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Periodontitis as the risk factor of chronic kidney disease: Mediation analysis

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Abstract

Aim: To determine sequences and magnitude of causality among periodontitis, diabetes and chronic kidney disease (CKD) by mediation analysis.

Methods: Ten-year-data were retrieved from the Electric Generation Authority of Thailand (EGAT) study. A cohort of 2,635 subjects was identified with no CKD at baseline. The interested outcome was CKD incidence defined as glomerular filtration rate <60 ml/min/1.73 m^2. The percentage of proximal sites with clinical attachment loss ≥5 mm was used to represent periodontitis. Mediation analysis with 1,000-replication bootstrapping was applied to two causal diagrams, *diagram A (Periodontitis → Diabetes → CKD)* and *diagram B (Diabetes → Periodontitis → CKD)*.

Results: The cumulative incidence of CKD was 10.3 cases per 100 persons during 10-year period. In *diagram A*, each increasing percentage of proximal sites with severe periodontitis increased the adjusted odds ratio of CKD 1.010 (95% CI: 1.005, 1.015) and 1.007 (95% CI: 1.004, 1.013), by direct and indirect effect through diabetes, respectively. In *diagram B*, diabetes increased the odds of CKD twofold, with 6.5% of this effect mediated via periodontitis.

Conclusions: Periodontitis had significant direct effect, and indirect effect through diabetes, on the incidence of CKD. Awareness about systemic morbidities from periodontitis should be emphasized.

KEYWORDS
chronic kidney disease, diabetes, mediation analysis, periodontitis

1 | INTRODUCTION

Chronic kidney disease (CKD) is a state of reduced glomerular filtration rate, increased urinary albumin excretion or both. It is an important cause of subsequent health burden, including end-stage renal disease, infection, cardiovascular disease and death. Despite therapeutic advances, the number of CKD patients continues to increase worldwide (Levin & Stevens, 2014).

Periodontitis, the most common oral disease, is generally described as an infectious disease. It causes the gum infection and destruction of tooth-supporting organs (Kassebaum et al., 2014). It has been identified as a novel and potentially modifiable risk factor for CKD (Chambrone et al., 2013). A potential biological pathway is that periodontal pathogens and inflammatory cytokines from the infected periodontium travel via the bloodstream and affect the endothelial function of nephrons (Kshirsagar et al., 2005). Associations between periodontitis and CKD have been suggested in cross-sectional studies from various ethnicities (Fisher et al., 2008; Ioannidou & Swede, 2011; Kshirsagar et al., 2005; Yoshihara, Iwasaki, Miyazaki, & Nakamura, 2016). Moreover, it has been identified as a factor in the decline of kidney function over time (Chang et al., 2017; Chen et al., 2015; Grubbs et al., 2015, 2016; Iwasaki et al., 2012; Shultis et al., 2007).

Diabetes is well established as another risk factor for CKD (Levin & Stevens, 2014; Shen et al., 2017). Simultaneously, diabetes associates bi-directionally with periodontitis (Taylor & Borgnakke, 2008). With the complexity and uncertainty of these causal pathways, diabetes could increase the risk of CKD partly through periodontitis or periodontitis could increase the risk of CKD partly through diabetes. Therefore, in this study, we aimed to assess the causal pathway of periodontitis, diabetes and CKD using a mediation analysis framework.

Clinical Relevance

Scientific rationale for the study: Periodontitis and diabetes are suspected risk factors of chronic kidney disease (CKD), but whether they were directly associated or mediated via each other are still unknown.

Principal findings: According to the mediation analysis, periodontitis had significant direct and indirect effects through diabetes on the incidence of CKD. Moreover, periodontitis was also an intermediate variable in the causal pathway of diabetes on CKD.

Practical implications: To reduce diabetes and CKD burden, awareness about systemic morbidities from periodontitis should be emphasized by public health practitioners.

2 | METHODS

This was performed as an observational cohort study that conformed with STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for reporting observational studies (Appendix S1).

The study used data from the Electric Generation Authority of Thailand (EGAT) project, an ongoing prospective cohort in Thailand aiming to examine non-communicable disease risk factors (Vathesatogkit et al., 2012). The EGAT employees were randomly selected and enrolled. All participants underwent a health survey every 5 years. Ethics clearance was provided by Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. In this study, data from the survey in 2003 (EGAT2/2) were taken as the baseline, and data from 2008 (EGAT2/3) and 2013 (EGAT2/4) were considered as follow-up visits at 5 and 10 years, respectively. Subjects were eligible if they participated in the health survey at least once in 2003, 2008 and 2013. Exclusions were those who had an estimated glomerular filtration rate (eGFR) <60 ml/min/1.73 m^2 at the baseline (EGAT2/2), absent from all periodontal examination due to refusal, had systemic conditions which required antibiotic prophylaxis or were fully edentulous.

A self-administered questionnaire was used to identify background characteristics, underlying diseases and health behaviours. Physical examination and routine blood tests under fasting conditions were performed by trained personnel from Ramathibodi Hospital. Periodontal examination was carried out by calibrated periodontists from the Department of Periodontology, Chulalongkorn University. Periodontal probing depth (PPD) and gingival recession (RE) were examined on all fully erupted teeth, except third molars and retained roots. PPD and RE were measured using an UNC15 probe on six sites per tooth. The clinical attachment level (CAL) was then calculated from the PPD and RE. Standardization for periodontal measurements was performed among 6–8 examiners before each survey. Weighted kappa (±1 mm) was used to determine the inter-examiner and intra-examiner agreements (Table S1).

The outcome of interest was CKD, as defined by an eGFR of less than 60 ml/min/1.73 m^2, measured in 2008 and 2013. The eGFR was calculated by the CKD Epidemiology Collaboration equation (CKD-EPI: 2009) as reported (Levin & Stevens, 2014). Serum creatinine was measured using the IDMS-standardized enzymatic assay on a Vitros 350 analyzer (Ortho-Clinical Diagnostics).

Periodontitis: The percentage of proximal sites with severe periodontitis (CAL ≥5 mm) was used to summarize periodontal status. Moreover, the 2007 Centers for Disease Control and Prevention and American Academy of Periodontology (CDC/AAP) case definitions, which categorized periodontitis by CAL and PPD, were alternatively used (Page & Eke, 2007).

Diabetes: Diabetes was diagnosed if an individual had a fasting blood glucose level of ≥126 mg/dl or took any type of anti-diabetic medications.

Other co-variables known to affect periodontitis, diabetes and CKD were included as follows: *age* (continuous), *gender* (male | female), *marital status* (single | married | widowed, divorced, separated), *education* (≤high school | vocational or diploma | ≥bachelor's degree), *income*

(<20,000 | 20,000–49,999 | ≥50,000 Baht/month), *exercise* (none | 1–2 times/week | ≥3 times/week), *smoking* (never | quit | current smokers), *alcohol drinking* (never | quit | current drinkers), *obesity* (Nishida, Ko, & Kumanyika, 2010) (waist–hip-ratio >0.9 (male) or waist–hip-ratio >0.85 (female)), *hypertension* (Chalmers et al., 1999) (HT; systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or took HT drugs), *dyslipidemia* (Catapano et al., 2016) (DLP; HDL <40 mg/dl (male) or HDL <50 mg/dl (female) or LDL ≥160 mg/dl or triglyceride ≥150 mg/dl or took any lipid lowering drug), *regular non-steroidal anti-inflammatory drugs (NSAIDs) use* (Yes | No), *family history of diabetes* (Yes | No) and *serum uric acid level* (continuous).

2.1 | Statistical analysis

Imputation: Among eligible subjects, missing data ranged from 0.38% to 18.30%. Assuming data were missing at random, multiple imputation using chain equation (MICE) for longitudinal data was performed for the within- and whole-wave missing data (Biering, Hjollund, & Frydenberg, 2015; Rubin & Schenker, 1991; White, Royston, & Wood, 2011). Twenty imputations of MICE were constructed using ordinal/multinomial logistic, or linear/interval/truncated regressions depending on the type of imputed data (Table S2).

Mediation analysis (Baron & Kenny, 1986; Iacobucci, 2012; Imai, Keele, & Tingley, 2010): Mediation models were constructed and analysed separately by two causal diagrams (Figure 1), *diagram A (Periodontitis → Diabetes → CKD)*, and *diagram B (Diabetes → Periodontitis→CKD)*.

For *diagram A*, periodontitis was fitted as the independent variable, diabetes was the mediator and the incidence of CKD was the outcome. Causal equations were constructed as follows:

$$M = a_0 + aX + \Sigma_k e_k z_k \quad \text{(path a)}$$

$$Y = b_0 + c'X + bM + \Sigma_k e_k z_k \quad \text{(path b)}$$

where X = independent variable; Y = outcome; M = mediator; z_k = confounders

The mediator was firstly regressed on the periodontitis (path a), and then the outcome variable was modelled with periodontitis and diabetes (path b). Other confounders for diabetes & CKD, whose *p*-values were less than 0.10 from univariate analysis, were simultaneously considered in the multivariate logistic regression using forward stepwise selection. Next, the generalized structural equation model (GSEM) was applied to construct these two equations simultaneously taking into account for within and between variations in the imputed data sets, and also longitudinal data.

A potential causal mediation effect was then estimated using the product of coefficients method (MacKinnon, Fairchild, & Fritz, 2007). A bootstrap analysis with 1,000 replications was applied to estimate the average causal mediation effects without requiring the assumption of normality (Preacher & Hayes, 2008). With a bias-corrected bootstrap technique, the total, direct and indirect (mediation) effects and their 95% CIs were estimated. Analyses for *diagram B* were performed in a similar manner to *diagram A*, except that diabetes was the independent variable, and periodontitis was the mediator. Data imputations and statistical analyses were performed using STATA 14.2 software. A *p*-value <0.05 was considered statistically significant.

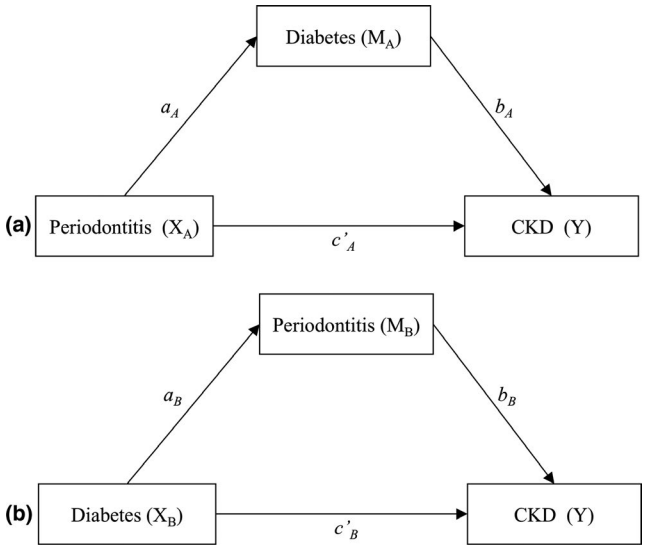

FIGURE 1 Structural causal diagrams

3 | RESULTS

A total of 2,795 subjects were eligible from the survey between 2003 to 2013. Among them, 1,821 (65%) subjects had complete attendance in all three waves, with 14% and 21% of subjects attending once and twice, respectively. In total, 126 subjects were excluded because they had CKD at baseline (EGAT2/2). Within the non-CKD cohort, six subjects were further excluded due to systemic conditions that prevented their periodontal examination. Fifteen subjects were fully edentulous, and 13 subjects refused to attend all periodontal examinations. This left 2,635 participants who were included in this analysis (Figure S1). Characteristics of the excluded cases are shown in Table S3. Almost all were similar to those included.

Baseline characteristics are shown in Table 1. The mean age was 47.7 ± 4.9 years, approximately three quarters were males and a half of them admitted to smoking and drinking alcohol. Prevalence of diabetes, HT and DLP at baseline were 7.7%, 27.3% and 68.6%, respectively. With the CDC/AAP periodontitis definition, the prevalence of moderate and severe periodontitis was ~50% and ~30%, respectively.

The total numbers of new CKD cases were 167 and 105 cases in the EGAT2/3 (2008) and 2/4 survey (2013), respectively. Overall, the cumulative incidence of CKD was 10.3 cases per 100 persons during 10-year period (95% CI: 9.1, 11.6). Additionally, this increased with the severity of periodontitis, where the CKD incidences among normal/mild, moderate and severe periodontitis were 7.2, 9.6 and 13.9 cases/100 persons, respectively.

TABLE 1 Characteristics of included subjects

Characteristics	EGAT 2/2 *n* = 2,532	EGAT 2/3 *n* = 2,183	EGAT 2/4 *n* = 1,948
Age (year)	47.7 ± 4.9	52.3 ± 4.6	56.9 ± 4.5
Gender			
Male	1848 (73.0)	1567 (71.8)	1,368 (70.2)
Female	684 (27.0)	616 (28.2)	580 (29.8)
Marital status			
Single	238 (9.5)	178 (8.2)	144 (7.5)
Married	2081 (83.0)	1801 (83.2)	1586 (82.2)
Divorced/ Widowed	189 (7.5)	187 (8.6)	200 (10.3)
Income (Baht/month)			
<20,000	312 (12.5)	135 (6.4)	189 (9.8)
20,000 – 49,999	1,318 (52.7)	634 (29.9)	248 (12.9)
≥50,000	872 (34.8)	1,347 (63.7)	1,488 (77.3)
Education			
≤Secondary school	688 (27.4)	488 (22.5)	357 (18.5)
Vocational/ Diploma	825 (32.8)	736 (33.9)	633 (32.8)
≥Bachelor's degree	999 (39.8)	946 (43.6)	940 (48.7)
Smoking			
Never smokers	1,340 (53.2)	1,138 (52.5)	1,047 (54.2)
Quit smoking	622 (24.7)	586 (27.0)	622 (32.2)
Current smokers	556 (22.1)	445 (20.5)	262 (13.6)
Alcohol			
Never drinkers	1,182 (47.0)	782 (36.1)	530 (27.5)
Quit drinking	211 (8.4)	312 (14.4)	433 (22.4)
Current drinkers	1,120 (44.6)	1,073 (49.5)	968 (50.1)
Exercise (times/week)			
None	728 (29.0)	1,055 (48.8)	622 (32.2)
1–2	670 (26.7)	351 (16.2)	272 (14.1)
≥3	1,112 (44.3)	756 (35.0)	1,035 (53.7)
NSAIDs use			
Yes	246 (9.8)	312 (14.4)	170 (8.8)
No	2,264 (90.2)	1855 (85.6)	1760 (91.2)
Height (cm)	163.6 ± 7.7	163.6 ± 7.7	163.5 ± 7.8
Weight (kg)	65.9 ± 11.5	66.2 ± 11.2	66.9 ± 11.9
Waist circumference (cm)	86.0 ± 9.9	87.1 ± 9.5	88.2 ± 10.1
Hip circumference (cm)	96.5 ± 6.6	95.7 ± 6.5	98.0 ± 6.9
BMI (kg/m^2)	24.6 ± 3.6	24.7 ± 3.6	25.0 ± 3.8
Waist to hip ratio	0.89 ± 0.07	0.91 ± 0.07	0.90 ± 0.07
Central obesity			
Yes	1,283 (51.4)	1,434 (66.4)	1,156 (60.1)
No	1,213 (48.6)	726 (33.6)	769 (39.9)
Diabetes mellitus			
Yes	194 (7.7)	254 (11.7)	305 (15.7)
No	2,329 (92.3)	1915 (88.3)	1641 (84.3)
Hypertension			
Yes	689 (27.5)	827 (38.2)	1,049 (54.4)
No	1815 (72.5)	1,338 (61.8)	881 (45.6)
Dyslipidemia			
Yes	1723 (68.5)	1640 (75.8)	1,468 (75.6)
No	791 (31.5)	525 (24.2)	473 (24.4)
Family history of DM			
Yes	929 (36.7)	853 (39.1)	780 (40.0)
No	1603 (63.3)	1,330 (60.9)	1,168 (60.0)
Total cholesterol (mg/dl)	234 ± 42	231 ± 42	218 ± 44
HDL (mg/dl)	53 ± 14	51 ± 12	58 ± 16
LDL (mg/dl)	152 ± 39	150 ± 30	145 ± 40
Triglyceride[a] (mg/dl)	126 (27, 1,362)	128 (31, 1,133)	121.5 (37, 1,280)
Creatinine (mg/dl)	1.01 ± 0.17	1.02 ± 0.19	0.98 ± 0.22
Uric acid (mg/dl)	5.6 ± 1.5	5.8 ± 1.5	6.1 ± 1.5
eGFR (mL/ min/1.73m^2)	83 ± 13	80 ± 14	80 ± 14
Periodontitis (CDC/AAP)			
Non-/ Mild periodontitis	429 (17.3)	227 (11.0)	272 (14.3)
Moderate periodontitis	1,268 (50.9)	1,092 (52.8)	1,002 (52.7)
Severe periodontitis	792 (31.8)	750 (36.2)	626 (33.0)
% sites with PPD ≥ 4 mm[a]	4.2 (0, 93.8)	4.2 (0, 88.7)	4.0 (0, 95.8)
% sites with PPD ≥ 6 mm[a]	0 (0, 63.6)	0 (0, 62.7)	0 (0, 75.0)
% sites with PPD ≥ 4 mm & CAL ≥ 5 mm[a]	0.9 (0, 91.7)	1.5 (0, 88.7)	1.2 (0, 95.8)
% proximal sites with CAL ≥ 3 mm[a]	41.7 (0, 100)	56.0 (0, 100)	55.1 (0, 100)
% proximal sites with CAL ≥ 5 mm[a]	2.7 (0, 100)	5.4 (0, 100)	4.4 (0, 100)

Note: Values are mean ± *SD* for continuous data, and frequency (%) for categorical data, except where specified.
Total numbers of subjects of each variable may be different depended on missing data.
Abbreviation: BMI, body mass index; CAL, clinical attachment level; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; FM-DM, family history of diabetes; FM-HT, family history of hypertension; HDL, high-density lipoprotein; LDL, low-density lipoprotein; N/A, not available; PPD, periodontal pocket depth.
[a]Median (range).

Missing data ranged from 0.38% to 18.30% with a median of 16.25% (Table S4). Most of the missing data were due to loss of patients in the follow-up. Twenty imputations were completely generated to fill in all missing observations. The actual and imputed data are compared in Table S5.

3.1 | Causal diagram A

Two equations, that is, mediation model (*Periodontitis* → *Diabetes*) and outcome model (*Periodontitis & Diabetes* → *CKD*), were constructed and adjusted for co-variables (Figure 1a). Univariate analysis was performed (Tables S6 and S7), which indicated that all the included factors were associated with diabetes and CKD, except for education and exercise in the CKD equation. The multivariate analysis suggested that periodontitis, diabetes, HT, income and the serum uric acid level remained associated with the CKD incidence, independently. The final model for diabetes indicated that periodontitis, age, education, obesity, HT, DLP and family history of diabetes were significant (Table 2).

These two final models were further considered simultaneously with the GSEM framework, where a 1,000-replication bootstrap was performed. The results revealed significant odds ratios (ORs) for mediated effects (*Periodontitis* → *Diabetes* → *CKD*) and direct effects (*Periodontitis* → *CKD*) of 1.007 (95% CI: 1.004, 1.013) and 1.010 (95% CI: 1.005, 1.015), respectively. The percentage mediated effect through diabetes was 42.4% (Table 3). Thus, every 1% increase in periodontitis extent increased the risk of diabetes and so increased the risk of CKD by 0.7%. In addition, it also directly increased the risk of CKD by 1.0%. If a subject had 50% of proximal sites with severe periodontitis, s/he would have a 50% higher risk of developing CKD directly from periodontitis than a subject with a normal periodontium. Moreover, this risk increased an additional 35% with the diabetes-mediated pathway.

3.2 | Causal diagram B

Periodontitis was considered as a mediator in this model (Figure 1b). The mediator model included risk factors of periodontitis, which included age, gender, income, education, marital status, exercise, smoking, alcohol, obesity, HT, DLP and diabetes. Similar to the diabetic model, all factors were found to be significantly related with periodontitis in the univariate analysis (Table S8). In the multivariate analysis, diabetes, age, gender, education, exercise and smoking remained significant (Table 4). After adjusting for co-variables, subjects with diabetes had the extent of severe periodontitis significantly higher than non-diabetes about 4.8%.

A 1,000-replication bootstrap yielded significant indirect effects (*Diabetes* → *Periodontitis* → *CKD*) and direct effects (*Diabetes* → *CKD*)

TABLE 2 Multivariate GSEM of mediation and outcome models of *Diagram A*

Factors	*b*	*SE*	*t*	*p*	95% CI LL	95% CI UL
DM Model [a]						
Periodontitis	0.011	0.002	4.81	<0.001	0.006	0.015
Age (year)	0.050	0.008	6.13	<0.001	0.034	0.066
Education						
≤High school	0.550	0.150	3.66	<0.001	0.255	0.844
Vocation/Diploma	0.494	0.135	3.66	<0.001	0.230	0.759
Obesity	1.076	0.124	8.69	<0.001	0.833	1.319
Family history of DM	0.938	0.116	8.09	<0.001	0.711	1.165
HT	0.833	0.106	7.82	<0.001	0.624	1.042
DLP	0.595	0.128	4.64	<0.001	0.343	0.846
CKD Model [b]						
Periodontitis	0.010	0.003	3.90	<0.001	0.005	0.015
DM	0.689	0.155	4.44	<0.001	0.385	0.994
Income (Baht/month)						
<20,000	0.278	0.175	1.59	0.112	−0.065	0.622
20,000–49,999	−0.476	0.155	−3.08	0.002	−0.780	−0.172
HT	0.748	0.141	5.31	<0.001	0.472	1.024
Uric acid (mg/dl)	0.467	0.044	10.51	<0.001	0.380	0.554

Abbreviation: b, coefficient; CI, confidence interval; CKD, chronic kidney disease; DLP dyslipidemia; DM, diabetes mellitus; HT, hypertension; *p*, *p*-value; SE, standard error; *t*, *t* test.
[a]DM model was adjusted for age, gender, income, education, marital status, exercise, smoking, alcohol, obesity, HT, DLP family history of DM and periodontitis.
[b]CKD model was adjusted for income, education, marital status, exercise, smoking, alcohol, obesity, NSAIDs use, HT, DLP, uric acid level, DM and periodontitis.

TABLE 3 Direct and indirect effects

Effects	*b*	*SE*	95% CI[a]	
			LL	UL
Diagram A				
Indirect (Periodontitis → Diabetes → CKD)	0.007	0.002	0.004	0.013
ORs of indirect effect	1.007 (1.004, 1.013)			
Per cent of indirect effect	42.4 (25.4, 65.4)			
Direct (Periodontitis → CKD)	0.010	0.003	0.005	0.015
ORs of direct effect	1.010 (1.005, 1.015)			
Per cent of direct effect	57.6 (34.6, 74.6)			
Diagram B				
Indirect (Diabetes → Periodontitis → CKD)	0.048	0.018	0.021	0.096
ORs of indirect effect	1.049 (1.022, 1.100)			
Per cent of indirect effect	6.5 (2.5, 13.8)			
Direct (Diabetes → CKD)	0.689	0.154	0.366	0.982
ORs of direct effect	1.993 (1.441, 2.671)			
Per cent of direct effect	93.5 (86.2, 97.5)			

Abbreviation: b, coefficient; CI, confidence interval; CKD, chronic kidney disease; SE, standard error.
[a]Bias-corrected bootstrapped

with an OR of 1.049 (95% CI: 1.022, 1.100) and 1.993 (95% CI: 1.441, 2.671), respectively. The total ORs of having CKD in subjects with diabetes was 2.091 (95% CI: 1.519, 2.828) compared to those without diabetes. Within these ORs, the percentage of the diabetes effect contributed through periodontitis as a mediator was 6.5% (Table 3).

3.3 | Sensitivity analysis

Sensitivity analysis was performed by considering various approaches for defining periodontitis, including the number of sites with CAL ≥5 mm, percentage of sites with PPD ≥4 mm & CAL ≥5 mm, and modified cumulative probing depth (i.e., the whole-mouth sum of pathologically increased probing depth >3 mm) (Dietrich, Jimenez, Krall Kaye, Vokonas, & Garcia, 2008). All definitions revealed the significant direct and mediation effects of periodontitis for the *diagram A* (*Periodontitis → Diabetes → CKD*), except using the modified cumulative probing depth where the effect of periodontitis on CKD was mediated through diabetes pathway only (Table S9). For *diagram B* (*Diabetes → Periodontitis → CKD*), the mediated effect of periodontitis was presented only for the percentage of proximal sites with CAL ≥5 mm, and percentage of sites with PPD ≥4 mm & CAL ≥5 mm (Table S10).

4 | DISCUSSION

The causal relationships between periodontitis, diabetes and CKD were constructed using mediation analysis, and indicated that periodontitis and diabetes were independent risk factors for the incidence of CKD. Both had a significant direct effect and indirect (mediation) effect, through each other. *Diagram A* suggested that for each increase of 1% in proximal sites with severe periodontitis, the OR of developing CKD were directly increased by 1.010, and indirectly increased through diabetes by 1.007. *Diagram B* suggested that diabetes increased the OR of CKD occurrence twofold, with 6.5% mediated via periodontitis.

Similar to previous cross-sectional (Fisher et al., 2008; Han et al., 2015; Ioannidou & Swede, 2011; Kshirsagar et al., 2005; Ricardo et al., 2015; Yoshihara et al., 2016) and cohort studies (Chang et al., 2017; Chen et al., 2015; Grubbs et al., 2015, 2016; Iwasaki et al., 2012; Shultis et al., 2007), our results showed a causative association between periodontitis and kidney function. Among previous cohort studies, half of them focused on the decline of kidney function, which both CKD and non-CKD cases were included (Chang et al., 2017; Chen et al., 2015; Iwasaki et al., 2012). Another three cohort studies focused on new cases of altered kidney function (Grubbs et al., 2015, 2016; Shultis et al., 2007) and revealed an independent effect of periodontitis. Shultis et al. (2007) showed that the incidences of macroalbuminuria were 2.0-, 2.1- and 2.6-fold higher in individuals with moderate or severe periodontitis or those who were edentulous, respectively. However, periodontal status was defined from the numbers of remaining teeth and radiography without clinical periodontal parameters. Likewise, the Osteoporotic Fractures in Men (MrOS) cohorts (Grubbs et al., 2016) applied the half-mouth protocol for periodontal examination,

TABLE 4 Multivariate GSEM of mediation and outcome models of *Diagram B*

Factors	*b*	*SE*	*t*	*p*	95% LCI	95% UCI
Periodontitis model[a]						
DM	4.801	1.131	4.24	<0.001	2.584	7.018
Age	0.636	0.044	14.51	<0.001	0.550	0.722
Gender: male	4.204	0.637	6.59	<0.001	2.954	5.453
Education						
≤High school	9.911	0.878	11.29	<0.001	8.190	11.632
Vocation/ Diploma	4.961	0.671	7.39	<0.001	3.645	6.277
Exercise (times/week)						
1–2	−1.607	0.616	−2.61	0.009	−2.814	−0.400
≥3	−1.273	0.576	−2.21	0.027	−2.403	−0.143
Smoking						
Quit smoking	3.748	0.764	4.90	<0.001	2.250	5.246
Current smokers	14.063	1.058	13.29	<0.001	11.988	16.137
CKD Model[b]						
Periodontitis	0.010	0.003	3.90	<0.001	0.005	0.015
DM	0.689	0.155	4.44	<0.001	0.385	0.994
Income (Baht/month)						
<20,000	0.278	0.175	1.59	0.112	−0.065	0.622
20,000–49,999	−0.476	0.155	−3.08	0.002	−0.780	−0.172
HT	0.748	0.141	5.31	<0.001	0.472	1.024
Uric acid (mg/dl)	0.467	0.044	10.51	<0.001	0.380	0.554

Abbreviation: b, coefficient; CI, confidence interval; CKD, chronic kidney disease; DLP, dyslipidemia; DM, diabetes; HT, hypertension; *p*, *p*-value; SE, standard error; *t*, *t* test.
[a]Periodontitis model was adjusted for age, gender, income, education, marital status, exercise, smoking, alcohol, obesity, HT, DLP and DM.
[b]CKD model was adjusted for income, education, marital status, exercise, smoking, alcohol, obesity, NSAIDs use, HT, DLP, uric acid level, DM and periodontitis.

which might have underestimated the prevalence of periodontitis (Eke, Thornton-Evans, Wei, Borgnakke, & Dye, 2010).

Conventional and mediation analyses are different in the way dealing with the third variable. For instance, in *diagram A*, the previous cohort studies considered diabetes as a confounder, meanwhile, our mediation analysis considered diabetes as an intermediate variable (mediator) between periodontitis and CKD. The later approach has an advantage in refining and understanding a possible pathway, since the mediation analysis can determine the process of how one variable effects the outcome (Wu & Zumbo, 2007). The previous mediation analysis (Fisher, Taylor, West, & McCarthy, 2011) found significant direct and indirect effects of periodontitis on CKD through diabetes duration and HT. However, this study outlined the pathways from the cross-sectional data, which CKD may not potentially true consequences of diabetes and periodontitis.

Various case definitions of periodontitis have been proposed for periodontal research (Beltran-Aguilar, Eke, Thornton-Evans, & Petersen, 2012), which largely impact on periodontitis prevalence, extent and its effects on systemic health (Costa et al., 2009; Ioannidou, Shaqman, Burleson, & Dongari-Bagtzoglou, 2010). Due to the lack of a universally accepted definition, the CDC in partnership with AAP proposed the standard case definitions (Page & Eke, 2007) for principally surveillance to determine the total prevalence of periodontitis. Some previous studies have applied these definitions assessing the relationship between periodontitis and kidney function (Grubbs et al., 2015, 2016; Ioannidou & Swede, 2011). However, we postulated that the plausible link between periodontitis and systemic disease was the cumulative periodontal inflammation. Using the CDC-AAP definition, the amount of inflammation in some cases seemed to be discrepancies within the same category. For example, subjects who had only two teeth with severe periodontitis would be grouped the same as subjects who had a whole-mouth of teeth with severe form. According to our sensitivity analysis, results from various periodontitis definitions were quite consistent, particularly in *diagram A*, which showed the direct and/or indirect effects of periodontitis on CKD incidence. However, using CDC/AAP definition did not show any significant effect on both diabetes and CKD (data not shown). It might imply to the limitation of discrimination from the standard definitions when applied to periodontal medicine. Therefore, in this study, we reflected the cumulative periodontal inflammation with the percentage

of proximal sites with severe periodontitis (CAL ≥5 mm). Only proximal sites were considered to rule out the attachment loss due to non-inflammation causes, such as traumatic brushing. Moreover, to capture periodontitis as the continuous data could be useful to identify the dose-dependency of periodontitis effect on incident CKD.

Systemic chronic inflammation from periodontitis is a risk factor not only for diabetes and CKD, but also for cardiovascular disease and all-cause mortality. Almost 50% of adults had periodontitis, with 10% having severe periodontitis (Eke et al., 2015). Large populations were at risk to develop the subsequent burdens from periodontitis. Prevention and treatment of periodontitis are effective and inexpensive modalities, however, awareness among patients and healthcare providers was low (Luo & Wu, 2017). Motivation of personal oral health care, routine dental check-up and professional cleaning are efficient in controlling the oral inflammation and minimizing the spreading of systemic inflammation from periodontitis.

Besides the advantages of mediation analysis, our study has other strengths. The causal relationship was obviously confirmed with the non-CKD cohort design. In addition, the MICE was used to dealing with missing data and attrition. Compared with the complete case analysis (actual data without imputation), the results from MICE were quite approximate in terms of significant factors and their coefficients, but MICE decreased the standard errors, and so increased the precision of the results. Finally, the periodontal status was examined with the gold standard protocol, full-mouth examination with six sites per tooth to minimize the misclassification of periodontitis.

However, there are limitations to this study. The presence of CKD was classified based on eGFR without any information on micro- or macro-proteinuria. Moreover, proteinuria by itself was also a worsening factor for kidney function, hence the CKD incidence and effect size of other co-variables might be biased. Second, the cohort had only three surveys with 5-year interval. With the large gaps between surveys, uncertainty of outcome and variables among visits were present. Thus, the time-varying co-variables analysis was used to compensate for this bias. Third, our studied population might not represent the general population, since EGAT employees represented older Thai adults with a higher education and income than average. Finally, other relevant diseases or behaviours might be the candidates of other mediators or moderators. Future investigation will be required to clarify.

In conclusion, periodontitis and diabetes had the significant direct and indirect effects via each other on increasing CKD incidence. Oral and systemic morbidities from periodontitis should be emphasized among nephrologists, general practitioners and patients. Its treatment and prevention should also be publicly promoted.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest in this study.

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