Serum levels of vitamin D and periodontal inflammation in community-dwelling older Japanese adults: The Otassha Study

RUNNING TITLE: Vitamin D and periodontal inflammation

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CLINICAL RELEVANCE

Scientific rationale for study: The potential nonlinear association of vitamin D status with periodontal inflammation has not been fully investigated.

Principal findings: Serum levels of 25-hydroxyvitamin D [25(OH)D] showed a nonlinear association with the periodontal inflamed surface area (PISA). In the lower range of serum 25(OH)D, the PISA value steeply increased with decreasing 25(OH)D levels; however, in the higher range, the association between serum 25(OH)D and PISA became modest and then almost plateaued.

Practical implications: Older Japanese adults with low vitamin D status may be at risk for periodontal inflammation; therefore, extra attention should be given to their periodontal care.

ABSTRACT

Aim: To evaluate the association between vitamin D status and periodontal inflammation as determined by the periodontal inflamed surface area (PISA) in community-dwelling older adults.

Materials and methods: This cross-sectional study included 467 Japanese adults (mean age=73.1 years) who underwent full-mouth periodontal examinations and measurements of serum levels of 25-hydroxyvitamin D [25(OH)D]. We used linear regression and restricted cubic spline models to analyze the association between exposure [serum 25(OH)D] and outcome (PISA).

Results: The linear regression model showed that, after adjusting for potential confounders, participants in the lowest quartile of serum 25(OH)D had 41.0 mm^2 more PISA (95% confidence interval=4.6–77.5) than that of the reference group (the highest quartile of serum 25(OH)D). The spline model showed that the association between serum 25(OH)D and PISA was nonlinear and restricted to the low 25(OH)D range. PISA initially sharply decreased as serum 25(OH)D increased, and then the decreasing trend slowed and plateaued. The inflection point with the minimum PISA value was a serum 25(OH)D level of 27.1 ng/ml, over which there was no decreasing trend in PISA with increasing serum 25(OH)D levels.

Conclusions: Low vitamin D status had an L-shaped association with periodontal inflammation in this cohort of Japanese adults.

KEYWORDS: cross-sectional studies; epidemiology; oral health; periodontal diseases; vitamin D

INTRODUCTION

Vitamin D is a pro-hormone and is mainly supplied by ultraviolet-induced skin synthesis. Serum 25-hydroxyvitamin D [25(OH)D] is the most commonly used indicator of vitamin D status (DeLuca, 2004). Traditionally, vitamin D is considered a key regulator of calcium-phosphorous homeostasis and bone metabolism (DeLuca, 2004). More recently, vitamin D was found to play a role in modulating inflammatory responses (Calton, Keane, Newsholme, & Soares, 2015).

Periodontitis is an inflammatory disease initiated by bacteria in the oral cavity. Persistent chronic inflammation related to dysbiosis of the periodontal microbiome results in destruction of the periodontal ligament, resorption of alveolar bone, and eventual tooth loss (Usui et al., 2021).

The bone-affecting properties of vitamin D have gained scientific attention. To date, several studies (Abreu et al., 2016; Dietrich, Joshipura, Dawson-Hughes, & Bischoff-Ferrari, 2004; Ebersole, Lambert, Bush, Huja, & Basu, 2018; Isola et al., 2020; Ketharanathan, Torgersen, Petrovski, & Preus, 2019; Laky et al., 2017; Zhou et al., 2021) have investigated the putative link between vitamin D status and periodontitis. Many studies (Abreu et al., 2016; Dietrich et al., 2004; Ebersole et al., 2018; Ketharanathan et al., 2019; Zhou et al., 2021) have defined periodontitis as involving clinical attachment loss (CAL), which reflects the destruction of periodontal tissues, including alveolar bone. Few studies have investigated periodontal inflammation (Machado, Lobo, Proença, Mendes, & Botelho, 2020). Thus, although vitamin D plays a critical role in modulating inflammatory responses, the association between vitamin D status and periodontal inflammation is unclear and requires further investigation. Moreover, a recent study (Zhou & Hyppönen, 2022) reported that the association between vitamin D status and systemic inflammation was restricted to the low vitamin D range, meaning that only individuals with low serum 25(OH)D levels exhibited elevated serum C-reactive protein (CRP) levels. Systemic low-grade inflammation, as reflected by elevated CRP levels, was reported to be involved in periodontitis progression (Pink et al., 2015). In light of these results, there may be a nonlinear relationship between vitamin D status and periodontal inflammation, which should be further tested.

The participants in the aforementioned studies on the association between vitamin D and periodontitis mainly comprised individuals aged <60 years. Thus, there is limited evidence on this association in older adults. In Japan, older adults retain more teeth than ever before, which means that an increasing number of teeth have the possibility of developing periodontitis. A national survey of dental disease in Japan found an increasing prevalence of periodontitis in the older age group (The Ministry of Health Labour and Welfare, 2016). Thus, more studies on periodontitis and its related factors are needed in this population.

Overall, the aim of this cross-sectional study was to evaluate the association between vitamin D status and periodontal inflammation, evaluated by the periodontal inflamed surface area (PISA), which quantifies the inflammatory burden posed by periodontitis (Nesse et al., 2008), in a cohort of Japanese community-dwelling older adults. Specifically, we hypothesize that a larger PISA value is observed among older adults with lower serum 25(OH)D levels.

MATERIALS AND METHODS

Design, setting, and participants

The study population comprised participants from the Otassha Study, an ongoing community-based cohort study with the aim of investigating the determinants of health and longevity among older adults. The design and protocol of the Otassha Study have been described in detail elsewhere (Fujiwara et al., 2013). Briefly, the Otassha Study was initiated in 2011 and invited adults aged ≥65 years who were listed in the basic resident register of areas surrounding the Tokyo Metropolitan Institute for Geriatrics and Gerontology in the Itabashi ward, northwest Tokyo, Japan. Follow-up surveys were conducted annually. At each follow-up, residents newly reaching 65 years of age were additionally recruited. However, recruitment of residents newly reaching 65 years of age was not conducted in 2020 due to the COVID-19 pandemic. Therefore, residents newly reaching 65 and 66 years of age were additionally recruited in 2021. At each survey, a comprehensive geriatric assessment was administered to enrolled participants. In addition to the annual assessment, a periodontal examination was conducted in 2021. Accordingly, data for the current analyses were obtained from the records of the 2021 Otassha Study.

The inclusion criteria were as follows: at least 65 years of age and able to read and understand Japanese. The exclusion criteria were as follows: having fewer than 2 teeth; taking antibacterial or anti-inflammatory drugs within the past 4 weeks; receiving periodontal therapy within the past 4 weeks; and having an incomplete dataset.

The present study was conducted in full accordance with the ethics principles of the Declaration of Helsinki and was approved by the ethics committee of the Tokyo Metropolitan Institute for Geriatrics and Gerontology (approval numbers R21-06, approval dates 27 August 2021). All the study participants provided written informed consent prior to being included in the study. As the current study was a secondary study of the 2021 Otassha Study, no sample size calculation was conducted. All available 2021 Otassha Study participants who met the eligibility criteria were included in the study.

Periodontal examination

Twelve calibrated dentists and dental hygienists recorded the probing pocket depth (PPD), gingival recession (GR), and bleeding on probing (BOP) at six sites around every tooth, except the third molars, using graduated periodontal probes and mouth mirrors (Williams Colorvue™ Probe and HD Mirrors, Hu-Friedy Mfg. Co., LLC., Chicago, IL, USA) under sufficient artificial illumination. Subsequently, CAL was calculated using the PPD and GR measurements. Prestudy calibration was conducted at Tokyo Metropolitan Institute for Geriatrics and Gerontology by examining volunteer patients to obtain acceptable reliability of periodontal measurements. All examiners achieved intraexaminer kappa values >0.8 for both PPD and GR measurements.

Measurement of serum levels of 25(OH)D

Twelve-hour fasting blood samples were collected at the time of examinations. Serum 25(OH)D concentration (ng/mL) measurement was performed with an electrochemiluminescence immunoassay (ECLIA) (Roche Elecsys Vitamin D total III, Roche Diagnostics, Mannheim, Germany) on an automated analyzer (Cobas 8000, e801, Roche Diagnostics, Mannheim, Germany) at the laboratory (BML General Laboratory, Saitama, Japan), which guaranteed that the intra- and interassay coefficients of variation were less than 15%. The limit of detection was 3 ng/ml.

Collection of other data

Data on age, sex, years of schooling, smoking status, oral health behaviors, height, and weight were obtained. Then, overweight and diabetes mellitus (DM) were defined. Details are included in the supplementary material.

Statistical analyses

The main exposure variable was serum 25(OH)D, which was categorized into quartiles. The outcome of interest was PISA, which was estimated by using CAL, GR, and BOP (Nesse et al., 2008).

The study population characteristics according to the serum 25(OH)D quartile were described using analysis of variance, the Kruskal–Wallis test, and the chi-squared test as appropriate. If the overall test was significant, post hoc comparisons were performed. The Shapiro–Wilk test was used to determine whether continuous variables were normally distributed.

Univariable and multivariable linear regression analyses were performed to assess the association between serum 25(OH)D levels (4^{th} quartile was set as the reference category) and PISA. The candidate covariates were determined a priori from known or suspected risk factors for periodontitis as follows: regular dental check-ups, toothbrushing frequency, interdental cleaning device use, age, sex, education level, smoking status, overweight, and DM (Borgnakke, 2016a, 2016b) . Any variable having a significant association with the outcome in univariable tests was selected and included in the multivariable model (Model 1). Furthermore, a fully adjusted model (Model 2) that included all the candidate covariates was constructed. For the multivariable model, multicollinearity was assessed using the variance inflation factor (VIF). Furthermore, effect modifications by sex and smoking status were evaluated by adding interaction terms in the regression models.

Then, restricted cubic splines (RCSs) were used to investigate the potential nonlinearity of the association between serum 25(OH)D and PISA. RCSs are the flexible and commonly used functions that can adequately model nonlinear associations (Harrell, 2001; Lusa & Ahlin, 2020). In the RCS, the non-linear association between exposure and outcome is described as a spline curve combining cubic polynomials and linear terms (Harrell, 2001). We specified four knots (5%, 35%, 65%, and 95% percentiles) which produced the best Bayesian Information Criterion values. The predicted values of the outcome (i.e., PISA) were plotted, with 95% confidence bands provided, against the primary exposure variable (i.e., serum 25(OH)D).

Other definitions for outcome and exposure were considered in the sensitivity analyses. First, logistic regression analyses were performed against periodontitis cases as the outcome variable, namely, stage III/IV periodontitis based on the 2018 European Federation of Periodontology/American Academy of Periodontology (EFP/AAP) case definitions (Tonetti, Greenwell, & Kornman, 2018). Furthermore, the RCSs with four knots was used to depict the relationship curve between serum 25(OH)D and stage III/IV periodontitis. A logistic regression model was applied to investigate the relationship between the exposure and the outcome. Odds ratios (ORs) were calculated relative to the median of serum 25(OH)D in the reference group (4^{th} quartile). Second, linear regression analyses were performed to investigate the association of the 3-category serum 25(OH)D variable (normal, insufficiency, and deficiency) with PISA. Detailed information about the periodontitis definition and serum 25(OH)D categorization is presented in the supplementary material.

RESULTS

Characteristics of the study participants

In August 2021, invitations were mailed to a total of 2477 older adults, including 1503 who had participated in previous health examinations (in 2011–2020) and 974 who had reached 65 or 66 years of age, and 642 agreed to participate in the onsite health examination conducted in September 2021. Among them, no participants had received periodontal therapy within the past 4 weeks; 23 who had fewer than 2 teeth and 21 who had taken antibacterial or anti-inflammatory drugs within the past 4 weeks were excluded, resulting in an eligible population of 598 participants for additional periodontal examination. A total of 127 of these participants refused to undergo a periodontal examination, and 4 had incomplete datasets. Thus, 467 adults (281 women and 186 men) were included in the present analysis (Figure 1). The age range was 65 to 92 years, with a mean [standard deviation (SD)] of 73.1 (6.3) years.

Table 1 presents the comparison of health characteristics according to the quartiles of serum 25(OH)D. Lower serum 25(OH)D was associated with a lower number of teeth, female sex, and fewer years of schooling.

Associations between serum 25(OH)D and periodontitis

Table 2 shows the crude associations of serum 25(OH)D and other characteristics with PISA. Compared to the reference group of the highest quartile of serum 25(OH)D, the group with the lowest quartile had 38.0 mm^2 more PISA [95% confidence interval (CI)= 1.1 to 74.9]. Additionally, regular dental check-ups, toothbrushing frequency, interdental cleaning device use, sex, overweight, and DM were significantly associated with PISA.

Table 3 shows the adjusted associations between serum 25(OH)D and PISA. For all models, all exposure variables had a VIF <5, indicating that there was no multicollinearity. The statistically significant association between serum 25(OH)D and PISA persisted after adjusting for possible confounders. Compared to the reference group, the group with the lowest quartile of serum 25(OH)D had 41.0 mm^2 more PISA (95% CIs 4.6 to 77.5) (Model 2). This association was independent of factors included as possible confounders in the statistical model.

In all the regression models, there was no significant interaction between serum 25(OH)D and sex or smoking status (data not shown).

Figure 2 depicts the relationship curve between serum 25(OH)D and PISA using the RCSs model. For visual simplicity and owing to sparse data, the x-axis (serum 25(OH)D) was truncated at the 1st (8.7 ng/ml) and 99th (36.0 ng/ml) percentiles of serum 25(OH)D. Overall, the PISA value initially sharply decreased as serum 25(OH)D increased, and then the decreasing trend slowed down and plateaued. The inflection point with the minimum PISA value was 27.1 ng/ml serum 25(OH)D. Over this inflection point, there was no decreasing trend in the PISA values with increasing 25(OH)D levels.

Table S1 presents the results of the sensitivity analysis that included stage III/IV periodontitis (2018 EFP/AAP case definition) as an outcome. Stage III/IV periodontitis was more prevalent among participants in the lowest quartile of 25(OH)D (odds ratio=2.01, 95% CI=1.11 to 3.63 in the fully adjusted model). The RCSs showed a statistically significant OR of stage III/IV periodontitis for serum 25(OH)D <16.0 ng/ml when the reference value was set to the median serum 25(OH)D in the 4th quartile (27.6 ng/ml; Figure S1). Table S2 presents the results of the sensitivity analysis that included 3 categories (normal, insufficiency, and deficiency) of serum 25(OH)D as an exposure. Although the deficiency group tended to have a larger PISA, 3-category serum 25(OH)D did not have a significant association with PISA.

DISCUSSION

The current cross-sectional study of community-dwelling older adults investigated the association between serum levels of vitamin D and periodontal inflammation. Compared to the participants in the highest quartile of serum 25(OH)D, those in the lowest quartile had a larger PISA. The RCSs model showed that serum 25(OH)D had a nonlinear association with PISA. We also observed that periodontitis cases were prevalent in the participants with lower serum 25(OH)D.

The findings of this study agree with those of previous studies showing a significant negative association between serum levels of vitamin D and BOP (Dietrich, Nunn, Dawson-Hughes, & Bischoff-Ferrari, 2005; Isola et al., 2020). The current findings are also in line with a recent meta-analysis (Machado et al., 2020) demonstrating lower serum 25(OH)D levels among individuals with chronic periodontitis than among those without chronic periodontitis. Furthermore, a recent study investigated serum 25(OH)D and periodontitis in the U.S. population using data from the 2013–2014 National Health and Nutrition Examination Survey (Zhou et al., 2021). Serum 25(OH)D had a nonlinear association with severe periodontitis based on the case definition developed by the Centers for Disease Control and Prevention and the American Academy of Periodontology. The range of serum 25(OH) D that is <102 nmol/L was negatively associated with severe periodontitis. Our study results are consistent with those of that other recent study in that they both show a nonlinear association between serum 25(OH)D and periodontitis.

This study has several strengths and novelties. First, we assessed periodontal inflammation, expressed as the PISA value (Nesse et al., 2008). We observed a nonlinear dose–response association of serum 25(OH)D with PISA. Most of the previous studies dichotomized the study participants as periodontitis or nonperiodontitis (Machado et al., 2020); therefore, they were unable to assess the dose–response association. Second, we performed a full-mouth periodontal examination, which is considered the gold standard for periodontitis diagnosis in population studies (Eke, Page, Wei, Thornton-Evans, & Genco, 2012). In addition to PISA values, we used the 2018 EFP/AAP case definitions (Tonetti et al., 2018), which are the most recent definitions and have been increasingly used in epidemiological settings. We observed that the association between serum 25(OH)D and stage III/IV periodontitis had the same direction as that between serum 25(OH)D and PISA, guaranteeing the consistency of our results. Third, our study participants were older than those in previous studies. Our results added new evidence that the association between serum vitamin D levels and periodontitis also exists in older populations.

Finally, we obtained rich data on the sociodemographic background and health characteristics of the participants. The association between serum 25(OH)D and PISA was adjusted for these data.

The observed associations between serum levels of vitamin D and periodontal inflammation appear to be biologically plausible. First, vitamin D status is involved in inflammation. Active vitamin D was reported to suppress proinflammatory cytokine production, including tumor necrosis factor-α and interleukin (IL)-1β, IL-6, IL-8, and IL-12, and promote the anti-inflammatory cytokine production of IL-10 (Charoenngam & Holick, 2020). A recent study using data from the UK Biobank (Zhou & Hyppönen, 2022) performed Mendelian randomization (MR) analyses and showed a causal association of low serum 25(OH)D with systemic low-grade inflammation, as reflected by elevated CRP levels. The findings from the Study of Health in Pomerania suggest that low levels of inflammatory markers are associated with a low risk for periodontitis progression (Pink et al., 2015). Moreover, a previous study using data from the UK Biobank (Zhou & Hyppönen, 2022) showed that serum 25(OH)D had an L-shaped association with CRP, where CRP levels decreased sharply with increasing 25(OH)D concentrations for participants within the deficiency range. Our study findings agree with those from the aforementioned studies. The RCSs model showed that in the lower range of serum 25(OH)D, the PISA value steeply increased with decreasing 25(OH)D levels; however, in the higher range, the association between serum 25(OH)D and PISA became modest and then almost plateaued. Second, vitamin D plays an essential role in maintaining a healthy mineralized skeleton (Charoenngam & Holick, 2020). A low serum 25(OH)D level was associated with low bone mass and an imbalance in bone metabolism by favoring bone resorption (Mendes, Charlton, Thakur, Ribeiro, & Lanham-New, 2020; Nakamura et al., 2008). A previous study showed an association between altered bone metabolism and periodontitis (Schulze-Späte et al., 2015). Third, vitamin D modulates the innate and adaptive immune systems (Charoenngam & Holick, 2020). Low serum 25(OH)D negatively affects immune function and is associated with infectious diseases (Charoenngam & Holick, 2020). Immune dysfunction has been suggested to play a role in the pathogenesis of periodontitis (Cekici, Kantarci, Hasturk, & Van Dyke, 2014). Overall, older adults with low 25(OH)D may be vulnerable to periodontal infection and inflammation because of systemic inflammation, altered bone metabolism, and/or deteriorated immune response.

On the other hand, the 3-category (normal, insufficiency, and deficiency) serum 25(OH)D variable did not have a significant association with PISA. There is still a lack of consensus on the cut-point to define states of deficiency, insufficiency and sufficiency for 25(OH)D status (Mendes et al., 2020). Current Japanese guidelines set the 25(OH)D cutoff point from the viewpoint of primary prevention for fractures and falls (N/A, 2017). Therefore, this cutoff point may not be optimal to preserve periodontal health.

It should be noted that recent MR studies (Baumeister et al., 2022; Hu, Zhou, & Xu, 2022; Li, Zheng, Xu, Zeng, & Deng, 2022) did not show a causal association between serum 25(OH)D and periodontitis. There are several potential explanations for the inconsistency between the results of our study and those of recent MR studies. First, previous studies with MR analysis did not include a sufficient number of Asian populations; therefore, the results may not be applicable to our study population of Japanese adults. Second, previous studies used the linear MR method (Baumeister et al., 2022; Hu et al., 2022; Li et al., 2022). Therefore, if the causal effect of vitamin D is truly nonlinear and restricted to the low 25(OH)D range, as suggested by our current analyses, previous studies with the linear MR method cannot capture this threshold effect. In fact, a previous study (Zhou & Hyppönen, 2022) found that nonlinear MR analysis supported the causal effect of serum 25(OH)D on CRP, but linear MR analysis did not.

The average PISA value in the older population ranged from 65.6 mm^2 to 92.6 mm^2 in earlier epidemiological studies conducted in Japan (Iwasaki et al., 2012, 2021; Miki et al., 2021). The corresponding value of our study population was within this range. On the other hand, there are racial, seasonal, and regional variations in 25(OH)D status (Hsu et al., 2020; Yoshimura et al., 2013); therefore, caution should be used in the comparison of the 25(OH)D status observed in our study with that of other studies. Nonetheless, one study was conducted in a similar area and season (i.e., in autumn) (Shimizu, Kim, Yoshida, Shimada, & Suzuki, 2015). Although that study included women (average age=78.6 years) only, the average 25(OH)D level was 22.1 ng/mL, which is not largely different from ours (20.3 ng/mL).

This study has some limitations. First, selection bias might have occurred, as the data were collected on volunteers. Of 2477 individuals who were invited to participate in the 2021 Otassha Study, 467 were included in the analyses. As discussed above, the PISA status and 25(OH)D level were similar to those of previous studies. However, we could not investigate whether there were differences in demographic and socioeconomic characteristics and medical conditions between our study population and the target population. Future studies are needed to verify whether the present findings can be applied to the broader population. Second, the current study had a cross-sectional design, thus preventing the establishment of any temporal associations or causality. Based on this single observational study, whether periodontal inflammation can be lowered by correcting low vitamin D status is still undetermined. Future longitudinal and intervention studies are necessary to obtain evidence of causality. Third, even though our analyses were adjusted for various potential confounding variables, residual confounding caused by nonmeasured factors such as dietary habits and physical activity or other unexpected confounding cannot be ruled out. Finally, although we included a larger number of participants than most of the studies that were synthesized to perform meta-regression for the association between serum vitamin D levels and periodontitis (Machado et al., 2020), we could not perform stratification analyses according to sex, smoking status, or other factors related to serum vitamin D levels and periodontitis due to the limited sample size. The limited sample size along with the heterogeneous oral health status of the study population could also be related to the wide CIs in our analyses. More accurate estimation could be obtained from future studies with larger sample sizes.

In summary, our study demonstrates that low vitamin D status has an L-shaped association with periodontal inflammation in this cohort of Japanese adults. Older Japanese adults with low vitamin D status may be at risk for periodontal inflammation; therefore, extra attention should be given to their periodontal care.

CONFLICTS OF INTEREST

Yasuyuki Sakata is an employee of Morinaga Milk Industry Co., Ltd.; the other authors have no conflicts of interest to declare.

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CONTRIBUTIONS

Research conception and study design, data analysis and interpretation, and statistical analysis: M.I.; data acquisition: M.I., K.M., M.S., M.H., Y.O., Y.M., A.E., H.K., Y.S., and K.I.; supervision: Y.F., Y.W., S.O., and H.H. Each author contributed important intellectual content during manuscript drafting or revision and accepts accountability for the overall work by ensuring that questions pertaining to the accuracy and integrity of any portion of the work are appropriately investigated and resolved. M.I. takes responsibility for the fact that this study has been reported honestly, accurately, and transparently, that no important aspects of the study have been omitted, and that any discrepancies from the study as planned have been explained.

DATA AVAILABILITY

The data are not publicly available due to ethical and legal restrictions imposed by the ethics committee of the Tokyo Metropolitan Institute for Geriatrics and Gerontology (data contain potentially identifying or sensitive patient information). Data may be made available from the corresponding author upon request.

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TABLES

Table 1. Characteristics of the study population according to the quartiles of serum 25(OH)D

	Total	Serum 25(OH)D level (ng/mL)				
		Q1 6.2–15.7	Q2 15.8–19.3	Q3 19.4–23.9	Q4 24.0–45.0	
	N=467	N=117	N=121	N=115	N=114	p value
PISA (mm^2)[*]	90.6 (142.8)	109.6 (182.5)	90.7 (116.4)	90.2 (144.8)	71.6 (116.5)	0.25
Stage III/IV periodontitis[†] (2018 EFP/AAP case definition)	251 (53.7%)	70 (59.8%)	63 (52.1%)	62 (53.9%)	56 (49.1%)	0.41
Average PPD (mm)[‡]	1.8 (1.6-2.2)	1.8 (1.6-2.3)	1.8 (1.5-2.1)	1.8 (1.5-2.1)	1.8 (1.6-2.2)	0.57
Average CAL (mm)[‡]	2.0 (1.7-2.5)	2.1 (1.8-2.6)	2.0 (1.7-2.3)	2.0 (1.7-2.4)	2.0 (1.7-2.4)	0.14
BOP (%)[‡]	3.0 (1.2-8.3)	3.6 (1.2-8.9)	3.6 (1.2-7.7)	3.0 (0.6-9.5)	2.4 (0.6-7.1)	0.42
Number of teeth[‡]	25 (21-27)	24 (19-26)[a]	26 (22-27)[b]	25 (21-27)[a, b]	26 (22-27)[b]	0.01
Regular dental check-ups[†]	292 (62.5%)	77 (65.8%)	76 (62.8%)	70 (60.9%)	69 (60.5%)	0.83
Tooth brushing ≥ 2 times/day[†]	156 (33.4%)	41 (35.0%)	38 (31.4%)	35 (30.4%)	42 (36.8%)	0.70
Use of interdental cleaning devices[†]	330 (70.7%)	81 (69.2%)	89 (73.6%)	81 (70.4%)	79 (69.3%)	0.87
Age[*]	73.1 (6.3)	73.4 (7.1)	73.0 (6.4)	73.3 (6.0)	72.7 (5.8)	0.84
Sex (male)[†]	186 (39.8%)	33 (28.2%)	37 (30.6%)	55 (47.8%)	61 (53.5%)	<0.01
Years of schooling[‡]	14 (12-16)	13 (12-16)[a]	14 (12-16)[a, b]	14 (12-16)[a]	16 (12-16)[b]	0.02
Current/past smoker[†]	173 (37.0%)	39 (33.3%)	38 (31.4%)	48 (41.7%)	48 (42.1%)	0.20
Overweight[†]	109 (23.3%)	31 (26.5%)	30 (24.8%)	24 (20.9%)	24 (21.1%)	0.68

Diabetes mellitus†	75 (16.1%)	25 (21.4%)	18 (14.9%)	16 (13.9%)	16 (14.0%)	0.35

*presented as the mean (SD)

†presented as n (%)

‡presented as the median (IQR)

25(OH)D, 25-hydroxyvitamin D; AAP, American Academy of Periodontology; BOP, bleeding on probing; CAL, clinical attachment loss; EFP, European Federation of Periodontology; IQR, interquartile range; PISA, periodontal inflamed surface area; PPD, periodontal probing depth; SD, standard deviation

Underlined text indicates data with significant adjusted standardized residuals.

The superscript letters [a] and [b] indicate statistically significant differences between groups.

Table 2. Crude associations of serum 25(OH)D and other characteristics with PISA (n=467)

	Linear regression analysis Outcome=PISA				
	Coefficient	95% CI			p value
Variables					
Quartiles of serum 25(OH)D (ng/mL)					
Q4 24.0–45.0	(reference)				
Q3 19.4–23.9	18.6	-18.5	to	55.6	0.32
Q2 15.8–19.3	19.1	-17.5	to	55.7	0.31
Q1 6.2–15.7	38.0	1.1	to	74.9	0.04
Regular dental check-ups	-44.2	-70.5	to	-17.8	<0.01
Tooth brushing ≥ 2 times/day	-28.3	-55.5	to	-1.1	0.04
Use of interdental cleaning devices	-38.8	-67.0	to	-10.7	0.01
Age (per one year increase)	-0.1	-2.2	to	1.9	0.89
Male (vs. female)	32.8	6.6	to	58.9	0.01
Years of schooling (per one year increase)	-2.6	-7.5	to	2.3	0.30
Current/past smoker (vs. never smoker)	10.6	-16.1	to	37.3	0.43
Overweight	73.9	44.2	to	103.7	<0.01
Diabetes mellitus	41.4	6.3	to	76.4	0.02

25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; PISA, periodontal inflamed surface area

Table 3. Adjusted associations between serum 25(OH)D and PISA (n=467)

	Linear regression analysis Outcome=PISA					
	Model 1*			Model 2*		
	Coefficient	95% CI	p value	Coefficient	95% CI	p value
Variables						
Quartiles of serum 25(OH)D (ng/mL)						
Q4 24.0–45.0	(reference)			(reference)		
Q3 19.4–23.9	19.2	-16.5 to 54.9	0.29	18.3	-17.6 to 54.1	0.32
Q2 15.8–19.3	22.6	-13.2 to 58.4	0.22	22.2	-13.7 to 58.1	0.22
Q1 6.2–15.7	41.1	4.7 to 77.4	0.03	41.0	4.6 to 77.5	0.03

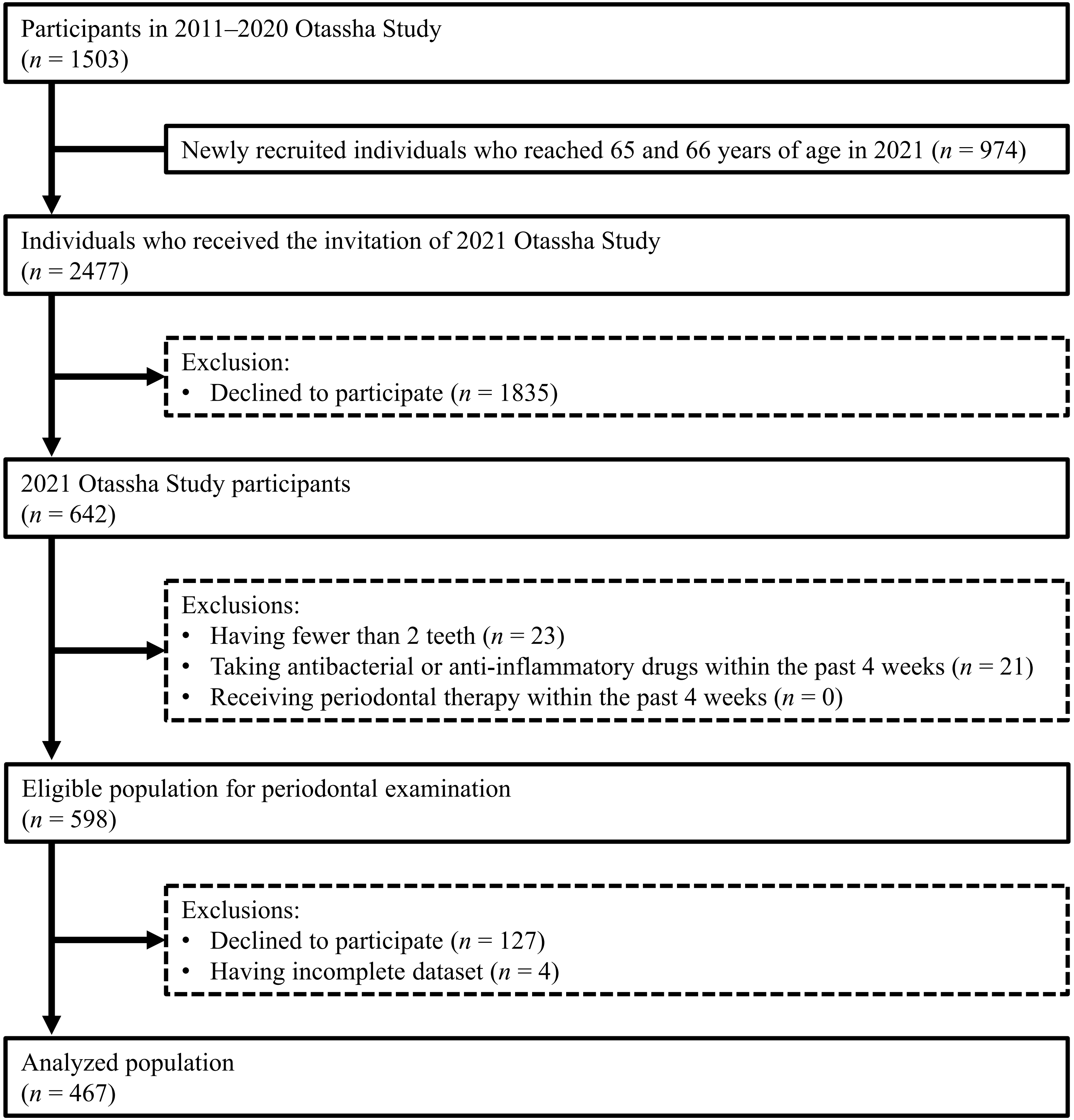
*Model 1 included variables having a significant association with the outcome in univariable tests as covariates (i.e., adjusted for regular dental check-ups, toothbrushing frequency, interdental cleaning device use, sex, overweight, and diabetes mellitus). Model 2 was a fully adjusted model (i.e., adjusted for regular dental check-ups, toothbrushing frequency, interdental cleaning device use, age, sex, education level, smoking status, overweight, and diabetes mellitus).

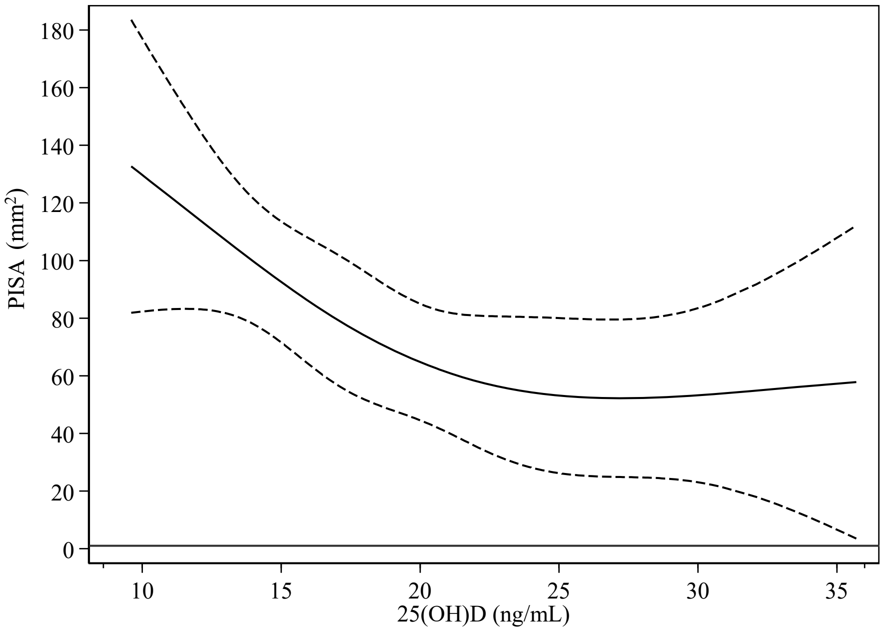
25(OH)D, 25-hydroxyvitamin D; CI, confidence interval; PISA, periodontal inflamed surface area

FIGURE LEGENDS

Figure 1. Flow diagram of our study.

Figure 2. Restricted cubic spline based on a multivariable-adjusted linear regression model of the association between serum 25(OH)D and PISA. The black solid line indicates the estimated values of PISA, and the dotted lines indicate the 95% confidence intervals. For visual simplicity and owing to sparse data, the x-axis (serum 25(OH)D) was truncated at the 1st (8.7 ng/ml) and 99th (36.0 ng/ml) percentiles of serum 25(OH)D.


Participants in 2011–2020 Otassha Study
(n = 1503)
Newly recruited individuals who reached 65 and 66 years of age in 2021 (n = 974)
Individuals who received the invitation of 2021 Otassha Study
(n = 2477)
Exclusion:
• Declined to participate (n = 1835)
2021 Otassha Study participants
(n = 642)
Exclusions:
• Having fewer than 2 teeth (n = 23)
• Taking antibacterial or anti-inflammatory drugs within the past 4 weeks (n = 21)
• Receiving periodontal therapy within the past 4 weeks (n = 0)
Eligible population for periodontal examination
(n = 598)
Exclusions:
• Declined to participate (n = 127)
• Having incomplete dataset (n = 4)
Analyzed population
(n = 467)



PISA (mm^2)
180
160
140
120
100
80
60
40
20
0
10
15
20
25
30
35
25(OH)D (ng/mL)