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Characterization of periodontitis in people with type 1 diabetes of 50 years or longer duration

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Abstract

Background: Periodontitis is more common and severe in people with diabetes than the general population. We have reported in the Joslin Medalist Study that people with type 1 diabetes of ≥50 years (Medalists) may have endogenous protective factors against diabetic nephropathy and retinopathy.

Methods: In this cross-sectional study, the prevalence of periodontitis according to the CDC/AAP classification in a subset (n=170, mean age=64.6±6.9 years) of the Medalist cohort and its associations to various criteria of periodontitis and diabetic complications were assessed.

Results: The prevalence of severe periodontitis in Medalists was only 13.5% which was lower than reported levels in diabetic patients of similar ages. Periodontal parameters, including bleeding on probing, plaque index, gingival index and demographic traits, including male sex, chronological age, and age at diagnosis were significantly associated with severity of periodontitis, which did not associate with diabetes duration, hemoglobin A1c, body mass index, and lipid profiles. Random serum C-peptide levels inversely associated with severity of periodontitis

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T. Shinjo, A. Ishikado, H. Hasturk, I. Wu, M. Matsumoto collected the data. T. Shinjo, D.M. Pober, L.J. Tinsley, S. M. Paniagua, H. Shah and H.A. Keenan conceived the analysis. T.E. Van Dyke, R.J. Genco gave crucial suggestions for the study. T. Shinjo prepared the manuscript. G.L. King supervised the study and participated in the writing of the manuscript. All authors reviewed/edited the manuscript.

Conflicts of interest

None.

(p=0.03), lower probing depth (p=0.0002) and clinical attachment loss (p=0.03). Prevalence of cardiovascular diseases (CVD) and systemic inflammatory markers, plasma IL-6 and serum IgG titer against *Porphyromonas gingivalis* (*P. gingivalis*) positively associated with severity of periodontitis (p=0.002 and 0.02, respectively). Antibody titer to *P. gingivalis* correlated positively and significantly with CVD, serum IL-6 and high-sensitivity C-reactive protein.

Conclusion: Some Medalists could be protected from severe periodontitis even with hyperglycemia. Endogenous protective factors for periodontitis could, possibly, be related to residual insulin production and lower levels of chronic inflammation.

A one-sentence summary describing the key findings from the study:

People with type 1 diabetes of 50 years or longer duration could be protected from severe periodontitis which are associated with serum C-peptide levels.

Keywords

Diabetes; Periodontitis; Periodontal medicine; Epidemiology; Cardiovascular diseases

Introduction

Periodontitis is more common and of greater severity in patients with diabetes mellitus (DM) than non-diabetic patients and can lead to significant tooth loss[1]. This excessive tooth loss can result in changes in diet and worsening of glycemic control in people with diabetes[1,2]. The prevalence of periodontitis is predicted to increase due to a dramatic elevation of DM in the U.S. population[3]. A meta-analysis and cohort study have reported that the prevalence of periodontitis is significantly increased, by approximately 1.7-fold, in patients with type 1 or type 2 diabetes mellitus (T1DM/T2DM)[4,5]. In addition, severe periodontitis in DM is associated with poor glycemic control and vascular complications, such as cardiovascular disease (CVD), diabetic nephropathy (DN), and decreased survival[6]. Most of the previous studies regarding the relationship between diabetes and periodontitis have focused on risk factors[5,7,8]. However, no study has been reported to evaluate potential factors that may delay or protect the progression of periodontitis in people with DM which required a survival cohort of very long duration of diabetes.

Recently, we have reported that people with T1DM of 50 years or longer (The Joslin Medalist Study), may have endogenous protective factors from the development of complications; such as, DN or retinopathy (DR) since the severity of these complications did not correlate to glycemic control (HbA1c)[9–12]. Using the Joslin Medalist Study, potential protective profiles in the retina and renal glomeruli have been characterized and replicated, suggesting that this long survival cohort can be used to identify protective factors.

Thus, we have studied a subset of the Medalist cohort, who has shown to have protection from other diabetic complications[9–12], to assess whether they are also protected from the development of periodontitis, a known complication of diabetes[13]. This long surviving cohort with T1DM can provide information on potential protective factors for periodontitis in an aging population with chronic T1DM.

Research design and methods

Design overview

We performed a cross-sectional study of individuals with T1DM for 50 years or longer who were enrolled in the Joslin 50-Year Medalist Study[9–11]. Periodontal examinations were performed by professionally-trained periodontists on Medalists from 2008 to 2010 at Joslin Diabetes Center (JDC). Examinations were carried out by trained and calibrated examiners. A high level of reproducibility and accuracy was verified prior to beginning the study (kappa factor ≥0.8). The study was approved by the Institutional Review Board of JDC (CHS #07–12) and conforms to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines[14].

Participants and data sources/measurement

A convenience sample of participants from the Joslin 50-Year Medalist Study, (Methods described[9–11]), were examined between 2008 and 2010 (n=170). Medalists were recruited by website, magazine, and physician referrals. Participants were followed until December 2015 or death. Written informed consent was provided at the time of initial study visit. At the time of study visit, clinical labs were done. HbA1c was measured by high-performance liquid chromatography*. Lipid profiles were measured by standard enzymatic methods and serum C-peptide was determined by radioimmunoassay† and validated at the Northwest Lipid Research Laboratory at the University of Washington as previously described[15]. High-sensitivity C-reactive protein (hsCRP) was measured by radioimmunoassay‡. Serum IL-6 levels were measured by enzyme-linked immunosorbent assay (ELISA)§. Oral hygiene and dental history were surveyed by response to questionnaire: 1) Frequency of dental floss usage in past 7 days, 2) Regular visit at dentist at least once per year and 3) Period from last visit at dentist.

Periodontal examination

- **Plaque assessment—**The presence of supragingival plaque was assessed[16] on both buccal and lingual sites of all teeth. The scoring criteria were: 0= visible plaque absent, 1= visible plaque present. Patient mean score were used for analysis.

- **Gingival assessment—**Gingival assessment was performed using the Modified Gingival Index[17] on six sites per tooth. Patient mean score were used for analysis.

- **Distances from the gingival margin to cemento-enamel junction—**The distance from the gingival margin (GM) to the cemento-enamel junction (CEJ), rounded to the next lowest whole mm value, was measured using a University of North Carolina probe. If the GM was apical to the CEJ, the value was negative. All teeth present in a given arch were measured, except those with a full crown or large restoration invading the CEJ. The

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†Beckman Coulter, Fullerton, CA
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§Human IL-6 ELISA kit: R&D System, Minneapolis, MN

sites for probe placement were distobuccal angled, midbuccal, mesiobuccal angled, distolingual angled, midlingual and mesiolingual angled.

- **Probing depth—**The distance from the GM to the base of the pocket or sulcus was the probing depth (PD). PD was measured with a University of North Carolina probe at six sites per tooth. All teeth present in each quadrant, except third molars, were measured. The sites for probe placement were the same as the distance from the GM to CEJ. Patient mean depth and proportion (%) of site with PD ≥5 or 6 mm were used for analysis.

- **Clinical attachment loss—**The amount of clinical attachment loss (AL) was calculated from the following formula: PD - distance from GM to CEJ (mm). Patient mean clinical AL and proportion (%) of site with clinical AL ≥5 mm were used for analysis.

- **Bleeding on probing—**Bleeding on probing (BOP) was recorded at 6 sites per tooth same as probing PD, using the following scoring: 0= No bleeding in 15 seconds after probing, 1= Bleeding in 15 seconds after probing. Patient mean score was used for analysis.

Case definitions of severity of periodontitis

Periodontitis severity was assessed according to the Centers for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) case definitions[18]. The criteria were as follows: No periodontitis: No evidence of mild, moderate, or severe periodontitis. Mild periodontitis: ≥2 interproximal sites with clinical AL ≥3mm, and ≥2 interproximal sites with PD ≥4mm (not on same tooth) or one site with PD ≥5mm. Moderate periodontitis: ≥2 interproximal sites with clinical AL ≥4mm (not on same tooth), or ≥2 interproximal sites with PD ≥5mm (not on same tooth). Severe periodontitis: ≥2 interproximal sites with clinical AL ≥6mm (not on same tooth) and ≥1 interproximal site with PD ≥5mm. Third molars were excluded from the case definition. The prevalence of periodontitis was calculated as the sum of all types of periodontal disease including mild, moderate, and severe periodontitis.

Determination of diabetic complication status

CVD status was determined by self-reported history of coronary artery disease, myocardial infarction, angina, prior cardiac or leg angioplasty, or bypass graft surgery[10]. Estimated glomerular filtration rate (eGFR) of <45mL/min/1.73 m^2 was defined as nephropathy[19]. Early Treatment Diabetic Retinopathy Study seven-field criteria were used for determination of proliferative diabetic retinopathy (PDR)[20]. The Michigan Neuropathy Screening Instrument was used to assess neuropathy; scores of ≥2 were considered positive[21]. Physical activity was assessed by the College Alumnus Questionnaire[22]. Hypertension was determined as systolic blood pressure >135mm Hg or diastolic blood pressure >85mm Hg at the visiting time and/or current report of blood pressure medications.

Measurement of serum IgG titer against Porphyromonas gingivalis

Serum IgG antibody titers against *Porphyromonas gingivalis* (*P. gingivalis*) were determined using compact chemical luminescent immunological automatic analyzer[‖]. Readouts were then calculated into specific IgG antibody titer against *P. gingivalis* (U/ml).

Statistical analysis

The association of periodontitis severity with parameters of interest was assessed using the Cochran-Armitage trend test (dichotomous variables) or Cuzick's test for trend (continuous variables). For the comparison of parameters between Medalists with and without a detectable non-fasting serum C-peptide (defined as C-peptide >0.05 ng/ml) general linear models were used for continuous outcomes and χ^2 tests were used for dichotomous outcomes. Linear regression by general linear model was used to assess the association of log-transformed serum IgG titer against *P. gingivalis* with continuous outcomes. Logistic regression by generalized linear model was used with dichotomous outcomes. To control for confounding, models were adjusted for relevant parameters. In the rare instance of missing data, those observations were excluded from the analysis. Values were shown as mean ± SD. Statistical analyses were completed using two different software packages[¶]. Where relevant, p-values were adjusted for multiple testing using Benjamini-Hochberg procedures controlling for false-discovery rate (FDR)[23]. An FDR <0.05 was deemed significant.

Results

Participants in the Joslin 50-Year Medalist Study between April 2008 and December 2010 were recruited (n=175) and five participants were excluded from the analysis due to incomplete dental examination or results (total n=170 for analysis). As described in Table 1, the subset of the Medalist cohort had mean age of 64.6±6.9, age of diagnosis at 11.0±6.5 years, and duration of T1DM for 53.4±4.3 years. As a group, the Medalists exhibited BMI of 26.0±3.9kg/m^2 and excellent glycemic control with HbA1c levels of 7.15±0.82%. These clinical characteristics are comparable to the whole Medalist cohort as previously published[24]. According to responses to questionnaires, 88.8% of Medalists regularly used dental floss as shown by an average usage frequency of 6.0±4.4 times in the past 7 days prior to study entry. Additionally, 87.1% of Medalists had a visit to a dental clinic within the last 6 months of our study entry (Table 1).

Prevalence of severity of periodontitis in Medalists

As shown in Table 2, the prevalence of mild, moderate and severe periodontitis in the Medalists (n=170) were 14.7%, 53.5% and 13.5%, respectively. The total prevalence of periodontitis was 81.8% in the Medalist cohort. The periodontitis severity prevalence was highest at moderate levels of severity.

Periodontal parameters

With the exception of the number of teeth present, other indicators of periodontitis, including mean levels of BOP, PI, GI score, PD and clinical AL, % of site with PD ≥5mm and % of site with clinical AL ≥5mm were all significantly associated with severity of periodontitis (FDR <0.05, Table 3). Interestingly, serum IgG titer against *P. gingivalis* also showed significant association with severity of periodontitis (p=0.02) with the biggest elevation occurring between the no to mild periodontitis groups (Table 3).

[‖]POCube: Sunstar, Osaka, Japan
[¶]SAS version 9.4: SAS Institute, Cary, NC and R version 3.4: R Foundation for Statistical Computing, Vienna, Austria

The relationship of severity of periodontitis with clinical parameters

Male gender and chronological age were significantly associated with severity of periodontitis (p=0.03 and 0.001, respectively, Table 3). Interestingly, age at diagnosis exhibited a significant association with severity of periodontitis (p=0.006), although duration of diabetes not showing a relationship with severity of periodontitis (Table 3). BMI, waist circumference, insulin dose, HbA1c, and lipid profiles were not associated with severity of periodontitis. As expected, history of smoking was associated to severity of periodontitis (p=0.01) and rate of regular dental visits at least once per year was inversely related to severity (p=0.007, Table 3).

Association of severity of periodontitis with other diabetic complications

History of CVD and presence of DN were associated with severity of periodontitis (p=0.01 and 0.08), whereas lipid profiles including total cholesterol, LDLc, HDLc and triglycerides, usage of lipid-lowering and hypertension medications were not associated with severity of periodontitis (Table 3). Similarly, DR and peripheral neuropathy were not associated with severity of periodontitis in the Medalist cohort (Table 3). Inflammatory cytokines, such as serum IL-6 levels but not hsCRP, were significantly associated with severity of periodontitis (p=0.002).

Association of serum C-peptide levels with periodontal assessments

Since the presence of serum C-peptide has been reported to decrease the risks for certain diabetic complications, we examined the association between detectable (>0.05ng/ml) C-peptide with periodontal measures. Interestingly, greater levels of mean PD and clinical AL in the Medalists were associated with undetectable C-peptide and was significantly higher than those with detectable C-peptide (p=0.0002 and 0.03, respectively). Mean BOP and % of site with clinical AL ≥5mm were lower in Medalists with detectable C-peptide (p=0.06 and 0.06, respectively). Serum IL-6 levels and IgG titers against *P. gingivalis* were not significantly associated with the presence of C-peptide (Table 4).

Association of the serum IgG titer against *P. gingivalis* with CVD and biomarkers

Since serum IgG titers against *P. gingivalis*, CVD and DN correlated with assessments of periodontal disease, we examined their relationship to each other. The results showed that the history of CVD, but not DN, in the Medalist cohort exhibited a significant association with serum IgG titer against *P. gingivalis* (p=0.0081, Odds-Ratio=1.35) even with adjustment for age, sex, BMI, HbA1c and smoking history. While LDLc showed a slight negative association with serum IgG titer against *P. gingivalis*, (p=0.035, Supplemental Figure 1) serum IL-6 and hsCRP had positive association with *P. gingivalis* antibody (p=0.01 and p=0.009) even after adjustment for age, sex, BMI HbA1c and smoking history. The addition of IL-6, hsCRP and LDLc in the adjusted model did not affect the significance of the relationship between IgG titers against *P. gingivalis* and CVD history. There was no relationship between detectable C-peptide and IgG titers against *P. gingivalis* (Table 5).

Discussion

The Medalist cohort allowed an excellent opportunity to characterize factors that are important for protecting or delaying periodontal diseases in people with T1DM since this cohort has one of the largest numbers of participants over the age of 65 and with extreme long duration of T1DM in existance[25,26]. Since the Medalist cohort is a survival cohort, it is not designed for the determination of risk factors, but it is very appropriate to evaluate factors which could be important for delaying or protecting against the development of various complications as we have done for DN and DR[11,13]. Our data showed that the Medalists had more frequent usage of dental floss and regular dental visit compared with age-matched population of NHANES[27] (Table 1), suggesting that the Medalists had better oral hygiene than populations of the same age group. In the Medalists the prevalence of overall periodontitis was comparable to the published age-matched general U.S. population (83.1%, per NHANES[27]) which was 2.3% higher than people without diabetes at comparable age (Table 2). However, our results showed that the prevalence of severe periodontitis in this cohort 13.5% is lower than the expected prevalence (approximately 23.3%) for the U.S. population, aged 65–74 years with diabetes (Table 2), which is based on the recent NHANES report from Eke et al on periodontitis among individuals with or without DM[5]. Eke et al using NHANES data from 2009–2012, which defined DM status by measuring fasting glucose and HbA1c levels, showed that both people with controlled and uncontrolled DM had 2.11 and 2.13-fold (average 2.12-fold) higher prevalence ratios of severe periodontitis compared with people without DM[5]. Therefore, we calculated the approximate prevalence of severe periodontitis as 23.3% by using prevalence ratio in DM (PR=2.12 fold) and the prevalence of severe periodontitis in non-DM (11.0%). We elected not to use an earlier report from the same group of authors that found the prevalence of severe periodontitis in people of 65–75 years old with DM was 10.8% and 11.0% in those without DM[27], because in this early report, DM status was derived from self-report, which, as stated in the paper, can significantly underestimate the number of people with DM. More than one-quarter (27.8%) of US individuals with DM are undiagnosed and therefore will not report to have DM[28]. To have a more precise comparison, further studies are needed to evaluate the prevalence of severe periodontitis in age-matched individuals with diagnosed DM in NHANES. Additional evidence that Medalists could be protected from severe periodontitis is that % of sites of both PD ≥6mm and CAL ≥5mm were found to be less than those in age-matched population of NHANES[27] including both DM and non-DM (Supplemental Table 1). It is very likely that the Medalists are careful about their health including glycemic control and oral health. However, it is also likely that Medalists were protected from severe periodontitis since the usual risk factors such as glycemic control did not correlate with severity of periodontitis. In addition, the lack of correlation of severity of periodontitis to glycemic control also suggested the presence of protective factors as observed in DN and DR. For people with DM, the major causal factors for periodontitis are specific bacterial infection and subsequently chronic inflammation[29]. Our findings indicate that the potential endogenous protective factors in the Medalist cohort against the development of severe periodontitis could be related to the amelioration of chronic inflammation. This conclusion is suggested by the findings that the major difference between the Medalists with and without periodontitis is the elevated risk for severe

periodontitis which is more likely to be attributed to the various steps for the resolution of inflammation due to the infection rather than initial phase which may be related more to the resolution of infection[30–32].

The Medalist cohort provides new insights on factors which may delay the progression of periodontitis by demonstrating a relationship between age of diabetes onset and residual C-peptide production with severity of periodontitis. These findings suggest the presence of protective factors against the progression of periodontitis since hyperglycemia and insulin resistances, which have been reported to increase the risk of periodontitis[33], did not correlate to the severity of periodontitis (Table 3). The finding that younger age of diabetes onset had less severe periodontitis (Table 3) is interesting and could be similar to previous report that children with the onset of hyperglycemia and T1DM pre-dating puberty enjoy a honeymoon period on incurring risks for vascular pathologies as reported for other microvascular complications[34]. The mechanism responsible for the relative protection against the toxic effects of hyperglycemia during the pre-pubertal period is not known. Physical activities may also be protective, and our results showed a trend of association of physical activity with severity of periodontitis although it did not reach significance (p=0.07, FDR=0.158, Table 3). Another interesting finding is that HbA1c levels which previous studies have shown to correlate to periodontitis[1], did not correlate with severity of periodontitis in the Medalists (Table 3). This lack of statistical association of HbA1c and periodontitis could indicate the presence of protective factors. However, it could also be related narrow range of HbA1c exhibited by the Medalists with very good glycemic control (mean 7.15%) compared to the national mean of HbA1c in T1DM (8.2% in overall and 7.6% in ≥50 years age)[35].

One potential protective factor for periodontitis in the Medalist cohort could be residual insulin production since random serum C-peptide levels showed a significant negative relationship with severity of periodontitis (Table 3). Serum C-peptide levels markers of endogenous insulin production. Previous studies have shown that residual beta cell function as measured by C-peptide in T1DM was related to better glycemic control and, indirectly, to decrease microvascular complications[9,10,15,36]. The presence of C-peptide levels in the plasma was not associated with the presence or absence of DN and DR[9–11] in the Medalist Cohort. However, it is still possible that the residual insulin production of the Medalist may have a direct protective effect on the gingival tissue. We have reported that gingiva tissue can respond to insulin by activating signaling pathways such as phospho-Akt (p-Akt) and eNOS to increase nitric oxide production[37]. With insulin resistance and diabetes, insulin signaling in the gingiva is selectively inhibited via the p-AKT pathway[37], which has been documented in many other vascular tissues, contributing to vascular pathologies[38,39]. Thus, it is possible that the residual insulin production in the Medalists could have positive actions on the gingiva to improve the immune response to bacterial infection and enhance the anti-inflammatory actions of the macrophages, which have been reported to respond to insulin[40]. This suggestion is supported by the fact that C-peptide levels were also negatively associated with BOP, PD, and clinical AL (Table 4) suggesting that insulin may have independent and protective actions on the gingiva. Our data showed a significantly inverse association of serum C-peptide levels with mean PD and clinical AL levels but not with % of site with either higher PD or clinical AL (Table 4). This indicates that serum C-peptide levels may be

a useful measure to predict the global periodontal condition and severity of periodontitis in T1DM of long duration.

Chronic inflammation has an important role in the pathogenesis of periodontitis which was confirmed in this study. Our results showed serum IL-6 levels, but not hsCRP levels, were associated with severity of periodontitis in Medalists (p=0.002, Table 3). It has been reported that IL-6 could induce CRP expression in liver, but IL-6 is known as a one of the driving factors of inflammation as well even without CRP: Lee et al showed that IL-6 but not CRP was found to be associated with the prevalence of chronic kidney disease[41]. Passoja et al. reported that in T1DM patients in a younger aged cohort (mean age 38.6 years), serum IL-6 level was associated with the extent of BOP and PD (>4mm at baseline)[42]. Since IL-6 levels are known to be associated with increased risks of CVD, it is not surprising that it also associated with CVD and periodontitis in this study. In T1DM, a major risk factor for CVD is the presence of DN[43]. Thus, the finding that CVD and DN in the Medalists are associated with periodontitis suggests common pathogenic factors such as chronic inflammation. One explanation for the association of DN and periodontitis being less robust than CVD (P<0.08) is the low prevalence of DN in the Medalist cohort (6.5%).

The results of serum IgG titer against *P. gingivalis* in the Medalists are interesting on several levels. First, the highest elevation in the titers to *P. gingivalis* occurred between no-mild stages of periodontitis supporting the role of *P. gingivalis* as a pathogen for periodontitis in T1DM as previously reported for other cohort of patients with T2DM[44]. Second, the association of *P. gingivalis* titers with severity of periodontitis (p=0.02, Table 3), CVD and levels of IL-6 in the Medalists even with adjustment for age, sex, BMI, HbA1c and smoking status (p=0.02 and 0.004 respectively, Table 5) suggest that in T1DM patients with long duration, severe periodontitis may due to unresolved *P. gingivalis* infection which may generate an elevation of systemic inflammation that can accelerate CVD. There are many reports showing association between chronic periodontitis and CVD, especially in T2DM[45–47]. However, the association of chronic infection and inflammation to periodontitis and CVD in T1DM has been scarce. Further studies are needed to confirm these findings and determine whether targeted treatments of periodontitis can reduce CVD outcomes in T1DM.

Limitations of the present study potentially include selection bias, due to convenience sampling of interested Medalist; however, these clinical characteristics of the studied Medalists did not differ significantly from the overall Medalist cohort on demographic categories. Another limitation is the cross-sectional nature of the study, which clearly limits the strength of the conclusion. In addition, since this special cohort is compared entirely of elderly people with T1DM, these finding may not be applicable to people with T2DM or T1DM of short duration. In addition, the comparison studies in the literature are mainly composed of people with T2DM and the Medalists are all T1DM, our comparative analysis may not be accurate. More research is needed to generalize these findings to other groups of people with diabetes. However, it will be difficult to characterized protective factors against any complications of diabetes using short duration of disease. Furthermore, self-report CVD introduces classification bias. Due to the nature of self-report, it is unlikely for misclassification of CVD to happen. This method of self-report has been validated by

several epidemiologic studies[48–50]. Lastly, this study cannot be used for studying risk factors since the Medalist cohort is a survival cohort which is selected against those with high risk of various complication including periodontitis.

Conclusions

Medalists may be protected from severe periodontitis despite of the presence of DM and hyperglycemia. HbA1c did not correlate with severity of periodontitis in the cohort. This is the first report on the prevalence and severity of periodontitis, and its relationship with systemic clinical parameters in individuals with T1DM of very long-duration. The endogenous protective factors for periodontitis could be present and similar to those for CVD. One possible protective factor could be endogenously produced insulin to neutralize chronic inflammation caused by residual infection with periodontal pathogens, such as *P. gingivalis*, in the gingival tissues.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1.

50-Year Joslin Medalist participants: Baseline Characteristics of the study

Value expressed with a plus/minus sign are the mean ± standard deviation. Numbers in parentheses shows the numbers of Medalists with the variables.

Variable	Participants n=170 Mean; %
Gender (Male)	50.6 (86)
Age (years)	64.4 ± 6.9
Age at Diagnosis (years)	11.0 ± 6.5
Duration (years)	53.4 ± 4.3
BMI (kg/m^2)	26.0 ± 3.9
Waist circumference (cm)	90.3 ± 12.7
Insulin dose/kilogram (unit/kg)	0.47 ± 0.16
HbA1c (%)	7.15 ± 0.82
Total Cholesterol (mg/dL)	163.3 ± 32.7
LDL (mg/dL)	80.4 ± 23.1
HDLc (mg/dL)	66.8 ± 20.8
Triglycerides (mg/dL)	77.6 ± 37.3
Random C-peptide (ng/mL)	0.14 ± 0.11
C-Peptide >0.05 ng/mL	60.0 (102)
eGFR (ml/min/1.73 m^2)	73.7 ± 18.1
ACR (mcg/mg)	27.3 ± 50.6
hsCRP (mg/L)	2.59 ± 7.55
IL-6 (ng/ml)	0.37 ± 0.89
insulin pump use	57.0 (98)
Regular physical activity	83.9 (141)
Current smoker	2.9 (5)
Former smoker	45.9 (78)
Never smoker	51.2 (87)
CVD	36.0 (59)
DN	6.5 (11)
PDR	45.1 (74)
Neuropathy (MNSI>=2)	82.1 (138)
Lipid lowering medication	68.1 (113)
HTN medication	61.2 (101)
IA2 or GAD65+	50.7 (78)
DR3 or 4+	97.0 (163)
Use of dental floss in past 7 days	
Yes	88.8 (151)
No	11.2 (19)
Frequency	6.0±4.4

Variable	Participants n=170 Mean; %
Last dental visit	
within 6 months	87.1 (148)
6–12 months	8.8 (15)
>12 months or never	4.1 (7)

BMI: body mass index, LDLc: low-density lipoprotein cholesterol, HDLc: high-density lipoprotein cholesterol, eGFR: estimated glomerular filtration ratio, ACR: albumin/creatinine ratio, hsCRP: high-sensitive C-reactive protein, CVD: cardiovascular disease, DN: diabetic nephropathy, PDR: proliferative diabetic retinopathy, MNSI: Michigan Neuropathy Screening Instrument, HTN: hypertension, IA2: insulinoma antigen-2, GAD65: glutamic acid decarboxylase-65, DR: diabetic retinopathy.

Table 2.

The relationship of gender to prevalence of periodontitis in the Medalist cohort

severity	number	%	Male	%	Female	%
No	31	18.2	13	15.1	18	21.4
Mild	25	14.7	7	8.1	18	21.4
Moderate	91	53.5	52	60.5	39	46.4
Severe	23	13.5	14	16.3	9	10.7
Total prevalence	139	81.8	73	84.9	66	78.6
Total	170		86		84	

Table 3.

50-Year Joslin Medalist participants: Characteristics and results of periodontal exam by severity of periodontitis

Value expressed with a plus/minus sign are the mean ± standard deviation. The Cochran-Armitage trend test (dichotomous variables) or Cuzick's test for trend (continuous variables).

Variable	No periodontitis n=31 Mean; %	Mild periodontitis n=25 Mean; %	Moderate periodontitis n=91 Mean; %	Severe periodontitis n=23 Mean; %	p-value	FDR
Gender (Male)	**41.9 (13)**	**28.0 (7)**	**57.1 (52)**	**60.9 (14)**	**0.03**	**0.076**
Age (years)	**61.5 ± 5.8**	**62.6 ± 7.6**	**65.5 ± 6.8**	**66.0 ± 7.1**	**0.001**	**0.007**
Age at Diagnosis (years)	**8.5 ± 4.5**	**9.4 ± 6.9**	**12.1 ± 6.8**	**11.7 ± 6.3**	**0.01**	**0.031**
Duration (years)	53.0 ± 3.3	53.2 ± 3.2	53.4 ±4.5	54.3 ± 5.4	0.77	0.828
BMI (kg/m^2)	25.8 ± 4.5	27.6 ± 4.0	25.6 ± 3.6	25.6 ± 4.3	0.57	0.681
Waist circumference (cm)	87.2 ± 11.8	93.3 ± 14.2	90.2 ±12.9	91.3 ± 11.0	0.23	0.341
Insulin dose/kilogram (unit/kg)	0.42 ± 0.09	0.54 ± 0.20	0.45 ± 0.17	0.52 ± 0.13	0.28	0.401
HbA1c (%)	7.15 ± 0.87	7.40 ± 0.81	7.07 ± 0.80	7.20 ± 0.86	0.34	0.472
Total Cholesterol (mg/dL)	171.7 ± 36.3	164.7 ± 25.6	159.2 ± 30.8	166.5 ± 40.3	0.2	0.319
LDLc (mg/dL)	85.2 ± 24.7	78.7 ± 20.0	78.3 ± 22.7	83.7 ± 25.5	0.52	0.651
HDLc (mg/dL)	70.3 ± 21.7	67.9 ± 18.8	65.6 ± 19.3	65.5 ± 27.4	0.19	0.319
Triglycerides (mg/dL)	75.4 ± 37.9	91.00 ± 44.9	73.6 ± 32.5	82.0 ± 43.3	0.75	0.827
Random C-peptide (ng/mL)	**0.16 ± 0.10**	**0.12 ± 0.09**	**0.14 ± 0.12**	**0.11 ± 0.08**	**0.03**	**0.076**
C-Peptide >0.05 ng/mL	**80.7 (25)**	**64.0 (16)**	**55.0 (50)**	**47.8 (11)**	**0.006**	**0.027**
eGFR (ml/min/1.73 m^2)	75.7 ± 16.7	73.1 ± 16.7	72.1 ± 19.0	77.9 ± 18.1	0.81	0.85
ACR (mcg/mg)	17.7 ± 21.1	23.3 ± 36.9	31.1 ± 59.2	30.2 ± 57.2	0.12	0.246
hsCRP (mg/L)	1.70 ± 1.99	2.97 ± 5.69	2.76 ± 9.37	2.75 ± 6.19	0.72	0.815
IL-6 (ng/ml)	**0.10 ± 0.23**	**0.21 ± 0.49**	**0.45 ± 1.06**	**0.65 ± 1.02**	**0.002**	**0.011**
Insulin pump use	67.7 (21)	56.0 (14)	52.7 (49)	60.9 (14)	0.4	0.534
Regular physical activity	93.6 (29)	87.5 (21)	81.1 (73)	78.3 (18)	0.07	0.158
Ever smoker *	**27.6 (8)**	**64.0 (16)**	**48.9 (44)**	**71.4 (15)**	**0.01**	**0.031**
CVD	**13.8 (4)**	**30.4 (7)**	**45.1 (41)**	**33.3 (7)**	**0.01**	**0.031**
DN	0	0	11.0 (10)	4.4 (1)	0.08	0.172
PDR	50.0 (15)	58.3 (14)	42.5 (37)	34.8 (8)	0.41	0.534
Neuropathy (MNSI>=2)	93.6 (29)	84.0 (21)	76.7 (69)	86.4 (19)	0.16	0.299
Lipid lowering medication	55.2 (16)	75.0 (18)	74.4 (67)	52.2 (12)	0.71	0.815
HTN medication	54.8 (17)	64.0 (16)	62.5 (55)	61.9 (13)	0.53	0.651
IA2 or GAD65+	65.5 (19)	57.1 (12)	44.4 (36)	47.8 (11)	0.13	0.254
DR3 or 4+	96.8 (30)	100 (25)	95.5 (85)	100 (23)	0.91	0.91
Frequency of flossing **	5.22±4.19	6.16±5.20	6.19±4.40	5.91±3.79	0.19	0.319
Regular dental visit ***	**100 (31)**	**100 (25)**	**95.6 (87)**	**82.6 (19)**	**0.007**	**0.027**
Last dental visit						
Within 6 months	93.5 (29)	92.0 (23)	83.5 (76)	87.0 (20)	0.2	0.319

Variable	No periodontitis n=31 Mean; %	Mild periodontitis n=25 Mean; %	Moderate periodontitis n=91 Mean; %	Severe periodontitis n=23 Mean; %	p-value	FDR
6–12 months	6.45 (2)	8.0 (2)	11.0 (10)	4.3 (1)	0.85	0.87
>12 months	0 (0)	0 (0)	5.5 (5)	8.7 (2)	0.06	0.143
Bleeding on probing	**0.14 ± 0.12**	**0.23 ± 0.16**	**0.20 ± 0.16**	**0.32 ± 0.21**	**0.002**	**0.011**
Plaque index	**0.44 ± 0.24**	**0.55 ± 0.25**	**0.61 ± 0.27**	**0.66 ± 0.22**	**<0.001**	**0.007**
Gingival index	**1.65 ± 0.31**	**1.67 ± 0.31**	**1.68 ± 0.35**	**1.85 ± 0.39**	**0.007**	**0.027**
Pocket depth	**1.89 ± 0.27**	**2.10 ± 0.21**	**2.19 ± 0.30**	**2.50 ± 0.30**	**<0.0001**	**0.001**
Site of PD≥5mm (%)	**0.02±0.11**	**0.31±0.37**	**0.77±1.44**	**5.56±4.92**	**<0.0001**	**0.001**
Clinical AL	**1.45 ± 0.31**	**1.47 ± 0.39**	**2.34 ± 0.66**	**2.98 ± 0.85**	**<0.0001**	**0.001**
Site of clinical AL≥5mm (%)	**0.24±0.42**	**0.23±0.43**	**5.25±8.28**	**17.5±14.7**	**<0.0001**	**0.001**
Number of teeth	26.1 ± 2.2	25.4 ± 2.3	25.2 ± 4	24.9 ± 3.3	0.22	0.338
Antibody titer (U/ml)	**245.7 ± 774.5**	**850.2 ± 3065.0**	**708.9 ± 3054.6**	**990.3 ± 2185.7**	**0.02**	**0.057**

FDR: false discovery rate, PD: pocket depth, clinical AL: clinical attachment loss.

Significance defined as <0.05;

*Ever-smoker comprises of former (n=78) and current smokers (n=5).

**Frequency of dental floss usage in past 7 days.

***Regular dental visits at least once per year.

Parameters with significance in p-value (p <0.05) and FDR (F <0.1: almost significant, F <0.05: significant) are shown in bold face type.

Table 4.

The relationship of random serum C-peptide with IL-6, clinical periodontal measurements and serum IgG titer against *P. gingivalis*

Value expressed with a plus/minus sign are the mean ± standard deviation. Bivariate analysis- t-test for continuous variables/for categorical variables chi-square test. Parameters with significance in p-value (p <0.05) and FDR (F <0.25: suggestive, F<0.05: significant) are shown in bold face type.

	C-peptide <0.05ng/ml	**C-peptide ≥0.05ng/ml**		
	n=68	**n=102**		
Variable	**Mean; %**	**Mean; %**	**p-value**	**FDR**
IL-6	0.47 ± 0.97	0.33 ± 0.84	0.43	0.61
Bleeding on probing	0.24 ± 0.19	0.19 ±0.15	0.06	**0.15**
Plaque index	0.61 ± 0.29	0.55 ± 0.24	0.14	0.23
Gingival index	1.75 ± 0.34	1.66 ± 0.36	0.11	**0.22**
Pocket depth	**2.28 ± 0.35**	**2.08 ± 0.30**	**0.0002**	**0.002**
Site % of PD≥5mm	1.34±3.04	1.13±2.48	0.61	0.66
Site % of PD≥6mm	0.55±1.78	0.42±1.28	0.59	0.66
Clinical AL	**2.31 ± 0.82**	**2.04 ± 0.77**	**0.03**	**0.15**
Site % of clinical AL≥5mm	7.08±11.9	4.04±7.77	0.06	**0.15**
Antibody titer	772.5± 3460	595.2 ± 1911.8	0.66	0.66

FDR: false discovery rate, PD: pocket depth, clinical AL: clinical attachment loss.

Table 5.

The relationship of serum IgG titer against *P. gingivalis* with CVD and various biomarkers

The correlation of serum IgG titer against *P. gingivalis* with CVD and biomarkers were assessed with adjustment for age, sex, BMI, HbA1c and smoking status. Linear regression by general linear model was used with continuous outcomes. Logistic regression by generalized linear model was used with dichotomous outcomes. Parameters with significance ($p < 0.05$) are shown in bold face type.

Adjusted for:	Outcome	Odds Ratio	95% Lower CL	95% Upper CL	p-value
Age, Sex, BMI, HbA1c, Smoking	**CVD**	**1.30**	**1.04**	**1.63**	**0.02**
Age, Sex, BMI, HbA1c, Smoking, IL6	CVD	1.30	1.00	1.71	0.05
Age, Sex, BMI, HbA1c, Smoking, CRP	**CVD**	**1.33**	**1.04**	**1.70**	**0.02**
Age, Sex, BMI, HbA1c, Smoking, LDLc	**CVD**	**1.30**	**1.04**	**1.63**	**0.02**
Age, Sex, BMI, HbA1c, Smoking, IL6, CRP	**CVD**	**1.34**	**1.01**	**1.82**	**0.04**
Age, Sex, BMI, HbA1c, Smoking, IL6, LDLc	CVD	1.30	0.99	1.69	0.06
Age, Sex, BMI, HbA1c, Smoking, CRP, LDLc	**CVD**	**1.33**	**1.00**	**1.71**	**0.02**
Age, Sex, BMI, HbA1c, Smoking, CRP, LDLc, IL6	CVD	1.33	0.99	1.80	0.06
Age, Sex, BMI, HbA1c, Smoking	DN	1.01	0.74	1.37	0.95
Age, Sex, BMI, HbA1c, Smoking	Detectable C-peptide	0.94	0.78	1.13	0.51

Adjusted for:	Outcome	Beta Estimate	95% Lower CL	95% Upper CL	p-value
Age, Sex, BMI, HbA1c, Smoking	LDLc	−1.46	−3.07	0.14	0.07
Age, Sex, BMI, HbA1c, Smoking	**IL-6**	**0.10**	**0.03**	**0.17**	**0.004**
Age, Sex, BMI, HbA1c, Smoking	**CRP**	**0.77**	**0.24**	**1.30**	**0.005**

CL: confidence limits