cause mortality across the severity of PD ($p < 0.001$). However, no significant associations were found between PD and

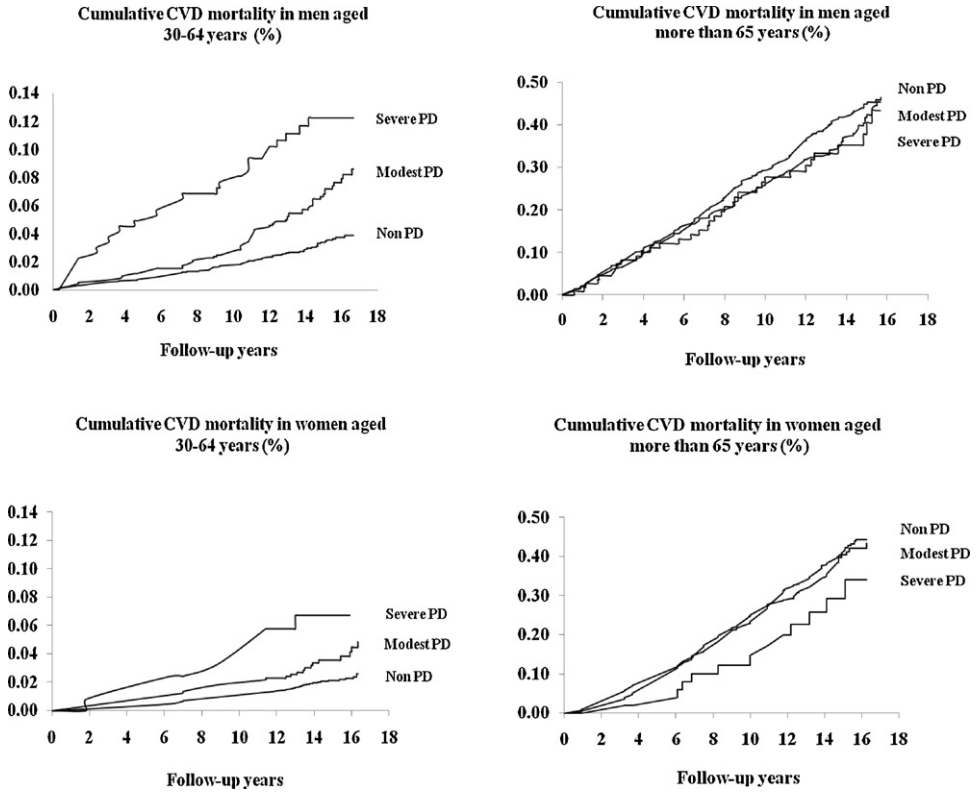

Fig. 1. Cumulative CVD mortality rates by PD in men and women.

Table 3
Unadjusted and multivariable adjusted hazard ratios (95% CI) of CVD and all-cause mortality by periodontal disease (PD).[a]

	CVD mortality				All-cause mortality			
	Non	Modest	Severe	*P* trend	Non	Modest	Severe	*P* trend
Men aged 30–64 years (n = 3732)								
No. of death/person-years	78/34,071	74/15,799	31/3584		251/34,071	217/15,799	81/3584	
Unadjusted	Referent	2.08 (1.51, 2.85)	3.83 (2.53, 5.81)	<0.001	Referent	1.89 (1.58, 2.27)	3.11 (2.42, 3.99)	<0.001
Adjusted	Referent	1.25 (0.89, 1.74)	2.13 (1.37, 3.31)	<0.001	Referent	1.21 (1.00, 1.47)	1.64 (1.25, 2.15)	<0.001
Men aged 65+ years (n = 1424)								
No. of death/person-years	237/6554	189/5662	36/1,132,475		545/6554	422/5662	86/1132	
Unadjusted	Referent	0.92 (0.76, 1.11)	0.88 (0.61, 1.23)	0.170	Referent	0.89 (0.78, 1.01)	0.90 (0.72, 1.13)	0.096
Adjusted	Referent	1.01 (0.83, 1.23)	1.12 (0.77, 1.67)	0.746	Referent	0.96 (0.84, 1.10)	1.07 (0.85, 1.37)	0.897
Women aged 30–64 years (n = 4259)								
No. of death/person-years	71/47,077	32/12,805	8/1764		239/47,077	124/12,805	22/1764	
Unadjusted	Referent	1.65 (1.09, 2.51)	3.03 (1.46, 6.30)	0.003	Referent	1.91 (1.54, 2.37)	2.48 (1.60, 3.84)	<0.001
Adjusted	Referent	0.91 (0.58, 1.42)	1.55 (0.74, 3.25)	0.636	Referent	1.19 (0.94, 1.49)	1.32 (0.84, 2.07)	0.087
Women aged 65+ years (n = 1434)								
No. of death/person-years	309/9613	147/5000	13/618		573/9613	299/5000	35/618	
Unadjusted	Referent	0.92 (0.75, 1.12)	0.82 (0.54, 1.23)	0.228	Referent	1.03 (0.89, 1.19)	0.93 (0.70, 1.24)	0.950
Adjusted	Referent	0.96 (0.79, 1.18)	0.92 (0.52, 1.62)	0.895	Referent	1.04 (0.90, 1.20)	1.16 (0.81, 1.65)	0.445

[a] Adjusted for age, race, household income, education, smoke, alcohol use, TC/HDL ratio, obesity, diabetes, hypertension and a history of CHD or stroke.

risk of CVD and all-cause mortality in men aged ≥65 and in women.

4. Discussion

Although a number of epidemiological studies have demonstrated that periodontal disease may significantly predict cardiovascular diseases, causality still needs to be confirmed. In this prospective analysis using national representative data, we observed significant associations between periodontal disease and CVD mortality and all-cause mortality among U.S. men aged 30–64 years after adjusting for demographic factors, traditional risk factors of CVD and other potential confounders. However, no significant associations were found in women and in men aged 65 years and over. Our findings are consistent with previous studies showing positive associations of poor oral health and CVD mortality and all-cause mortality in young men [19–21]. More recently, a large prospective study with 7674 subjects and a median follow-up period of 12 years presented a dose-dependent relationship among number of teeth and CVD and all-cause mortality [22]. On the contrary, a large 6-year follow-up study of 44,119 male health professionals suggested that periodontal disease was not an independent predictor of subsequent cardiovascular disease in middle-aged to elderly men [12]. Similarly in the Physicians' Health Study of 22,071 U.S. male physicians, no association was observed between periodontal disease and subsequent CHD [11]. However, these two large cohort studies had no oral examination data, but instead relied on participant reports of periodontal disease (no medical records to confirm the reports).

The gender differences in CVD and metabolic syndrome risk factors have received a lot of attention [23,24]. Gender has a significant influence on the cause, clinical manifestation and prognosis of CVD. Modifiable risk factors, such as smoking, physical activity, show differences between the sexes. Sex hormones and sex-specific genetic factors are also likely to be involved in the pathogenesis of CVD [24].

Several possible pathways relating periodontal disease and CVD including bacteremia, inflammation and vascular injury have been discussed in previous studies [7,10,25]. The bacteria may contribute to the vascular pathology either directly through their cytotoxicity or indirectly by inducing or exacerbating inflammation. They are present in atherosclerotic plaques and may play an important role in the development and progression of atherosclerosis leading to CVD [26]. Inflammation is a normal response to many physical states including fever, injury and infection. Laboratory evidence and findings from clinical and population studies suggest that a chronic low-grade inflammation, such as periodontal disease, may be involved in the process of development of atherosclerosis for CVD [27,28]. Inflammation (triggered by environmental factors or genetic influences) causes a sequence of actions in the artery such as, plaque rupture, thrombus formation and immobilization into the blood vessels, therefore increasing plaque build up and contributing to diminished flow in arteries [29]. The inflammatory biomarkers teleologically plays an important role in the body's immune response and has been associated with increased risk of CVD [30]. Additionally, previous studies have been shown that the systemic inflammation markers: C-reactive protein, IL-6, TNF-α and fibrinogen were elevated in patients with PD [31,32]. Our multivariable analyses verified that PD cases had higher levels of hsCRP, white cell count and fibrinogen than non-cases. Therefore, our results also support that inflammation may be an important pathway linking PD with CVD.

This is the first fairly large retrospective cohort study with a long follow-up period using U.S. national representative data in which PD was associated with an increased risk of CVD and all-cause mortality in men aged 30–64 years. The NHANES III baseline and dental

examination data were collected based on standard procedures. Age, as the most important factor of mortality, was adjusted carefully in analyses. First, we separated the analyses by age groups (30–64 and ≥65 years), and then age as well as all known traditional CVD risk factors were also adjusted in separate multivariable models. Our findings were less likely to be interpreted as residual confounding effects. Moreover the present study provided multiple indexes of inflammation to consistently show the association between PD and inflammation.

Unlike other measures of PD, such as gingival bleeding, periodontal attachment loss and pocket depth are generally considered irreversible, can provide a cumulative record of current and past disease. A recent review of analytical epidemiology revealed a conspicuous lack of uniformity in the definition of periodontitis in epidemiological studies [15]. In 2003 a working group appointed by the Centers for Disease Control and Prevention (CDC) and the American Association of Periodontology (AAP) defined moderate periodontitis as two or more interproximal sites with >4 mm CAL, not on the same tooth, or two or more interproximal sites with probing depths >5 mm, not on the same tooth. Severe periodontitis was defined as two or more interproximal sites with CAL ≥6 mm, not on the same tooth, and one or more interproximal sites with probing depths PD ≥5 mm [33]. These definitions are expected to serve as the standard definitions of moderate and severe periodontitis in surveillance and epidemiological studies. However, a recent validation study demonstrated that NHANES III partial mouth periodontal examination protocols using CDC/AAP definition substantially understated the prevalence of periodontitis by 50% or more [33]. Therefore, we used a different criterion to measure the severity of PD to reduce misclassification due to the half-mouth examination in NHANES III. The prevalence of severe PD in this study was 5.1%, which is consistent with a recent validation study using a subsample of NHANES III [33]. The prevalence of severe PD was 4.8% based on a full-mouth "gold standard" examination.

Our study has several limitations. A large number of participants were excluded from the periodontal examination and many potential confounding factors were based on self-report. Also, using the National Death Index to determine our outcomes may result in misclassification due to the inaccuracies of death certificates. Moreover, we failed to exclude the subjects with transient inflammatory disease or trauma which may increase the levels of inflammatory biomarkers since no detailed information was available in NHANES III. However, the subjects with recent infection or trauma might be less likely to participate in the survey. Finally, PD is a condition that undergoes exacerbations and remissions, and recent studies supported that periodontal treatment may improve inflammatory markers and vascular function [34]. Our study was based on one-time assessment of the periodontal status and we have no data on subsequent treatments. Thus we cannot evaluate possible varying effects of active inflammation and clinical markers over follow-up period.

In conclusion, this study provides support for the hypothesis of the association of periodontal disease with CVD and all-cause mortality in men younger than 65, and for a potential oral-infection-inflammation pathway for CVD using national representative data in United States. Our results will furnish novel information about oral health and CVD and general health, thus have large potential public health implications.

Author contributions

FX and BL defined the research question, did the statistical analysis and manuscript writing.

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Conflict of interest

None declared.

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