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Original Article

Correlation of plasma osteopontin and osteocalcin with lower renal function in dental patients with carotid artery calcification and tooth loss

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ABSTRACT

Objectives: To investigate plasma osteopontin (OPN) and osteocalcin (OCN) levels in dental patients with carotid artery calcification (CAC) and determine the correlations between these proteins and renal function and tooth loss.
Methods: The health parameters and number of teeth of 99 participants were recorded. Panoramic radiographs were taken for CAC evaluation, and OPN and OCN levels were measured.
Results: None of the participants had overt kidney disease, and 14 (14.14%) had CAC. The age, sex, and health profiles of patients with CAC were not different from those without CAC. The OPN and OCN levels in participants with CAC were higher than in those without ($p = 0.026$ and $p = 0.025$, respectively). The OPN levels were correlated with the estimated glomerular filtration rate (eGFR) ($p = 0.021$) and tooth loss ($p = 0.027$). The OCN levels were correlated with the eGFR ($p = 0.002$), tooth loss ($p = 0.023$), blood urea nitrogen ($p = 0.040$), and creatinine levels ($p = 0.031$). The median tooth loss in individuals with an eGFR <60 mL/min/1.73 m^2 was higher than that of individuals with an eGFR ≥60 mL/min/1.73 m^2 ($p = 0.033$). In individuals with CAC, tooth loss correlated more strongly with the eGFR, and the correlation between OPN and OCN levels was more apparent.
Conclusion: Dental patients with CAC and increased tooth loss have a greater tendency for decreased renal function, which may be associated with OPN and OCN; thus, these patients should be referred for investigation.

1. Introduction

Vascular calcification (VC) appears with increased prevalence in atherosclerosis, cardiovascular disease (CVD), and chronic kidney disease (CKD). Recent investigations have shown that VC is a complicated pathological process in which one of the main factors is low-grade inflammation, which contributes to endothelial dysfunction [1]. Several proteins and factors are associated with VC. Osteopontin (OPN) and osteocalcin (OCN), both bone-related proteins, are involved in vascular remodeling and calcification at ectopic sites and demonstrate a close interaction with the glomerular filtration rate. An overexpression of OPN and OCN in CKD patients has been reported, and these proteins are possible predictive biomarkers in VC, atherosclerosis, CKD, and CVD [2,3].

Osteopontin regulates numerous biological activities, including bone matrix remodeling, tissue calcification, production of various pro–inflammatory cytokines, and it can also promote macrophage adhesion, migration, and vascular smooth muscle cell proliferation [4]. Osteopontin is present in the extracellular matrix of

Abbreviations: BUN, Blood urea nitrogen; CAC, Carotid artery calcification; eGFR, Estimated glomerular filtration rate; OCN, Osteocalcin; OPN, Osteopontin.

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mineralized tissues and in extracellular fluids at the site of inflammation. It is secreted by many different cell types, including macrophages, lymphocytes, epithelial cells, adipocytes, osteoblasts, osteoclasts, chondrocytes, and vascular smooth muscle cells [4]. Therefore, OPN plays many important roles in calcification, inflammation, and the immune response [4,5]. The combination of elevated OPN levels and high C-reactive protein levels is significantly associated with an increased risk of CVD mortality [6]. However, the possibility that OPN could be used as a VC serum marker is still under investigation [1].

Osteocalcin, expressed by osteoblasts, is mostly found in the bone matrix; OCN plays an essential role in energy metabolism and supports β-cell proliferation, insulin secretion, and insulin sensitivity. The main functional role of OCN is its pro-osteoblastic effect or bone-building function [3]. Although it is known to promote the differentiation and mineralization of vascular smooth muscle cells, it remains unclear how OCN regulates extra bone mineralization and vascular function. Few studies have assessed the association of VC with OCN, and the results are controversial [7–10].

The findings that OPN and OCN are involved in inflammation and mineralization have generated interest in the roles of OPN and OCN in carotid artery calcification (CAC). Our previous study revealed the association between CAC and tooth loss in dental patients [11]. Investigating the relationship among OPN, OCN, CAC, tooth loss, and renal function in dental patients is another important area for research. A close relationship between circulating OPN and OCN levels and the presence of CAC or tooth loss may allow early identification of asymptomatic CKD or CVD subjects. We found no publications that examined the relevance of OPN and OCN in dental patients with CAC but without CVD. Therefore, the objective of the present study was to compare plasma OPN and OCN levels in dental patients with and without CAC to determine whether OPN and OCN are associated with renal function. Another objective was to determine whether tooth loss and CAC detected on dental radiographs were correlated with altered renal function. Such insights could lead dentists to screen and prevent the development of CKD and CVD in the future.

2. Material and methods

2.1. Study design

This study was carried out at the Golden Jubilee Medical Center, Mahidol University. The study was approved by the Ethics Committee of Mahidol University and Tokyo Medical and Dental University, and it also conformed with the Declaration of Helsinki (reference number: MU–IRB 2011/134.3006, TMDU–IRB 2012/860). Subjects fulfilling the required inclusion criteria were enrolled after providing written informed consent.

2.2. Study population

This study investigated the same group of Thai patients who had their annual health check-up as in our previously published study [11]. There were 99 participants, with an age range of 35–76 years, who fulfilled the following inclusion criteria: No systemic diseases; not receiving medications including antithrombogenic agents, lipid-lowering drugs, and anti-osteoporosis drugs; and/or without a history of the presence of other infections. Height and body weight were measured to calculate participants' body mass index (BMI). Triglyceride (TG), high-density lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C), total cholesterol, fasting plasma glucose (FPG) levels, and blood pressure (BP) were evaluated from medical chart records. Data on serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), blood urea nitrogen (BUN), creatinine, and uric acid were also collected. Routine procedures for biochemical tests for TG, HDL-C, LDL-C, total cholesterol, FPG, SGOT, SGPT, BUN, creatinine and uric acid were used, and a standard mercury sphygmomanometer (Matsuyoshi, Tokyo, Japan) was used for BP measurements. Patients with systemic diseases, those who had received medications or had a history and/or the presence of other infections and systemic antibiotics, immunosuppressive drugs, or periodontal treatment in the 6 months prior to the recording were excluded. Demographic data were also collected.

2.3. Assessment of carotid artery calcification (CAC)

Members of the CAC group had been diagnosed from panoramic extra-oral radiographs (PAN) in the previously published study [11]. Carotid artery calcification was defined as a vertico-linear, nodular (round, oval), or heterogeneous radiopaque mass located 1.5–2.5 cm infero-posteriorly to the mandibular angle, and adjacent to the cervical vertebrae at the level of the lower margin of the third, and including the whole fourth, cervical vertebrae [12] (Fig. 1).

2.4. Assessment of renal function

Renal function was evaluated from medical chart records and was determined by the estimated glomerular filtration rate (eGFR). In order to initially evaluate abnormal renal function, participants were divided into two groups according to whether their eGFR levels were higher or lower than 60 mL/min/1.73 m^2 [13].

2.5. Assessment of tooth loss

The number of teeth lost was recorded by one examiner (ST).

2.6. Assessment of osteopontin (OPN) and osteocalcin (OCN)

Blood was collected and centrifuged to obtain plasma on the same date that venipuncture was performed for blood chemistry, and a 1 mL aliquot was frozen and stored at −80 °C for the measurement of bone-related protein levels.

Plasma was thawed at room temperature for protein measurement by enzyme-linked immunosorbent assay (ELISA). The human OPN and OCN commercial ELISA kits (ab100618 and ab195214; Abcam®, Chuo-ku, Tokyo, Japan) were used to measure OPN and OCN, respectively. Each plasma sample was analyzed in duplicate, and all assays were conducted according to the manufacturer's instructions.

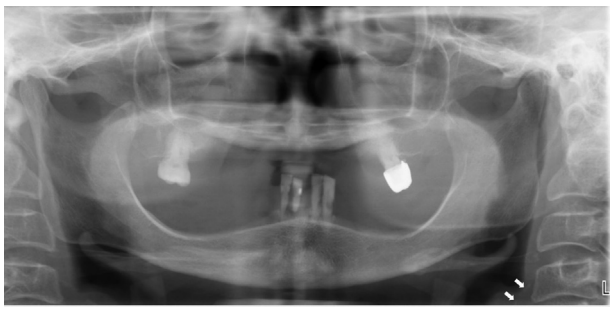

Fig. 1. Presentation of carotid artery calcification. Radiopaque mass as a vertico-linear and oval nodule located infero-posteriorly to the mandibular angle and adjacent to the cervical vertebrae.

The plasma samples were diluted 5–10 fold with the assay diluent provided in the kit to obtain an absorbance value within the range of the standards used. Recombinant human OPN samples ranging from 0 to 18 ng/mL were used as standards. Exactly 100 μL of diluted plasma sample or standard was added to a microplate pre-coated with anti-human OPN antibody. The microplate was sealed with the cover provided in the kit and incubated for 2.5 h at room temperature on a plate shaker set to 400 rpm. The solution was discarded, and the wells were washed four times with the wash buffer, inverted, and blotted off the plate against clean paper towels. Then, 100 μL of biotinylated OPN detection antibody was added, and the microplate was incubated for 1 h at room temperature on a plate shaker set to 400 rpm. The solution was discarded, and the microplate was washed four times with wash buffer and blotted off the plate using paper towels. After addition of 100 μL of diluted HRP-conjugated streptavidin, the microplate was incubated for 45 min at room temperature with gentle shaking. The microplate was then washed four times, and 100 μL of tetramethylbenzidine (TMB) substrate was added followed by incubation for exactly 30 min at room temperature in the dark on a plate shaker set to 400 rpm. Finally, 50 μL of stop solution (2 M sulfuric acid) was added, and the intensity of the color was measured immediately. The absorbance of each well was read using a microplate reader (SOFTMax™ Molecular Devices Corp., CA, USA) at a wavelength of 450 nm. A standard curve was prepared with software in log–log mode, and a linear graph was plotted. The amount of OPN present in the plasma samples was calculated from the regression equation obtained from the curve.

Similarly, plasma OCN levels were determined with Simple-Step ELISA® (Abcam®, Cambridge, UK), which employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via the immunoaffinity of an anti-tag antibody coating the well. Samples of 50 μL, at 100-fold dilution, or 0–10 ng/mL standards were added to the wells, followed by 50 μL of the antibody cocktail, and were incubated for 1 h at room temperature on a plate shaker set to 400 rpm. After three washes, 100 μL of TMB substrate was added to each well and incubated for 10 min in the dark on a plate shaker set to 400 rpm. Finally, 100 μL of stop solution was added to each well, the microplate was placed on a plate shaker for 1 min to mix, and the optical density was recorded at 450 nm. A four-parameter logistic provided the curve fit, and the amount of OCN present in the plasma samples was calculated.

2.7. Data analyses

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp, Armonk, NY, USA). Descriptive statistics were used to present information on all variables. Continuous data are expressed as median and interquartile range, and categorical data are expressed as frequency and percentage. The results are reported according to the presence or absence of CAC, and comparisons of categorical variables between the groups were performed using the Fisher's exact test. All continuous variables were assessed for normality using the Kolmogorov–Smirnov test. Serum OPN and OCN levels were compared between study groups using Mann-Whitney U test. Spearman's correlation coefficients were used to determine the correlation between OCN and OPN levels and clinical and biochemical parameters. Two-sided significance and a value of $p < 0.05$ were used taken to represent a statistically significant difference for all analyses.

3. Results

Age, sex, and other health profiles of patients with CAC were not significantly different from those without CAC. The participants with CAC had higher plasma OPN and OCN levels than those without CAC ($p = 0.026$ and $p = 0.025$, respectively) (Table 1). In all subjects, the plasma levels of OPN were positively correlated with the plasma levels of OCN ($r = 0.248$, $p = 0.009$) (Fig. 2). Moreover, the levels of OPN were negatively correlated with the eGFR ($r = -0.207$, $p = 0.021$) and the number of teeth lost ($r = 0.199$, $p = 0.027$) (Fig. 3a and b). The OCN levels were also adversely correlated with the eGFR ($r = -0.301$, $p = 0.002$) and the number of teeth lost ($r = 0.217$, $p = 0.023$) (Fig. 3c and d). In addition, BUN ($r = 0.234$, $p = 0.040$) and creatinine levels ($r = 0.206$, $p = 0.031$) were correlated with the OCN levels.

The number of teeth lost was negatively correlated with the eGFR ($r = -0.194$, $p = 0.030$); the median number of teeth lost in individuals with an eGFR ≥60 mL/min/1.73 m^2 was 3 (1, 6), while that of individuals with an eGFR < 60 mL/min/1.73 m^2 was 9 (3, 18) ($p = 0.033$) (Fig. 4). When the 14 individuals with CAC were analyzed separately, the number of teeth lost was more strongly correlated with the eGFR ($r = -0.640$, $p = 0.014$), and the positive correlation between the OPN and OCN levels was more prominent ($r = 0.709$, $p = 0.007$).

4. Discussion

Our study revealed a plausible role of OPN and OCN in the pathogenesis of VC, in that higher levels of OPN and OCN were found in participants with CAC than in those without CAC. We also demonstrated a significant correlation between plasma OPN and OCN and lower renal function, as well as tooth loss, in participants without incident CVD. Additionally, increased tooth loss was observed in individuals with lower renal function.

Osteopontin is implicated in both the inflammation and mineralization processes that lead to VC. It is also associated with vascular function and has been reported as a surrogate marker of atherosclerotic lesions, especially in calcified plaques, and contributes to the pathogenesis of asymptomatic atherosclerosis and coronary artery disease [3]. Our findings suggest that OPN might play a role in the pathogenesis of CAC; this is consistent with previous reports that have shown that high plasma levels of OPN were independently associated with the extent and severity of arterial stiffness, coronary atherosclerosis, and VC in patients with asymptomatic coronary artery disease [6,14,15]. Chen et al. found that the OPN levels of patients with coronary heart disease were significantly higher than those of a healthy control group [16]. Furthermore, a study by Abdalrhim et al. revealed that OPN levels were significantly associated with CVD outcomes in patients with stable coronary artery disease after a median follow-up of 4.8 years (hazard ratio [HR] = 1.24); they also reported that higher OPN levels were associated with a higher incidence of heart failure hospitalizations (HR = 2.04) [17]. In their study, the median OPN level at baseline was higher than that of our study; this may be due to variation in the stage of CVD at which patients without CVD were examined in the current study. However, the results of the current study were comparable to previous reports which found elevated OPN levels in patients with established VC, although the effects of elevated OPN in VC still require further investigation.

No clear association was established between plasma OCN and VC or atherosclerosis, although the presence of OCN-positive cells and histological staining had a consistent positive correlation with calcification or atherosclerosis [18]. Higher concentrations of OCN are associated with carotid intima-media thickness or known CVD risk factors in children from families with metabolic syndrome [7].

Table 1
Characteristics of participants according to carotid artery calcification.

Variables	Carotid artery calcification		p^*
	Absence (n = 85)	Presence (n = 14)	
Age (years)	47 (42, 55)	54 (45, 66)	0.052
Sex			0.338
Male	26 (26.3)	2 (2.0)	
Female	59 (59.6)	12 (12.1)	
Body mass index (kg/m^2)	23.73 (22.06, 26.08)	23.86 (21.83, 27.17)	0.896
Systolic blood pressure (mmHg)	124 (113, 135)	129 (126, 139)	0.078
Diastolic blood pressure (mmHg)	78 (72, 86)	78 (72, 81)	0.418
Fasting plasma glucose (mg/dL)	94 (90, 101)	95 (87, 97)	0.536
Total cholesterol (mg/dL)	198 (183, 234)	204 (177, 244)	0.829
High-density lipoprotein cholesterol (mg/dL)			
Male	52 (42, 61)	47 (36, 58)	0.529
Female	55 (43, 65)	64 (42, 73)	0.256
Low-density lipoprotein cholesterol (mg/dL)	133 (111, 160)	130 (94, 178)	0.841
Triglyceride (mg/dL)	103 (73, 177)	137 (70, 248)	0.782
Serum glutamic oxaloacetic transaminase (SGOT) (unit/L)	19 (16,23)	21 (16, 31)	0.611
Serum glutamic pyruvic transaminase (SGPT) (unit/L)	19 (13, 29)	20 (14, 30)	0.662
BUN (mg/dL)	11 (9, 14)	11 (9, 13)	0.805
Creatinine (mg/dL)	0.7 (0.6, 0.9)	0.7 (0.6, 0.8)	0.516
Uric acid (mg/dL)	5.0 (4.2, 6.4)	5.6 (4.5, 6.4)	0.592
Osteopontin levels (ng/mL)	**1.76 (0.92, 4.98)**	**4.71 (1.79, 10.48)**	**0.026**
Osteocalcin levels (ng/mL)	**303.36 (205.57, 374.26)**	**410.76 (281.80, 546.21)**	**0.025**
Tooth loss (n)	**3 (1, 5)**	**12 (3, 15)**	**0.003**

All data are presented as the median (25th, 75th percentile) or frequency (%).
Bold denotes statistical significance.
*Mann-Whitney U test for quantitative data or chi-squared test for qualitative data.

Similar to the present study, Okura et al. reported that higher concentrations of OCN were related to CAC in patients with hypertension [8]. Their study, which investigated OCN to determine whether there was an association with calcification in the carotid artery, is the only other study to research this area apart from the current study. Choi et al. studied 162 Korean men and women with asymptomatic CVD and found that higher OCN concentrations were associated with CAC independent of conventional cardiovascular risk factors and bone mass density in men, although this association was not found in women [10]. In contrast, Zhang et al. reported conflicting results by showing that OCN levels were significantly negatively associated with intima-media thickness [9]. A study by Kanazawa et al. also showed opposing results between the baseline and final measurements. Although they demonstrated that initially, total OCN was significantly and positively correlated with carotid plaque score, at the end of the study OCN was negatively correlated with changes in plaque score [19]. They hypothesized that while atherosclerotic plaques may initially promote OCN secretion, eventually the increased level of OCN may suppress the progression of atherosclerosis or calcification. These conflicting results were reviewed by Millar et al. who suggested that they could be explained by the subject characteristics, type of OCN measured, type of calcification, and the involvement of systemic diseases [18]. However, their results indicate that OCN is closely associated with VC, although the exact mechanism of association has to be elucidated.

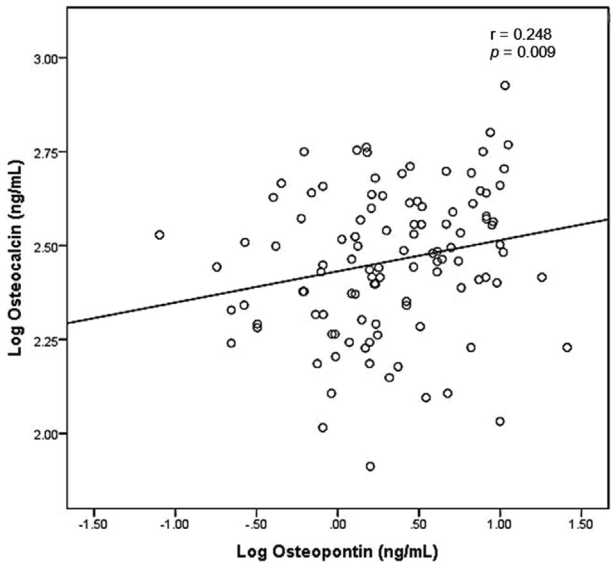

Fig. 2. Positive correlation between plasma osteopontin and plasma osteocalcin.
Plasma osteopontin and osteocalcin levels (ng/mL) were log transformed ($p = 0.009$*).
* Spearman's rank correlation.

Vascular calcification, a common finding in CVD, is often detected in patients with CKD. Various studies have identified circulating proteins that may be responsible for extraskeletal calcification and dysfunction in mineral metabolism, both of which are features of CKD-mineral bone disorder [20]. Osteopontin is produced by a variety of tissues, including the kidney, where it has a role in renal clearance and demonstrates a close interaction with the glomerular filtration rate. Lorenzen et al. reported that in patients with CKD, OPN levels increase with a progressive decline in the eGFR, so that the increase in OPN reflects the severity of renal impairment [21]. Furthermore, in a study conducted by Rossi et al., it was shown that arterial stiffness is greater in individuals with impaired renal function compared to those with an eGFR $\geq$60 mL/min. Patients with coronary artery disease and impaired renal function had higher levels of OPN compared to those with coronary artery disease alone. Vascular stiffness is associated with cardiovascular mortality across all stages of CKD, and Gluba et al. reported that OPN is a powerful biomarker of VC in patients with CKD [22,23]. Chen et al. also revealed that OPN levels were proportional to renal function in patients with coronary heart disease [16]. Renal impairment may aggravate vascular disease through its effects on inflammation [22]. Indeed, our study also suggests that OPN levels

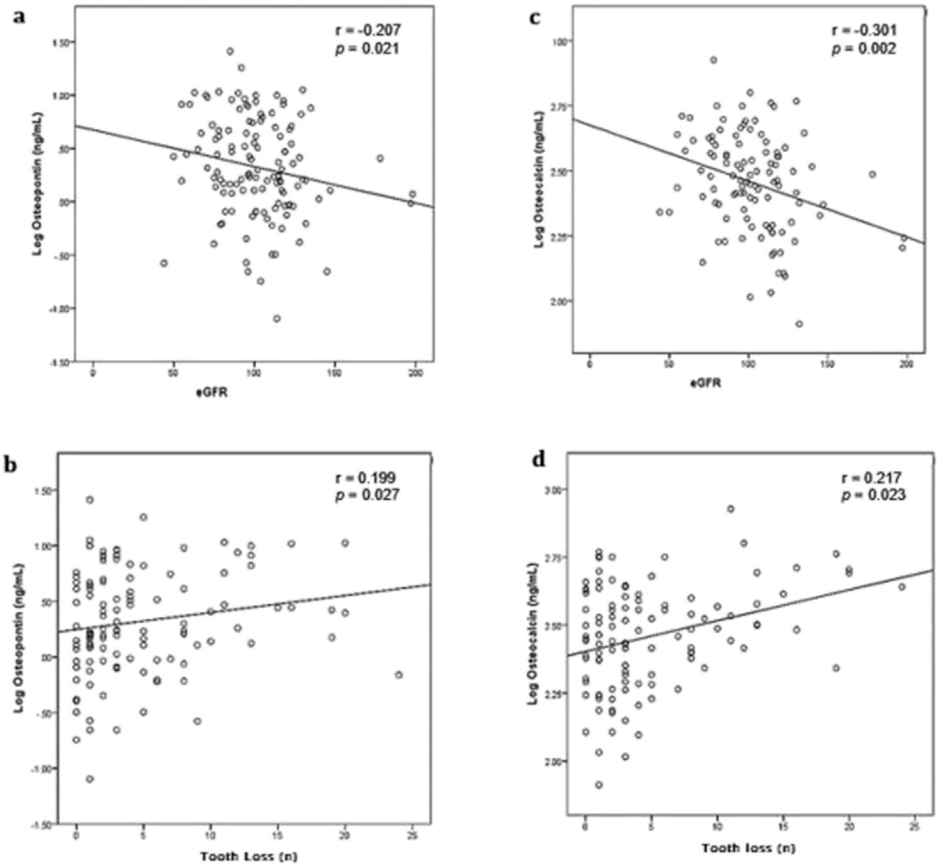

Fig. 3. Correlation of plasma osteopontin and osteocalcin with the estimated glomerular filtration rate (eGFR) and the number of teeth lost. Plasma levels of osteopontin and osteocalcin decreased, while the eGFR (mL/min/1.73 m^2) increased **(a and c)**. Plasma levels of osteopontin and osteocalcin increased with the number of teeth lost **(b and d).*** Spearman's rank correlation.

are further increased in patients with decreased renal function, and thus may contribute to the development of vascular disease in this group.

Our results regarding plasma OCN are consistent with the study of Mihai et al. which revealed an overexpression of OCN and OPN in CKD patients versus the control group. Circulating OCN levels were increased 4.6-fold in stage 4 CKD, and 2-fold and 1.3-fold in stage 3 and 2 CKD, respectively [2]. OPN levels were also increased more than 2-fold in stage 2 CKD, rising to 4-fold in stage 3 CKD, and 7-fold in stage 4 CKD [2]. In another study, OCN levels were highest in dialysis patients, and gradually decreased to the lowest level in patients with mild kidney disease [23]. As in the current study, Mihai et al. found strong correlations between the eGFR and the following: OPN, eGFR and OCN, and OPN and OCN [2]. Moreover, serum OCN did not correlate with BMI, BP, HBA1c, or cholesterol, indicating that OCN levels are associated with renal function; these findings were also consistent with our study [8]. The association between OPN and OCN might reflect the severity of the vascular changes in CKD and predict the progression of the disease, thus encouraging further investigation in this area.

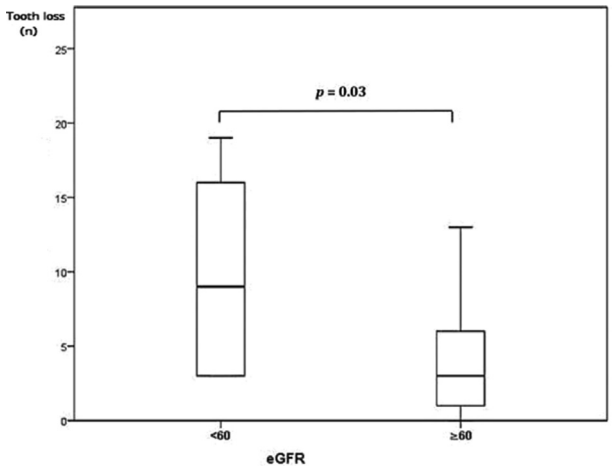

Fig. 4. Median number of teeth lost between participants with and without abnormal renal function. The median number of teeth lost in participants with an eGFR <60 mL/min/1.73 m^2 was significantly higher than in participants with an eGFR ≥60 mL/min/1.73 m^2 ($p = 0.033$*). * Mann–Whitney U test.

Our study disclosed a positive correlation between tooth loss and plasma OPN and OCN levels, in that the greater the number of teeth lost, the higher the levels of these proteins. To the best of our knowledge, no previous study has reported an association between plasma OPN and OCN levels and tooth loss. Some studies have investigated plasma OPN levels in subjects with chronic periodontitis, a chronic bacterial infection that results in the destruction of alveolar bone and subsequent tooth loss [24,25]. Comparable results have been reported showing that OPN levels, both in gingival crevicular fluid and proportionally in plasma, increased progressively from health to periodontitis [24,25]. With extensive periodontal destruction, there comes a substantial increase in OPN concentration in the gingival crevicular fluid, which is associated with an increase in plasma OPN concentration. Moreover, serum OCN levels were also positively associated with

the severity of periodontal disease, as determined by a clinical attachment level ≥6 mm [26].

Potentially significant associations between periodontitis and renal function have been reported [13,27,28]. For example, the severity of tooth loss was considered an independent risk indicator for CKD, and Choi et al. showed that tooth loss was inversely associated with the presence of CKD, as defined by an eGFR <60 mL/min/1.73 m^2; this finding is comparable with that of the current study [13]. Similarly, a significant negative relationship between patients with an eGFR <30 mL/min/1.73 m^2 and the mean tooth loss has been demonstrated [27]. Fisher et al. also found that adults who had periodontal disease and were edentulous were twice as likely to have CKD as adults without periodontal disease [28]. In contrast, a recently published systematic review reported that one in every five patients with end-stage renal disease on dialysis was fully edentulous [29]. Moreover, the study by Yoshihara et al. indicates that CKD and periodontal disease can have reciprocal effects, and that a bidirectional relationship exists between CKD and periodontal disease [26]. Periodontal disease can contribute to the overall systemic inflammatory burden reflected by elevated CRP levels, and thus has the potential to affect renal function. In contrast, CKD can greatly affect bone metabolism and renal function and increase OCN levels; therefore, patients with CKD have a higher probability of periodontal disease [26]. According to a recent study, the 10-year all-cause mortality rate for individuals with CKD increased from 32% to 41% with the addition of periodontitis; this study showed a strong association between periodontitis and increased mortality in individuals with CKD. Moreover, from previous prospective evaluation, individuals with CKD and periodontal disease had a 35% increased risk of CVD mortality when compared to those without [30]. Therefore, periodontitis, as a source of chronic systemic inflammation, may be an important contributor beyond the traditional risk factors for CVD mortality in patients with CKD [31]. Further longitudinal studies should be conducted to validate OPN and OCN as markers of periodontal destruction and as risk factors for CKD and CVD.

A high prevalence of VC and a high incidence of cardiovascular events are two key complications of CKD [32]. An eGFR <60 mL/min/1.73 m^2 was significantly associated with an increased risk of cardiovascular mortality independent of traditional risk factors [33]. The pattern of the relationship between the eGFR and cardiovascular mortality is largely consistent across age categories. Furthermore, the risk gradient for CVD was similar among age, sex, and racial subgroups, and a lower eGFR was associated with an increased risk of CVD [34]. Chronic kidney disease markedly accelerates atherosclerosis development, and has been demonstrated to exacerbate VC. Therefore, screening for VC is suggested, as it is considered to be a cardiovascular risk marker associated with a several-fold increase in morbidity and mortality risk, both in the general population and in CKD patients, increasing gradually through the stages of CKD, and peaking in stage 5 CKD patients [2]. Additionally, recent studies have shown that CAC appears to improve cardiovascular risk assessment in patients with CKD [20]. Hu et al. also demonstrated an association between tooth loss and an increased risk of all-cause mortality (CVD, stroke, and dementia) [35]. Our recent study concurred that dental patients with CAC were at intermediate risk of CVD, independent of health status [11]. When all aspects are considered, the current study found increased levels of OPN and OCN, lower renal function, and increased tooth loss in patients with CAC compared to those without. Therefore, tooth loss and CAC, as determined from a panoramic radiograph, offer the promise of improved screening and management of dental patients with early renal dysfunction. However, more studies are needed to establish the underlying pathophysiologic mechanisms involved. This finding might also suggest a future role for plasma OPN and OCN levels to risk-stratify patients with lower renal function, CVD, or both, for either lifestyle modification or strict follow-up.

This study has some limitations. Its cross-sectional design precludes inferences about the contribution of renal impairment on CAC and the effect on future cardiovascular risk. Another limitation is the small study size because of the rigorous inclusion criteria. We were only able to include a small number of patients with CAC, although all patients underwent measurement of eGFR. Future studies involving a larger number of subjects are required to improve the statistical power. Because the study participants were involved voluntarily, extending our results to a general population might be difficult. All patients were Thai, limiting the power to assess individuals of non-Asian ancestry. Future studies should confirm the predictive value of OPN and OCN in a larger cohort to establish reliable markers of renal function and oral inflammation to avoid the errors of the medical evaluation of CVD. Furthermore, future studies pertaining to circulating levels of OPN and OCN in human pathology should be considered in terms of their influence on renal function and oral inflammation.

5. Conclusion

Our study found significantly increased plasma levels of OPN and OCN in dental patients with CAC, and a correlation between plasma OPN and OCN levels and increased tooth loss and lower renal function. Dental patients with increased tooth loss and CAC may benefit from prediction of lower kidney function and higher CVD risk based on non-invasive panoramic extra-oral radiographs, and increased plasma OPN and OCN levels might play an important role in these associations. The early detection of decreased renal function can help to prevent the progression of renal disease or eventual cardiovascular events.

CRediT authorship contribution statement

Supanee Thanakun: Conceptualization, Funding acquisition, Formal analysis, Writing - original draft, Writing - review & editing. **Chantida Pawaputanon Na Mahasarakham:** Funding acquisition, Formal analysis, Writing - review & editing. **Suchaya Pornprasertsuk-Damrongsri:** Funding acquisition, Formal analysis, Writing - review & editing. **Yuichi Izumi:** Writing - review & editing.

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Ethical statement

The study was approved by the Ethics Committee of Mahidol University and Tokyo Medical and Dental University, and it also conformed with the Declaration of Helsinki (reference number: MU–IRB 2011/134.3006, TMDU–IRB 2012/860).

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.job.2019.06.004.

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