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Periodontitis and risk of prevalent and incident coronary heart disease events

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Abstract
Objective: To investigate periodontitis as a risk factor for prevalent and incident coronary heart disease (CHD) in a group of middle-aged men from Northern Ireland.
Methods: A representative sample of 1400 dentate men had a comprehensive periodontal examination between 2001 and 2003. Prevalent and incident CHD events were validated by independent cardiologists. Logistic regression was used to assess the cross-sectional relationship between periodontitis and prevalent CHD and Cox's proportional hazards analysis to assess the longitudinal relationship between periodontitis and incident CHD.

Results: The mean age of the men at baseline was 63.7 (SD 3.0) years. Of the 1400 men examined, 126 (9%) had prevalent CHD. After adjusting for confounding variables, men with highest mean CAL (Q4) had an odds ratio of 2.15 (95% CI 1.15–4.02), \( p = 0.02 \) for prevalent CHD in comparison to men with the lowest CAL (Q1). During a median follow-up of 12.7 years, 137 (10.8%) of the 1274 men free of CHD at baseline had an incident CHD event. After adjusting for confounding variables, the hazard ratio for incident CHD in men in Q4 versus Q1 CAL categories was 1.36 (95% CI 0.81–2.29), \( p = 0.24 \).

Conclusions: In this group of dentate men, periodontitis was associated with prevalent CHD. However, there was no association with incident CHD.

KEYWORDS
coronary heart disease, inflammation, periodontitis

1 | INTRODUCTION

Coronary heart disease (CHD) remains the global leading cause of death and morbidity (Lozano et al., 2012). Although CHD mortality has declined in the United Kingdom (UK) (Bhatnagar et al., 2016), the burden of multimorbidity and comorbidity in patients with incident non-fatal CHD has increased (Tran et al., 2018). In the UK, CHD prevalence is around 3% in England and 4% in Scotland, Wales and Northern Ireland (Bhatnagar et al., 2016). Epidemiological studies have helped identify a range of what are now considered classic risk factors for cardiovascular disease (including CHD) such as age, smoking, hypertension and hypercholesterolaemia (Yusuf et al., 2001). Identification of such risk factors has led to the development of risk-prediction algorithms and established cardiovascular risk models for men and women (Lloyd-Jones Donald, 2010). These conventional risk factors, however, cannot fully explain excess cardiovascular risk, with at least 25% of all future events occurring in individuals with only one of the classical risk factors (Vilahur et al., 2014).
Periodontitis is a multifactorial inflammatory disease associated with dysbiotic dental plaque biofilms and characterised by progressive destruction of the tooth-supporting structures (Papapanou et al., 2018). Globally, periodontitis is one of the most prevalent non-communicable diseases, with severe periodontitis affecting an estimated 11.2% of the population (Kassebaum et al., 2014). In the UK, approximately 45% of all adults have some form of periodontitis and prevalence increases with age (White et al., 2012).

Several direct and indirect pathways have linked periodontitis with CHD. Atherosclerosis represents the most common pathological substrate of CHD, and the characterisation of the disease as a chronic low-grade inflammatory condition is now largely accepted (Golia et al., 2014). Periodontitis has been shown to represent a source of low-grade chronic inflammation, which contributes to the cumulative systemic inflammatory burden (Paraskevas et al., 2008; Winning et al., 2015). Due to evidence implicating chronic inflammation in the aetiology of CHD (Ridker et al., 2000), an aetiological relationship between periodontitis and CHD has been hypothesised. Furthermore, the direct involvement of periodontal pathogenic bacteria in atherosclerosis has also been proposed as a potential explanation for the putative relationship between periodontitis and CHD (Mougeot et al., 2017).

Successful periodontal therapy has a number of beneficial effects on surrogate markers of CHD including the following: CRP (D’Aiuto et al., 2013; Paraskevas et al., 2008; Teeuw et al., 2014), endothelial function (Tonetti et al., 2007), and carotid intima-media thickness (Orlandi et al., 2014). Despite these reported improvements in surrogate markers, there is no reliable evidence that periodontal therapy results in reduced primary or secondary occurrence of incident CHD (Li et al., 2017; Liu et al., 2019; Sanz et al., 2020).

At an epidemiological level, several systematic reviews have supported an association between periodontitis and incident CHD (Bahekar et al., 2007; Blaizot et al., 2009; Dietrich et al., 2013; Humphrey et al., 2008; Sanz et al., 2020). A meta-analysis, which included 15 studies with 230,406 participants, found that individuals with periodontitis had a significantly increased risk of incident CHD (risk ratio: 1.19; 95% confidence interval [CI] 1.13–1.26, \( p < 0.001 \)), independent of known confounders (Leng et al., 2015). However, not all studies have found the association to be consistent, particularly when the cohort is stratified by age (Dietrich et al., 2008), and there appears to be little evidence for any association in individuals older than 65 years (Dietrich et al., 2013). The implication of this may be highly relevant because any interventional trial will target high-risk groups, and since age is the most important predictor of CHD will therefore include older individuals. Further epidemiological studies have been recommended to clarify if periodontitis represents a risk for incident CHD in older individuals.

Acknowledging the lack of evidence, the aim of this study was to determine whether baseline periodontitis was associated with prevalent and incident CHD in a cohort of men, aged 58–72 years at study entry, in Northern Ireland.

### Materials and Methods

The men were participants in the Northern Ireland PRIME study (Prospective Epidemiological Study of Myocardial Infarction), which is a longitudinal cohort study of cardiovascular disease in Northern Ireland, and forms part of the MORGAM (MONICA Risk Genetics Archiving and Monograph) Project (Evans et al., 2004). From 1991 to 1994, 2748 men were recruited from local industry, the civil service and general medical practices. The sample was recruited to broadly match the social class structure of the population and represented ~5% of 50- to 60-year-old men in the greater Belfast region (Yarnell, 1998). From 2001 to 2003, 2010 surviving men attended for re-screening with their date of attendance taken as the baseline for this study which comprised 1400 (69.7%) of the men who were dentate and underwent a comprehensive periodontal examination.

Participants also completed questionnaires gathering information on their medical history, family history of myocardial infarction, social circumstances, demographic background and tobacco use. A physical examination assessed anthropometric measures including weight and height. Fasting blood samples were also obtained at the time of examination. Please see Supplementary Information for further details of all covariates examined.

A cross-sectional analysis was initially performed on the 1400 dentate men to explore the association between periodontitis and prevalent CHD. Prevalent CHD included men with a previous diagnosis of a CHD event defined as evidence of a myocardial infarction (MI), coronary artery bypass grafting (CABG), or percutaneous coronary intervention (PCI). Accurate information on a history of CHD was available from the main study database (as this is the primary outcome under investigation in the PRIME study, with men having already been under observation for 10 years prior to entry to the current study). Subsequently, a prospective analysis of incident CHD was performed on the 1274 CHD-free men (excluding 126 men with prevalent CHD; Figure 1).

Approval was obtained from the Research Ethics Committee of the Faculty of Medicine, Queen’s University Belfast, and the Office
for Research Ethics Committees (Northern Ireland) reference number 06/NIR02/107. Participation was voluntary, and all men provided informed, written consent.

2.1 | Periodontal examination

Comprehensive periodontal examinations were completed by four calibrated dental hygienists. Regular monthly meetings took place to ensure inter- and intra-examiner consistency and reproducibility (Linden et al., 2009). Clinical measurements were made at the mesial, distal, buccal and palatal/lingual aspects of all teeth excluding third molars. Additionally, number of teeth (excluding third molars), was also recorded at this baseline examination.

Gingival recession was measured as the distance between the gingival margin and the cemento–enamel junction. Periodontal probing depth (PPD) was measured as the distance between the gingival margin and the base of the periodontal pocket. Measurements were made to the nearest millimetre, and when any doubt existed, the lower value was scored. Clinical attachment loss (CAL) was calculated as periodontal pocket depth minus gingival recession. Mean CAL was then derived, and the cohort was divided into four categories by CAL quartiles (Q1 being men within the lowest quarter for mean CAL, Q4 being men in the highest) as the main exposure variable. CAL quartiles were initially defined based on the 1400 men for the cross-sectional analysis and subsequently re-defined based on the 1274 men for the prospective analysis.

Ancillary exposure variables considered were mean CAL as a continuous variable, mean PPD as continuous variable and divided into categories by quartiles, and number of teeth. Periodontal status was also defined according to Centres for Disease Control and Prevention and the American Academy of Periodontology (CDC/AAP) case definitions (Page & Eke, 2007).

2.2 | Cohort follow-up

The study commenced on the day each man attended for his periodontal examination (2001–2003) and the end of follow-up was 1st April 2015. The men were followed up by postal questionnaires circulated in 2007, 2011 and 2015 and asked to complete a clinical event questionnaire. If the subject did not respond, phone contact was established with the man and a further questionnaire sent. Self-reported information on any potential CHD events, provided as responses to the postal questionnaires or phone contacts, was subject to extensive validation using information from hospital and medical records. Reports on any imaging performed were retrieved. Diagnostic data for all CHD events included ECGs, biomarkers, surgical interventions, revascularisation procedures and any other treatment or special investigations. The Business Services Organization (BSO), an agency of the Department of Health, uses information from the Northern Ireland Registrar General together with data from GMP registrations and pensions to identify all deaths in Northern Ireland. The BSO provided six monthly updates on deaths of participants in PRIME, who are flagged on their system. Death certificates and post-mortem information were retrieved to determine the cause of death. If necessary, the circumstances of death were obtained from GMPs or the family.

All the relevant information for each fatal or non-fatal coronary event was examined by a validation committee. The events were
further classified into subtypes according to the criteria detailed in the PRIME manual. The validation committee included consultants who specialise in coronary disease and were independent of the researchers in the study. Incident CHD events included myocardial infarction, defined by one of the following sets of conditions: (a) new diagnostic Q-wave or other fresh typical electrocardiographic signs of necrosis; (b) typical or atypical pain symptoms and new (or increased) ischaemia and myocardial marker levels higher than twice the upper limit and (c) post-mortem evidence of fresh myocardial infarction or thrombosis. Incident CHD events also included CABG and PCI.

A 'time to event' was generated based on the date the participant had their baseline periodontal examination and the date of their incident CHD event. For those who did not complete the study to the defined end date, ‘date of last contact’ was taken as the last date a man had returned a follow-up questionnaire or death date, and data were accordingly censored at this point. Study participation rates (of questionnaires returned) are detailed in Supplementary Information.

2.3 | Statistical analysis

Comparisons of baseline characteristics (using either the independent samples t-test for continuous variables or the chi-square test for categorical variables) were made between those who did/did not present with prevalent CHD and also those who had/had not an incident CHD event.

Multiple logistic regression analysis was utilised to determine odds ratios for the association between prevalent CHD and quarters of the CAL distribution, Q2, Q3 and Q4 being compared with Q1 as the reference category. A series of models was fitted to adjust for potential confounding variables. Model 1 included adjustment for age, BMI, smoking, total cholesterol and triglycerides; Model 2 included additional adjustment for socio-economic conditions, living status and education; Model 3 included adjustment also for tooth brushing frequency and dental attendance; finally Model 4 added adjustment for diabetes, hypertension and family history of incident myocardial infarction. Diabetes and hypertension may have potential mediating as well as confounding effects, so were therefore added in the final model. A test for trend in odds ratios (ORs) for prevalent CHD across the four CAL categories was also performed.

A Kaplan–Meier plot was used to display the cumulative incidence of CHD events by periodontal status (in CAL categories). Cox’s proportional hazards analysis was used to estimate the hazard ratio (HR) for incident CHD events in CAL categories Q2, Q3 and Q4 versus Q1 as reference category. A series of models were fitted with adjustment for the same confounding variables used in the multiple logistic regression. A test for trend in hazard ratios for incident CHD across CAL categories was also performed. The same modelling was also carried out for other exposure variables including the following: CDC/AAP case definitions of periodontitis, mean CAL, mean PPD, mean PPD in groups defined by quartiles and number of teeth. Hazard proportionality was assessed using plots of −log(−log(survival)) against time and with tests on Schoenfeld residuals.

The level of statistical significance was set at \( p < 0.05 \). Analyses were performed using SPSS version 25 (IBM Corp) and Stata release 14 (StataCorp). Power calculation is reported in Supplementary Information.

3 | RESULTS

3.1 | Periodontitis and prevalent CHD

A total of 1400 men were analysed as part of the cross-sectional analysis. The mean age of the men was 63.7 (SD 3.0) with a range of 58–72 years. Characteristics of men by prevalent CHD status are reported in the left side of Table 1.

Multiple logistic regression analysis showed a significant relationship between periodontitis and prevalent CHD (Table 2). The unadjusted OR for prevalent CHD in men with the highest CAL (Q4) compared to those with the lowest CAL (Q1) was \( OR = 2.64 \) (95% CI 1.51–4.64) \( p < 0.001 \). Following sequential adjustment for confounding variables, this OR attenuated but remained significant at \( OR = 2.15 \) (95% CI 1.15–4.02), \( p = 0.02 \). A test for trend across CAL quartiles gave \( p = 0.001 \) in the unadjusted model, whereas in the fully adjusted this attenuated to \( p = 0.02 \).

3.2 | Periodontitis and incident CHD

The 1274 men who were CHD-free were included in the incident CHD analysis, with a median follow-up time of 12.7 years (interquartile range [IQR] 10.6–13.3). Of the 1274 men, 137 (10.8%) had an incident coronary event, consisting of non-fatal myocardial infarction \( (n = 59) \), PCI \( (n = 30) \) and cardiac death \( (n = 9) \). There were 200 (15.7%) deaths in men with no coronary events during follow-up, 862 men (67.7%) completed the full study with no coronary event, and 75 men (5.9%) were lost to follow-up. Characteristics of men by incident CHD status are reported in the right side of Table 1.

The Kaplan–Meier plot (Figure 2) shows the cumulative incidence of CHD event in the four CAL categories. The unadjusted HR for incident CHD in men with the highest mean CAL (Q4) compared to those with the lowest mean CAL (Q1) was \( HR = 1.58 \) (95% CI 0.97–2.58), \( p = 0.07 \) (Table 3). After sequential adjustment for confounding variables this HR attenuated to \( HR = 1.36 \) (95% CI 0.81–2.29), \( p = 0.24 \). A test for trend across CAL categories did not reach statistical significance in the unadjusted \( (p = 0.16) \) or in the fully adjusted model \( (p = 0.47) \). The testing of other exposure variables for periodontitis (Table 4) in similar models failed to show any significant associations.

4 | DISCUSSION

The main finding of this prospective cohort study was that periodontitis was not a statistically significant risk factor for incident CHD during a 12.7-year follow-up of a large group of 58- to 72-year-old
|                              | Prevalent CHD event | Incidence CHD event<sup>a</sup> |
|------------------------------|--------------------|---------------------------------|
|                              | Yes (n = 126)      | No (n = 1274)                  | p     | Yes (n = 137)      | No (n = 1137)                  | p     |
| Age, years, mean (SD)        | 64.6 (2.9)         | 63.6 (3.0)                     | <0.001| 63.6 (2.8)         | 63.6 (3.0)                     | 1.00  |
| Number of teeth, mean (SD)   | 17.8 (6.8)         | 19.6 (6.0)                     | 0.005 | 19.0 (6.7)         | 19.7 (5.9)                     | 0.25  |
| Moderate/severe periodontitis, n (%) | 57 (45.2)         | 534 (41.9)                    | 0.47  | 58 (42.3)          | 476 (41.9)                     | 0.92  |
| Mean CAL, geometric mean (IQR) | 2.5 (1.8-3.3)     | 2.1 (1.6-2.8)                  | <0.001| 2.3 (1.7-3.0)     | 2.1 (1.6-2.8)                  | 0.05  |
| Mean PPD, geometric mean (IQR) | 2.2 (1.9-2.5)     | 2.1 (1.8-2.4)                  | 0.03  | 2.2 (1.8-2.5)     | 2.1 (1.8-2.4)                  | 0.26  |
| BMI, kg/m², mean (SD)        | 28.3 (3.9)         | 27.4 (3.6)                     | 0.02  | 27.4 (3.5)         | 27.4 (3.6)                     | 0.85  |
| CRP, geometric mean (IQR)    | 1.8 (1.0-2.9)      | 1.7 (1.0-2.4)                  | 0.82  | 1.8 (1.0-3.3)      | 1.7 (1.0-2.4)                  | 0.32  |
| Total cholesterol, mmol/L, mean (SD) | 4.73 (0.86)    | 5.49 (0.95)                    | <0.001| 5.52 (0.97)        | 5.48 (0.95)                    | 0.58  |
| Triglycerides, mmol/L, geometric mean (IQR) | 1.57 (1.09-2.11) | 1.54 (1.09-2.12)              | 0.76  | 1.68 (1.12-2.38)   | 1.53 (1.07-2.14)               | 0.03  |
| HDL cholesterol, mmol/L, mean (SD) | 1.26 (0.31)    | 1.34 (0.35)                    | 0.02  | 1.31 (0.34)        | 1.34 (0.35)                    | 0.36  |
| LDL cholesterol, mmol/L, mean (SD) | 2.69 (0.77)   | 3.38 (0.84)                    | <0.001| 3.39 (0.86)        | 3.38 (0.84)                    | 0.86  |
| Statin medication use, n (%)  | 96 (76/2)          | 134 (10.5)                     | <0.001| 19 (13.9)          | 115 (10.1)                     | 0.18  |
| Smoking, n (%)               |                   |                                |       |                   |                                |       |
| Never                        | 31 (24.6)          | 524 (41.1)                     | <0.001| 46 (33.6)          | 478 (42.0)                     | 0.08  |
| Former                       | 78 (61.9)          | 532 (41.8)                     |       | 60 (43.8)          | 472 (41.5)                     |       |
| Current                      | 17 (13.5)          | 218 (17.1)                     |       | 31 (22.6)          | 187 (16.4)                     |       |
| Hypertension, n (%)          | 56 (44.4)          | 375 (29.5)                     | <0.001| 40 (29.4)          | 335 (29.5)                     | 0.99  |
| Diabetes, n (%)              | 13 (10.3)          | 71 (5.6)                       | 0.03  | 6 (4.4)            | 65 (5.7)                       | 0.52  |
| Family history of incident myocardial infarction, n (%) | 38 (30.2)          | 208 (16.3)                     | <0.001| 30 (21.9)          | 178 (15.7)                     | 0.06  |
| Socio-economic conditions, n (%) |                   |                                |       |                   |                                |       |
| Low                          | 40 (31.7)          | 418 (32.8)                     | 0.27  | 38 (27.7)          | 380 (33.5)                     | 0.41  |
| Medium                       | 23 (18.3)          | 302 (23.7)                     |       | 35 (25.5)          | 267 (23.5)                     |       |
| High                         | 63 (50.0)          | 553 (43.4)                     |       | 64 (46.7)          | 489 (43.0)                     |       |
| Education, years, mean (SD)  | 11.5 (3.0)         | 11.6 (2.9)                     | 0.58  | 11.4 (3.0)         | 11.6 (2.9)                     | 0.43  |
| Lives alone, n (%)           | 12 (9.5)           | 148 (11.6)                     | 0.48  | 15 (10.9)          | 133 (11.7)                     | 0.80  |

(Continues)
men from Northern Ireland. Utilising other exposure variables of periodontitis consistently demonstrated little support for an association with incident CHD in fully adjusted Cox proportional hazard analysis. There was, however, a significant association between baseline periodontitis and prevalent CHD. This finding is in line with the reported outcomes of a range of observational studies undertaken over the past 25 years (Stewart & West, 2016). A consensus statement and review by the American Heart Association concluded that while observational studies supported an association between periodontal disease and atherosclerotic vascular disease independent of known confounders they did not support a causative relationship (Lockhart et al., 2012). The highest level of evidence of a causative link would be provided by randomised controlled trials; however, the practical limitations are immense given that the current standard of cardiovascular care is so successful that populations have low event rates (Libby et al., 2011). Cohort studies will therefore provide the highest available form of evidence.

The finding from this study that periodontitis is not significantly associated with incident CHD contrasts with the finding of an association with prevalent CHD. It is also not in line with the available prospective cohort studies, the majority of which have reported an association between periodontitis and incident CHD (Leng et al., 2015). However, our results partially corroborate the findings of Dietrich et al. (2008) who reported an age-dependent association between periodontitis and CHD. The study examined a group of 1203 men aged 21–84 years enrolled in the United States

### TABLE 1 (Continued)

| Frequency (percentage) | Prevalent CHD event | Incident CHD event<sup>a</sup> | p | p | p |
|------------------------|---------------------|-----------------|----|----|----|
| Yes (n = 126)          | 79 (62.7)           | 95 (69.3)       | 0.39| 0.52|
| No (n = 1274)          | 873 (68.6)          | 778 (68.5)      | 0.39| 0.52|
| **Dental attendance, n (%)** |                    |                 |    |    |
| ‘Regular’              |                     |                 |    |    |
| 13 (10.3)              | 116 (9.1)           | 9 (6.6)         | 0.39| 0.52|
| ‘Occasional’           |                     |                 |    |    |
| 34 (27.0)              | 284 (22.3)          | 107 (9.4)       | 0.39| 0.52|
| ‘Only when in trouble’|                     |                 |    |    |
| 5 (4.0)                | 55 (4.4)            | 3 (2.2)         | 0.39| 0.52|
| **Tooth brushing <=2/day, n (%)** |                 |                 |    |    |
| 5 (4.0)                | 55 (4.4)            | 3 (2.2)         | 0.39| 0.52|

Note: Statistical significant p < 0.05 values are indicated in bold. Hypertension n = 1 missing (in Event group). Socio-economic conditions n = 1 missing (in No event group). Dental attendance n = 1 missing (in No event group). Tooth brushing n = 18 missing (1 in Event group, 17 in No event group). Abbreviations: BMI, body mass index; CAL, clinical attachment level; CHD, coronary heart disease; CRP, C-reactive protein; HDL, high-density lipoprotein; IQR, interquartile range; LDL, low-density lipoprotein; n, number of men; PPD, periodontal probing depth; SD, standard deviation. <sup>a</sup>Prevalent cases excluded.

### TABLE 2 Multiple logistic regression analysis for baseline association of prevalent coronary heart disease with periodontitis status before and after adjustment for confounders in 1344 men with complete data

| Frequency (percentage) | Prevalent CHD event | Incident CHD event<sup>a</sup> | p |
|------------------------|---------------------|-----------------|----|
| Yes (n = 126)          |                     |                 |    |
| No (n = 1274)          |                     |                 |    |
| **Dental attendance, n (%)** |                 |                 |    |
| ‘Regular’              |                     |                 |    |
| 13 (10.3)              | 116 (9.1)           | 9 (6.6)         | 0.39|
| ‘Occasional’           |                     |                 |    |
| 34 (27.0)              | 284 (22.3)          | 107 (9.4)       | 0.39|
| ‘Only when in trouble’|                     |                 |    |
| 5 (4.0)                | 55 (4.4)            | 3 (2.2)         | 0.39|
| **Tooth brushing <=2/day, n (%)** |                 |                 |    |
| 5 (4.0)                | 55 (4.4)            | 3 (2.2)         | 0.39|

Note: Statistical significant p < 0.05 values are indicated in bold. Model 1 = adjusted for age, body mass index, smoking, total cholesterol and triglycerides. Model 2 = Model 1 + socio-economic conditions, marital status and education years. Model 3 = Model 2 + tooth brushing frequency and dental attendance. Model 4 = Model 3 + diabetes, hypertension and family history of incident myocardial infarction. Abbreviations: CAL, clinical attachment level; CI, confidence interval; OR, odds ratio; Q1, Q2, Q3 and Q4, quarters of distribution.
Department of Veteran Affairs Normative Aging Study. Utilising Cox proportional hazard models with adjustment for a range of covariates, they reported a significant independent association between periodontitis and incident CHD (HR = 2.12, 95% CI 1.26–3.60) in men <60 years of age. However, no association was found in men ≥60 years of age (Dietrich et al., 2008). The mean age of men at baseline in the present study was 63.6 years (SD 3.0), with 93.4% of the cohort aged ≥60 years. This may explain why we observed a significant association between periodontitis and prevalent CHD but not with incident CHD in the present study. Other studies have also reported similar age-dependent associations between periodontitis and incident CHD (DeStefano et al., 1993; Geismar et al., 2006), and periodontitis and an ischaemic cerebrovascular accident (Grau et al., 2004; Joshipura et al., 2003).

Periodontitis at a younger age is a marker of higher disease susceptibility (Genco & Borgnakke, 2013). Therefore, the findings from previous studies reporting a positive association between periodontitis and incident CHD in younger cohorts suggest a
hypothetical that common pro-inflammatory susceptibility factors may explain a large degree of the association pattern (Dietrich et al., 2008). Related to this, evidence is emerging for genetic pleiotropy between periodontitis and atherosclerotic cardiovascular diseases (Aarabi et al., 2017). To date, 4 genetic loci have been shown to be shared between atherosclerotic cardiovascular diseases and periodontitis: CDKN2B-AS1 (ANRIL; Schaefer et al., 2009); a conserved noncoding element within CAMTA1 upstream of VAMP3 (Bochenek et al., 2013); PLG (Schaefer et al., 2015); and a haplotype block at the VAMP8 locus (Munz et al., 2018). These shared genes provide support for the possibility that periodontitis is not causally related to atherosclerotic diseases, but rather both conditions are sequelae of similar aberrant inflammatory pathways. Therefore, in epidemiological studies investigating a link, an association will more likely be observed in younger cohorts where there may be a stronger genetic influence on the development of both periodontitis and atherosclerotic cardiovascular disease.

The main exposure variable of interest was mean CAL based on the original design of the PRIME study in the late 1990s and a corresponding power calculation (see Supplementary Information). CAL represents cumulative periodontal tissue damage and is derived from measures of pocket probing depth and gingival recession. A drawback of the use of mean CAL as a measure of periodontitis is that it fails to account for tooth loss and there is a recognised potential for bias as tooth loss increases (Albandar, 2011). We therefore included analysis on a range of ancillary exposure variables including PPD, number of teeth, and the more widely accepted CDC/AAP case definitions of periodontitis (Page & Eke, 2007). The CDC/AAP case definition utilises a combination of the CAL and PPD based on defined disease thresholds and has been recommended for use in epidemiological studies (Holtfreter et al., 2015).

Although no evidence for an association between periodontitis and incident CHD events was found in this study, the influence of periodontitis on the development of atherosclerosis cannot be completely excluded. In an earlier follow-up of the same PRIME cohort, we found that periodontitis was an independent risk factor for incident type 2 diabetes mellitus (T2DM) (Winning et al., 2017). It is well established that diabetes is a prominent risk factor for cardiovascular disease, but the aetiology is not completely understood and goes beyond classic hyperglycaemic effects on tissues (Dokken, 2008). Given the shared inflammatory pathophysiology of both cardiovascular disease and diabetes, the low-grade contribution periodontitis may make to the systemic inflammatory burden means a potential role should not be dismissed (Schenkein & Loos, 2013). Active periodontitis, by enhancing systemic inflammation, might consequently contribute to the development of atherosclerosis short of its expression in incident CHD events.

The strengths of this study include the homogeneity of the sample: White Western Europeans, of limited age range (58–72 years), who at original recruitment, were representative of a working male population in Northern Ireland (Yarnell, 1998). Ethnicity has previously been shown to have a significant impact on the development of both periodontitis (Delgado-Angulo et al., 2016) and incident CHD (D’Agostino et al., 2001). It should be emphasised that the homogeneity of the sample adds robustness to the analysis. The exposure variable was comprehensively assessed by a detailed full-mouth periodontal examination. The fact that all other various exposure variables used in the analysis (Table 4) yielded the same result, strengthens the finding that periodontitis was not associated with incident CHD events in this group of men. The long prospective follow-up (media n = 12.7 years) and relatively small number of men who were lost during follow-up (5.9%) add confidence to findings. Men that were lost to follow-up were appropriately censored at ‘date of last contact’ (date of last questionnaire

| TABLE 4 | Summary table of Cox’s proportional hazard analysis for risk of incident coronary heart disease event by other measures of chronic periodontitis exposure before and after adjustment for confounders in 1211 men with complete data |
|-----------------|-----------------|-----------------|
|                         | Crude model      | Fully adjusted model |
|                         | HR (95% CI) p    | HR (95% CI) p     |
| Moderate/severe periodontitis (ref. no/mild) | 1.05 (0.74, 1.48) 0.79 | 1.00 (0.70, 1.43) 0.99 |
| Mean CAL (per doubling) | 1.30 (1.00, 1.69) 0.047 | 1.23 (0.93, 1.63) 0.15 |
| Mean PPD (per doubling) | 1.35 (0.83, 2.17) 0.23 | 1.28 (0.77, 2.12) 0.34 |
| PPD (ref Q1) |         |         |
| Q2            | 0.57 (0.34, 0.98) 0.30 | 0.57 (0.34, 0.98) 0.43 |
| Q3            | 1.05 (0.66, 1.65) 0.14 | 1.02 (0.64, 1.63) 0.34 |
| Q4            | 1.10 (0.70, 1.74) 0.30 | 1.07 (0.66, 1.73) 0.30 |
| Number of teeth (per tooth increase) | 0.98 (0.95, 1.01) 0.14 | 0.98 (0.96, 1.01) 0.30 |

Note: Values in bold signify statistical significance p < 0.05.
Abbreviations: CAL, clinical attachment level; CI, confidence interval; HR, hazard ratio; PPD, periodontal probing depth; Q1, Q2, Q3 and Q4, quarters of distribution.
*Fully adjusted model = adjusted for age, body mass index, smoking, total cholesterol, triglycerides, socio-economic conditions, lives alone, education years, tooth brushing frequency, dental attendance, diabetes, hypertension and family history of incident myocardial infarction.
*Variable transformed prior to analysis using logarithms to base 2.
*Test for trend across categories.
returned) in the analysis. Due to the design of the PRIME study, with its main aim to investigate putative risk factors for the development of cardiovascular disease, we were able to make use of a range of baseline data on potential confounding factors.

There are several limitations with this study. Firstly, the study was conducted in a male-only cohort and we therefore cannot draw a conclusion on any potential association between periodontitis and incident CHD events in women. This does highlight a broader problem of under-representation of women in cardiovascular research (Vitale et al., 2017). The PRIME study was designed in the late 1980s to investigate risk factors for the development of cardiovascular diseases, which were significantly more prevalent in men at that time (Jousilahti et al., 1999). The participants consented to long-term follow-up and we took the view that it was important to make use of the CHD data as it was the case in comparable studies such that of Dietrich et al. (2008).

Secondly, the study is underpowered based on the number of actual events in the incident CHD analysis (n = 137) being lower than the number of anticipated events in the power calculation (n = 185; see Supplementary Information). This reflects a trend in developed countries towards declining incident CHD rates (Bhatnagar et al., 2016; Sanchis-Gomar et al., 2016). A retrospective power calculation based on the 137 actual events still achieved an 85% power at a significance level of 0.05 to detect a linear trend in incidence rate across CAL quarters of 14% versus 11.7% versus 9.3% versus 7.0% (a top to bottom quarter risk ratio of 14%/7% = 2.0).

Thirdly, men underwent a one-off periodontal examination (baseline of the present study) with no intervention being performed. Those with periodontitis were informed and advised to attend with a dentist. It is unknown whether participants went on to have any periodontal intervention performed, suffered tooth loss, or what influence this might have had on their CHD risk during follow-up. Similarly, men initially classified as not having periodontitis may have developed periodontitis during the observation time. Both scenarios have the potential to dilute a potential association between periodontitis and CHD.

Fourthly, some of the men recruited as part of the original representative cohort (1991–1994) did not attend the rescreening that was the baseline for this analysis (2001–2003). Given that for the included men to have had an oral examination they had to have survived nearly 10 years since cohort inception, it is possible that those with the most severe disease are not represented in our examined sample and we surmise, therefore, that the associations observed might be somewhat biased towards the null. Alternatively, for any collider stratification bias to have affected our estimates, one would have to suppose that a factor downstream or distal to CHD (e.g. treatment) affected the manifestation of periodontal disease and that we had inadvertently conditioned or adjusted for such a variable but we consider this unlikely (Richiardi et al., 2019).

Fifthly, as in most cohort studies, follow-up for the men who were lost or died from non-cardiac causes was censored, so their risk of CHD was assumed to be no different from men with similar characteristics that remained in the cohort. It is difficult to assess what impact this might have on the findings.

Finally, in common with all observational studies, the possibility of residual confounding or failure to account for other relevant confounders, such as diet, stress or genetic predisposition, may have had some influence on the association between periodontitis and CHD. This would also include undiagnosed diabetes or hypertension as these were assessed by self-report rather than baseline HbA1c/glucose levels or blood pressure measurements.

## 5 | CONCLUSION

In conclusion, baseline periodontitis was associated with prevalent CHD in a group of 58- to 72-year-old men in Northern Ireland. However, periodontitis was not a statistically significant independent risk factor for incident CHD over a 12.7-year follow-up period. Our findings do not support the hypothesis that periodontitis is an important independent risk factor for incident CHD. Larger, prospective cohort studies in diverse populations are necessary to further confirm or refute periodontitis as a risk factor in incident CHD.

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## CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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