The Relationship between Periodontal Disease and Vitamin D

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Background: As vitamin D is known to have functions in bone homeostasis and immune system regulation, it is plausible that vitamin D levels could affect the progression of periodontal disease. Currently, there is conflicting evidence regarding the role of vitamin D in the progression of periodontal disease. The purpose of this study was to explore the relationship between plasma 25(OH)D concentration and periodontal disease measured by gingival inflammation (GI) and loss of attachment (LOA). It is hypothesized that lower 25(OH)D levels will be associated with higher measures for GI and LOA.

Methods: This cross-sectional study used data from the Canadian Health Measures Survey (CHMS) on subjects aged 13 – 79 years of age. Vitamin D status was determined by measuring plasma 25(OH)D concentrations in nmol/L. Periodontal disease was measured by assessing (GI) using Löe's gingival index and by calculating (LOA). Student's t-tests and chi-square tests were used to perform a bivariate analysis between potential independent variables including 25(OH)D and the dependent variables of GI and LOA. Multiple logistic regression analyses were then performed for GI and LOA to determine the adjusted correlation between 25(OH)D and GI and 25(OH)D and LOA and to control for potential confounding variables.

Results: Plasma 25(OH)D concentration less than 50 nmol/L and less than 75 nmol/L, but not mean plasma 25(OH)D concentration, were associated with GI at the bivariate level. However, no association was found between mean plasma 25(OH)D concentration, 25(OH)D concentration less than 50 nmol/, or 25(OH)D concentration less than 75 nmol/L and GI in the multiple regression analysis. None of mean plasma 25(OH)D concentration, 25(OH)D concentration less than 50 nmol/L, or 25(OH)D concentration less than 75 nmol/L were associated with LOA at the bivariate level. A statistically significant association was observed between 25(OH)D concentration less than 75 nmol/L and LOA in the multiple regression analysis, but not mean plasma 25(OH)D concentration or 25(OH)D concentration less than 50 nmol/L.

Conclusion: Vitamin D status was inversely associated with GI at the bivariate level, but not at the multivariate level. Conversely, vitamin D status was not associated with LOA at the bivariate level, but it was inversely associated with LOA at the multivariate level. These results provide modest evidence supporting a relationship between low plasma 25(OH)D concentrations and periodontal disease as measured by GI and LOA.

INTRODUCTION

Periodontitis is a chronic inflammatory condition of the periodontium initiated by microbial biofilms that form on the teeth[1]. Bacterial products themselves as well as the host's immune response to these products result in destruction of the tissues that support the teeth including alveolar bone. Because of this tissue destruction, periodontitis is a major cause of tooth loss in adults.[2, 3] Prevention of this disease is important because tooth loss can affect one's nutritional status[4] as well as one's quality of life.[5] Periodontitis has also been associated with systemic conditions such as cardiovascular disease[6] and type II diabetes mellitus.[7]

Vitamin D is a fat-soluble vitamin obtained from exposure to sunlight, from the diet, and from dietary supplements.[8] Vitamin D from the skin and diet is metabolized in the liver to 25-hydroxyvitamin D [25(OH)D], which is then metabolized in the kidneys to its active form, 1,25-dihydroxyvitamin D [1,25-$(OH)_2D$], by the enzyme D-1α-hydroxylase.[8] The former, 25(OH)D, is the major circulating metabolite of this vitamin in the blood and it is used to determine a patient's vitamin D status.[8] Currently, there is no consensus on optimal levels of 25(OH)D; however, most experts define 25(OH)D levels of less than 50 nmol/L (20ng/mL) as vitamin D deficiency.[8] Recent evidence suggests that 25(OH)D levels may have to be as high as 75 nmol/L (30ng/mL) to achieve vitamin D sufficiency.[8]

Vitamin D is involved in regulating calcium absorption from the gut, in regulating plasma calcium concentration, and in regulating bone mineralization.[9] In the gut, 1,25-$(OH)_2D$ enters intestinal epithelial cells and binds the vitamin D-receptor (VDR). One function of the VDR is to stimulate the expression of the calcium binding protein gene. Calcium binding protein ameliorates calcium absorption in the small intestine. In the absence of vitamin D, only 10 to 15% of dietary calcium is absorbed in the gut.[8] The interaction of 1,25-$(OH)_2D$ with the VDR improves this absorption to 30 to 40%.[8] 25(OH)D indirectly affects bone mineralization and plasma calcium concentration through its inverse plasma relationship with parathyroid hormone (PTH).[9] As 25(OH)D levels decrease below 75 nmol/L, the parathyroid gland is stimulated to secrete more PTH.[9] PTH in turn stimulates bone resorption and this bone resorption results in increased plasma calcium concentrations.[9] PTH also has an effect on vitamin D levels. PTH stimulates the conversion of 25(OH)D to 1,25-$(OH)_2D$ in the kidney.[8] As more vitamin D is converted to its active form, more calcium can be absorbed by the intestine. Because vitamin D regulates calcium absorption, plasma calcium concentration, and bone mineralization, it follows that this vitamin would affect bone health. Indeed there is much evidence linking sufficient vitamin D levels with bone health.

Studies have found significant positive associations between 25(OH)D levels and bone mineral density[10] as well as between vitamin D supplementation and a lower risk of fractures.[11]

More recent evidence indicates that vitamin D also has a regulatory function on the immune response, stimulating the immune response at times, while inhibiting it at others. One study[12] demonstrated that after being exposed to antigens, macrophages upregulated the expression of the VDR and D-1α-hydroxylase, resulting in increased production of the antibacterial proteins cathelicidin and beta-defensins. The authors concluded that the ability to produce active vitamin D improved bacterial killing. There are many examples of vitamin D's ability to inhibit the immune response. In vitro studies have shown that 1,25-$(OH)_2$D inhibits the proliferation, maturation, and differentiation of dendritic cells from monocytes.[13] The active form of vitamin D has also been shown to inhibit the production of inflammatory cytokines in monocytes including IL-1, IL-6, IL-8, IL-12, and TNF-α.[13] Some studies have also reported that 1,25-$(OH)_2$D has the ability to suppress the proliferation and cytokine production of T-lymphocytes.[13]

Because periodontitis is characterized by bone loss and because vitamin D regulates bone mineralization, it is plausible that vitamin D could affect the progression of periodontal disease by its effects on bone. Similarly, because the immune response is responsible for much of the tissue destruction in periodontitis and because vitamin D is thought to regulate the immune response, vitamin D could affect the progression of periodontal disease by its effects on the immune system. Not surprisingly, studies are emerging that are investigating the relationship between vitamin D and periodontal disease.[14-19] Two large cross-sectional studies[14, 15, 17] have found an association between low vitamin D levels and markers of periodontal disease. However, the largest prospective study to date[19] as well as the most recent cross-sectional study[20] that examined the relationship between vitamin D and periodontal disease found no relationship between these two entities. It is clear that further research is needed to determine what impact, if any, vitamin D status has on the progression of periodontal disease. Furthermore, most of the research to date examining the relationship between vitamin D and periodontal disease has been conducted in the United States; there is no Canadian evidence on this relationship. It is plausible that this relationship could be stronger in a Canadian sample because Canadians are subjected to longer winter seasons and may obtain less vitamin D from sun exposure. Thus, a larger proportion of Canadians may be vitamin D-deficient. This study aims to explore the relationship between plasma 25(OH)D concentration and periodontal disease measured by GI and LOA

using cross-sectional data from the CHMS. It is hypothesized that lower 25(OH)D levels will be associated with higher measures for GI and LOA.

MATERIALS AND METHODS

Study Sample

Data for this study were obtained from subjects 6 - 79 years of age participating in Cycle 1 of the CHMS. Cycle 1 of the CHMS was undertaken from 2007 to 2009 and was a national, cross-sectional survey conducted by Statistics Canada, which was a representative sample of 97% of the Canadian population.[21] Data collection involved both direct physical measurements and interviews of participants (the household questionnaire) living across all provinces and territories. A total of 5,604 subjects completed both the household questionnaire and the physical measurements.[21] All subjects provided informed consent. The CHMS excluded residents of First Nations Reserves or Crown land, residents of institutions, full-time members of the Canadian Forces, and residents of certain remote regions of Canada.

Statistics Canada used a probability sampling approach, incorporating aspects of stratification and cluster sampling. From a potential of 257 identified sites, 15 were selected and stratified by region. Overall, there was one site in Atlantic Canada, four sites in Quebec, six in Ontario, two in the Prairies, and two in British Columbia. Approximately 350 respondents were sampled at each site, stratified by age group (five age groups: 6-11, 12-19, 20-39, 40-59, 60-79 years). For the purposes of this investigation, the authors restricted the analysis to subjects 13 - 79 years of age for the analysis of GI and 20 - 79 years of age for the analysis of LOA.

Clinical Oral Exam

Dental examinations were completed by 14 Canadian Forces dentists that were calibrated to World Health Organization (WHO) standards. An initial central calibration of the dentists took place as well as recalibration on the first day at each new examination site. All examiners achieved high agreement (Cohen's Kappa ≥ 0.6) initially at all site locations. The examiners recorded GI on the buccal, lingual, mesial, and distal surfaces of each of six indicator teeth, which were 1.6, 1.2, 2.4, 3.6, 3.2, and 4.4. If a permanent index tooth was missing, but the corresponding deciduous tooth was present, GI was recorded for the deciduous tooth. GI was scored using Löe's gingival index that scored 0 if inflammation was absent, 1 if it was mild, 2 if it was moderate, 3 if it was severe, and 4 if the tooth was absent. For the purpose of this investigation, the highest score for GI for each subject was used and then GI was dichotomized into "no or mild inflammation" (groups 0 and 1 combined) and "moderate to severe inflammation" (groups 2 and 3 combined). The Williams probe was used to measure LOA, which was

defined as the distance from the cemento-enamel junction to the bottom of the periodontal pocket, at six sites on each of the WHO's indicator teeth that were present. These indicator teeth included teeth 1.7, 1.6, 1.1, 2.6, 2.7, 3.7, 3.6, 3.1, 4.6, and 4.7. Examiners recorded the highest LOA measurement for each sextant. For the purpose of this study, the highest score for LOA for each subject was used and then LOA was grouped into three categories: slight (≤ 3 mm), moderate (4 – 5 mm), and severe (> 5 mm). Examiners recorded plaque using Green and Vermillion's simplified oral hygiene index that scored 0 if no debris or stain was present, 1 if less than 1/3 of the tooth surface was covered with soft debris or extrinsic stain, 2 if between 1/3 and 2/3 of the tooth surface was covered with soft debris or extrinsic stain, and 3 if more than 1/3 of the tooth surface was covered with soft debris or extrinsic stain. Examiners recorded plaque on the labial surfaces of maxillary teeth and mandibular incisors and the lingual surfaces of mandibular molars on the same indicator teeth used for LOA, once again recording the highest score for each sextant. For the purpose of this investigation, a mean plaque score was calculated for each subject.

Assessment of Plasma 25(OH)D

Plasma vitamin D levels were measured by a chemiluminescence assay, the LIAISON 25-Hydroxyvitamin D TOTAL assay (Diasorin, Ltd.).[21] Two different vitamin D level cut-offs were examined in this investigation: 50 nmol/L (based on the Institute of Medicine's threshold for vitamin D sufficiency) as well as 75 nmol/L (based on emerging evidence for vitamin D sufficiency. Mean 25(OH)D levels were also examined.

Data on Other Covariates

To account for other confounding factors for GI and LOA, additional independent variables were considered. Smoking was one of these variables and it was accounted for using the household questionnaire. Respondents were classified as never smokers (smoked < 100 cigarettes in their lifetime), former smokers (smoked ≥ 100 cigarettes in their lifetime, but not currently smoking), and current smokers (smoked ≥ 100 cigarettes in their lifetime and are currently smoking). These self-reported smoking measures have been used previously and have been found to correlate with plasma concentrations of cotinine, the major metabolite of nicotine.[14] For the analysis, current smokers and former smokers were grouped together and compared to never smokers. Smoking was also analysed using pack years. This statistic was calculated by taking the number of cigarettes smoked each day times the number of years smoked divided by twenty. This statistic has also been used before and it has been found to correlate with attachment loss.[22]

Diabetes status was determined based on the self-reported responses to the household questionnaire. Diabetes status has been shown previously to be correlated with gingival inflammation[23] as well as with extent and severity of periodontal disease.[24-26] An analysis of the percentage of glycosylated hemoglobin (HbA1c) was also performed using the phlebotomy component of the CHMS. HbA1c is considered to be a measure of long-term diabetic control and values indicative of poor diabetic control have been previously correlated with prevalence, severity, and extent of periodontitis.[26, 27] Values of $\leq 7.0\%$ were considered to be good control while values of $> 7.0\%$ were considered to be moderate to poor control.

Body mass index (BMI) was also considered in the analysis. This variable was already present in the CHMS data and had been calculated from measurements taken at the clinical exam. The authors analysed mean BMI values measured in kg/m^2 as well as BMI categories. In the analysis of GI, BMI values of < 18.5 kg/m^2 were considered underweight, values $18.5 - 24.9$ kg/m^2 were considered normal, values 25.0 - 29.9 kg/m^2 were considered overweight, and values of ≥ 30.0 kg/m^2 were considered to be obese. In the analysis of LOA, when four categories of BMI were used, the data was suppressed due to not enough subjects in the underweight category. To render the data releasable, only three categories of BMI could be used for this analysis, which were < 25 kg/m^2 (underweight and normal), $25 - 29.9$ kg/m^2 (overweight), and ≥ 30 kg/m^2 (obese). It was speculated that combining the underweight and normal categories would not adversely affect the analysis for LOA as previous studies have only correlated higher mean BMI values with markers of periodontal disease.[28, 29]

The effect of total household income was explored in categories which were < $20,000, $20,000 - $60,000, and > $60,000. Other independent variables that were considered using responses from the household questionnaire included daily vitamin D supplement use, daily multivitamin supplement use, frequency of visits to a dental professional (once a year or not), tooth-brushing frequency (twice a day or not), flossing frequency (once a day or not), age, and sex.

Statistical Methods

Data were accessed and analyzed within the Research Data Centre (RDC) at the University of Manitoba using SPSS 20 (IBM, Armonk, NY), SAS 9.2 (Cary, NC), and Stata 13 MP (StataCorp LP, College Station, TX). As per RDC restrictions, original sample sizes were suppressed. Bootstrap weights for variance estimation and weighted results are presented with degrees of freedom fixed to 11. Descriptive statistics included means and frequencies with 95% confidence intervals (CI). Chi-square tests were used to determine the unadjusted correlation of each categorical independent variable with GI and LOA. Student's t-tests were performed to determine the unadjusted correlation of each continuous

independent variable and GI and LOA. Multiple logistic regression models for GI and for LOA were developed to determine the adjusted correlation between 25(OH)D levels and GI as well as between 25(OH)D and LOA, controlling for potential confounding variables. Three multiple logistic regression models were used for GI and LOA: Model A used 25(OH)D concentration of less than 50 nmol/L, Model B used 25(OH)D concentration less than 75 nmol/L, and Model C used mean 25(OH)D concentration. For choosing variables to include in the multiple logistic regression analysis for GI and LOA, variables with a p value of less than or equal to 0.075 were considered with the exception of plasma vitamin D concentration and known risk factors for periodontal disease such as smoking. A p value ≤ 0.05 was considered to be statistically significant.

RESULTS

The mean plasma 25(OH)D concentrations with 95% CI in the GI and LOA samples were 90.8 (77.5 – 104.2) and 85.6 (74.6 – 97.2) nmol/L, respectively. Although the mean 25(OH)D levels were well above the thresholds for vitamin D sufficiency, 63% of each sample had plasma 25(OH)D concentrations below the 75 nmol/L threshold while 25% of each population had 25(OH)D levels below the Institute of Medicine threshold of 50 nmol/L.

Results of the bivariate analysis of GI are summarized in Table 1. Several variables were found to be statistically significant in this analysis including 25(OH)D concentration less than 50 nmol/L and 25(OH)D concentration less than 75 nmol/L. Subjects with 25(OH)D concentrations of less than 50 nmol/L and less than 75 nmol/L had significantly increased odds of having more GI (OR = 1.63 and 1.44 respectively). Those taking vitamin D supplements had significantly lower odds for GI (OR = 0.56), while those with diabetes were at increased odds of having moderate to severe GI (OR = 1.33). Mean BMI was significantly higher among those with the worst GI. Meanwhile, those who reported frequenting a dental professional one or more times per year, those who reported brushing their teeth at least twice daily, and those who reported flossing daily had significantly lower odds for GI. Increased scores for the plaque index were associated with increased odds for moderate to severe GI. Males had increased odds for GI compared to females, while those in higher income categories had lower odds for GI than those in lower income categories.

However, when confounding variables were controlled for in the multiple regression analysis of GI (Table 2), only plaque and sex remained as being significantly associated with GI. Females had lower odds of having moderate to severe GI, while high values for the plaque index increased the odds of

having moderate to severe GI. No statistically significant relationship between 25(OH)D and GI was observed in Models A, B, or C in the multiple logistic regression analysis of GI.

Several variables were found to be statistically significant in the bivariate analysis of LOA (Table 3). Surprisingly, taking a multivitamin or a vitamin D supplement was associated with increased odds of having more severe LOA. Higher mean HbA1c values were associated with increased odds of more severe LOA as was HbA1c of > 7%. As to be expected, older age was associated with increased odds of having more severe LOA, while having an income of greater than $60,000 was associated with lower odds of having more severe LOA. No statistically significant association was found between 25(OH)D levels and LOA in the bivariate analysis.

Results of the multiple regression analysis of LOA appear in Table 4. In general, few variables were found to be significantly and independently associated with more severe LOA. Age and smoking were found to be statistically significant with increased age and former or current smoking status increasing the relative risk of having moderate versus slight LOA. Plasma 25(OH)D concentration < 75 nmol/L was also found to be statistically significant ($p = 0.05$) with 25(OH)D levels below this threshold being associated with an increased relative risk (RRR = 2.09) of having severe versus slight LOA.

DISCUSSION

In this study, which was the first to report associations between plasma 25(OH)D levels and markers of periodontal disease in a Canadian population, there were mixed observations supporting the hypothesis that lower 25(OH)D levels would be associated with higher measures for GI and LOA. While significant associations between low 25(OH)D thresholds and increased odds of GI were identified, these relationships were not observed after undertaking the multiple regression analysis. Conversely, while no significant associations were found between 25(OH)D levels and LOA in the bivariate analysis, we did observe a significant association between the 25(OH)D threshold of < 75 nmol/L and increased relative risk for greater LOA in the multiple regression analysis. One must exercise caution in interpreting this latter finding; however, as it may or may not represent a true association. Since 25(OH)D levels were a key independent variable of interest, they were included in the various logistic regression models for LOA even though they were not associated with LOA at the bivariate level. Furthermore, it was not possible to perform backwards elimination in the multiple regression analysis using the available software while using a bootstrapping command. Had backwards elimination been used, the 25(OH)D variables may have been among the first variables to be eliminated as they had the largest p values in

the bivariate analysis of any of the variables included. The fact that stronger associations between 25(OH)D levels and GI or LOA were not observed may seem counter-intuitive based on vitamin D's roles in bone homeostasis and immune system regulation. However, currently there is conflicting evidence in the literature regarding the relationship between vitamin D and periodontal disease.

One of the first studies to support an association between 25(OH)D levels and periodontal disease was that of Dietrich et al, 2004[14]. This was a cross-sectional study with a sample size of 11,202 that used data from the National Health and Nutritional Examination Survey III (NHANES III) to examine the relationship between 25(OH)D levels and attachment loss. This study concluded that there was an inverse relationship between 25(OH)D levels and attachment loss in subjects 50 or older and this relationship was independent of possible confounding variables. This same group performed a separate analysis in 2005 on a sample of 6700 from the NHANES III to investigate the relationship between 25(OH)D levels and gingival inflammation.[15] This second analysis found that sites in subjects in the lowest 25(OH)D quintile were 20% less likely to bleed on gingival probing than sites in subjects in the highest 25(OH)D quintile.

Another study that found an association between 25(OH)D levels and periodontal disease was a cross-sectional study by Millen et al. in 2013.[17] This study's sample consisted of 920 postmenopausal women and it assessed the extent of periodontal disease by measuring the parameters of alveolar crestal height, tooth loss, clinical attachment level, probing depth, and percent bleeding on gingival probing. The study categorized subjects as vitamin D adequate if they had a plasma 25(OH)D concentration of $\geq$ 50 nmol/L and vitamin D inadequate if this concentration was < 50 nmol/L. Similar to the findings of Dietrich et al., Millen et al. found that vitamin D status was inversely associated with periodontal disease as measured by bleeding on probing and clinical categories that incorporated probing depth as a parameter.

Millen et al.[19] have also published the largest and longest longitudinal study to date analysing the relationship between vitamin D and periodontal disease. This 5-year cohort study on a sample of 655 postmenopausal women measured plasma 25(OH)D concentrations at baseline and follow-up as well as multiple periodontal parameters. Unlike the previous studies, including the cross-sectional study by this same group, this latter study by Millen et al. found no statistically significant associations between baseline 25(OH)D concentrations and change in periodontal disease measures at five years. A similar result was found by Antonoglou et al. in 2015[20] in a cross-sectional study assessing the

relationship between vitamin D and periodontal disease. This study, which was conducted on a sample of 1262 Finnish subjects, assessed the extent of periodontal disease by counting the number of probing depths ≥ 4 mm and the number of bleeding sextants per subject. Unlike the other cross-sectional studies discussed, Antonoglou et al. found no associations between serum 25(OH)D and their markers of periodontal disease.

As the results of this present study contain mixed evidence supporting an association between low 25(OH)D levels and periodontal disease, they are in agreement with the results of some of the studies discussed, while they are at odds with the results of others. The observation of associations between low 25(OH)D thresholds and increased odds of GI at the bivariate level in this present study is consistent with the studies supporting a relationship between 25(OH)D levels and periodontal disease including the cross-sectional studies by Dietrich et al. and Millen et al. Likewise, the observation of a significant association between the 25(OH)D threshold of < 75 nmol/L and increased relative risk for greater LOA in the multiple regression analysis of this study is also consistent with the cross-sectional studies by Dietrich et al. and Millen et al. Conversely, the observation of no association between low 25(OH)D thresholds and GI after undertaking the multiple regression analysis in this present study is more in agreement with the studies not supporting a relationship between 25(OH)D levels and periodontal disease including the longitudinal study by Millen et al. and the study by Antonoglou et al. Likewise, the finding of no association between low 25(OH)D thresholds and LOA at the bivariate level is also consistent with these later two studies.

Limitations of the present study included factors associated with a cross-sectional design as well as factors related to how the markers of periodontal disease were defined. Measurements for GI and LOA were performed on only 6 and 10 indicator teeth respectively. Furthermore, the worst score for each subject was then used to categorize subjects into one of the categories for GI or LOA. Thus, a greater potential existed in this study to overestimate or underestimate the severity and extent of periodontal disease than if for example, full-mouth probing had been used. Similarly, because a gingival index was used in the assessment of GI, one could argue that more subjectivity was present in this assessment than if bleeding on probing had been used. Thus, once again, this subjectivity could lead to the overestimation or underestimation of periodontal disease as measured by GI. The cross-sectional design of this study was another limitation, which does not permit the determination of causality or the determination of 25(OH)D levels at the time when attachment loss occurred. The possibility that

residual confounding variables may have been present that weren't accounted for is another limitation of this study.

Strengths of this study included the large sample size and the representative nature of the sample under investigation and the fact that dental examinations were performed by well calibrated dentists. Another advantage was the availability of actual patient plasma 25(OH)D concentration, which is the recognized gold standard in determining an individual's overall vitamin D status, instead of relying on dietary intake estimates.

While the cross-sectional studies by Dietrich et al. and Millen et al. have provided strong evidence supporting a relationship between vitamin D status and periodontal disease, the largest and longest longitudinal study as well as the recent cross-sectional study by Antonoglou et al. failed to find an association between these two entities. The results of this present study, performed on a representative sample of Canadian adults, provide modest evidence supporting a relationship between low 25(OH)D concentrations and periodontal disease as measured by GI and LOA. Prospective studies with longer follow-up are likely required to fully elucidate what effect, if any, vitamin D levels have on the progression of periodontal disease.

CONCLUSIONS

Vitamin D status was inversely associated with GI at the bivariate level, but not at the multivariate level. Conversely, vitamin D status was not associated with LOA at the bivariate level, but it was inversely associated with LOA at the multivariate level. These results provide modest evidence supporting a relationship between low plasma 25(OH)D concentrations and periodontal disease as measured by GI and LOA.

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TABLES

Table 1: Bivariate Analysis for Gingival Inflammation (GI)				
Variable	**Proportion None – Mild GI (95% CI)**	**Proportion Moderate – Severe GI (95% CI)**	**p value**	**Unadjusted Odds Ratio (95% CI)**
[25(OH)D][a] • < 50 nmol/L • ≥ 50 nmol/L	 0.60 (0.51 - 0.68) 0.71 (0.64-0.77)	 0.40 (0.32 - 0.49) 0.29 (0.24 - 0.36)	0.010	1.63(1.15 - 2.30)
[25(OH)D][a] • < 75 nmol/L • ≥ 75 nmol/L	 0.65 (0.58 - 0.72 0 0.73 (0.67 - 0.78)	 0.35 (0.28 - 0.42) 0.27 (0.22 - 0.33)	0.014	1.44 (1.09 - 1.90)
Mean [25(OH)D][b] (nmol/L)	88.75 (79.63 - 97.87)	94.80 (71.57 - 118.04)	0.43	1.00 (0.999 - 1.001)
Vitamin D supplement use[a] • Yes • No	 0.81 (0.73 - 0.87) 0.70 (0.64 - 0.75)	 0.19 (0.13 - 0.27) 0.30 (0.25 - 0.36)	0.007	0.56 (0.37 - 0.85)
Multivitamin or vitamin D supplement use[a] • Yes • No	 0.73 (0.68 - 0.79) 0.69 (0.62 - 0.75)	 0.26 (0.21 - 0.33) 0.31 (0.25 - 0.38)	0.087	0.78 (0.58 - 1.05)
Smoking[a] • Former or current • Never	0.59 (0.50 - 0.68) 0.69 (0.61 - 0.75)	0.41 (0.32 - 0.50) 0.31 (0.25 - 0.39)	0.057	1.51 (0.97 - 2.35)
Mean pack years of smoking[b]	11.12 (9.64 - 12.60)	13.72 (10.23 - 17.21)	0.17	1.01 (1.00 - 1.03)
Diabetes[a] • Yes • No	 0.62 (0.53 - 0.70) 0.68 (0.62 - 0.74)	 0.38 (0.29 - 0.47) 0.32 (0.26 - 0.38)	0.036	1.33 (1.01 - 1.74)
Mean glycosylated hemoglobin[b] (%)	5.6 (5.4 - 5.7)	5.6 (5.5 - 5.7)	0.23	Unable to calculate
Glycosylated hemoglobin[a] • ≤ 7% • > 7%	 0.69 (063 - 0.74) 0.58 (0.45 - 0.71)	 0.31 (0.26 - 0.37) 0.42 (0.29 - 0.55)	0.10	1.61 (0.90 - 2.89)
Mean body mass index[b] (kg/m^2)	25.71 (25.11 - 26.32)	26.37 (25.76 - 26.98)	0.049	1.02 (1.00 - 1.05)
Body mass index[a] • < 18.5 kg/m^2 • 18.5 - < 25 kg/m^2 • 25 - < 30 kg/m^2 • ≥ 30 kg/m^2	 0.65 (0.51 - 0.77) 0.69 (0.64 - 0.74) 0.70 (0.62 - 0.78) 0.63 (0.54 - 0.71)	 0.35 (0.23 - 0.49) 0.31 (0.26 - 0.36) 0.30 (0.22 - 0.38) 0.37 (0.29 - 0.46)	0.15	 0.84 (0.49 - 1.43) 0.80 (0.46 - 1.37) 1.11 (0.63 - 1.95)
Visits dental professional once/yr. or more[a] • Yes • No	 0.73 (0.66 - 0.79) 0.52 (0.45 - 0.59)	 0.27 (0.21 - 0.34) 0.48 (0.41 - 0.55)	0.000	0.40 (0.31- 0.53)
Brushes twice/d or more[a] • Yes • No	 0.72 (0.65 - 0.78) 0.56 (0.50 - 0.62)	 0.28 (0.22 - 0.35) 0.44 (0.38 - 0.50)	0.000	0.50 (0.40 - 0.63)
Flosses once/d or more[a] • Yes • No	 0.72 (0.66 - 0.78) 0.56 (0.48 - 0.63)	 0.28 (0.22 - 0.34) 0.44 (0.37 - 0.52)	0.000	0.47 (0.37 - 0.62)
Plaque • 0	 0.93 (0.86 - 0.97)	 0.07 (0.03 - 0.14)	< 0.001	

• 1 • 2 • 3	0.73 (0.65 - 0.80) 0.51 (0.40 - 0.62) 0.28 (0.21 - 0.37)	0.27 (0.20 - 0.35) 0.49 (0.38 - 0.60) 0.72 (0.63 - 0.79)		5.13 (2.80 - 9.39) 13.33 (5.28 - 33.65) 35.43 (13.17 - 95.30)
Mean age[b] (yrs.)	40.94	40.63	0.67	1.00 (0.99 - 1.00)
Sex[a] • Male • Female	 0.63 (0.57 - 0.69) 0.73 (0.65 - 0.79)	 0.37 (0.31 - 0.43) 0.27 (0.21 - 0.35)	0.012	0.65 (0.47 - 0.89)
Income[a] • < $20,000 • $20,000 - $60,000 • > $60,000	 0.49 (0.40 - 0.59) 0.64 (0.56 - 0.72) 0.73 (0.67 - 0.79)	 0.51 (0.41 - 0.61) 0.36 (0.28 - 0.44) 0.27 (0.21 - 0.33)	0.001	 0.54 (0.39 - 0.74) 0.36 (0.22 - 0.56)

a - χ^2 test

b - t test

	Table 2: Multiple Logistic Regression for Moderate – Severe versus None – Mild Gingival Inflammation			
	Variable	Adjusted Odds Ratio	95% Confidence Interval	p Value
Model A: Vitamin D < 50 nmol/L	Vitamin D < 50 nmol/L	1.12	0.77 – 1.62	0.53
	Vitamin D supplement Use	0.95	0.67 – 1.34	0.75
	Smoking (former or current)	1.18	0.70 – 1.98	0.51
	Diabetes	1.14	0.75 – 1.75	0.51
	Plaque			
	• 1	3.67	1.80 – 7.50	0.00
	• 2	8.44	3.45 – 20.70	0.00
	• 3	23.57	7.04 – 78.91	0.00
	Sex (female)	0.61	0.38 – 0.99	0.05
	Income			
	• $20,000 - $60,000	0.60	0.33 – 1.10	0.09
	• > $60,000	0.50	0.22 – 1.14	0.09
	Mean BMI (kg/m^2)	1.02	0.99 – 1.05	0.16
Model B: Vitamin D < 75 nmol/L	Vitamin D < 75 nmol/L	1.06	0.74 – 1.51	0.73
	Vitamin D supplement Use	0.94	0.67 – 1.33	0.71
	Smoking (former or current)	1.18	0.70 – 1.98	0.50
	Diabetes	1.13	0.74 – 1.72	0.54
	Plaque			
	• 1	3.70	1.78 – 7.67	0.00
	• 2	8.47	3.44 – 20.87	0.00
	• 3	23.78	6.97 – 81.08	0.00
	Sex (female)	0.61	0.38 – 0.98	0.04
	Income			
	• $20,000 - $60,000	0.60	0.33 – 1.07	0.08
	• > $60,000	0.49	0.22 – 1.08	0.07
	Mean BMI (kg/m^2)	1.02	0.99 – 1.05	0.15
Model C: Mean Vitamin D (nmol/L)	Mean Vitamin D nmol/L	1.00	1.00 – 1.00	0.74
	Vitamin D supplement Use	0.93	0.66 – 1.30	0.64
	Smoking (former or current)	1.18	0.70 – 1.98	0.50
	Diabetes	1.13	0.74 – 1.73	0.53
	Plaque			
	• 1	3.73	1.78 – 7.78	0.00
	• 2	8.56	3.47 – 21.15	0.00
	• 3	24.14	7.00 – 83.25	0.00
	Sex (female)	0.61	0.38 – 0.97	0.04
	Income			
	• $20,000 - $60,000	0.60	0.33 – 1.07	0.08
	• > $60,000	0.49	0.22 – 1.06	0.07
	Mean BMI (kg/m^2)	1.02	0.99 – 1.05	0.14

Table 3: Bivariate Analysis for Loss of Attachment (LOA)					
Variable	**Proportion Slight LOA**	**Proportion Moderate LOA**	**Proportion Severe LOA**	**p value**	**Unadjusted Odds Ratio (95% CI)**
[25(OH)D][a]					
• < 50 nmol/L	0.83 (0.78 - 0.87)	0.11 (0.09 - 0.13)	0.06 (0.03 - 0.10)	0.21	0.80*
• ≥ 50 nmol/L	0.82 (0.76 - 0.86)	0.13 (0.10 - 0.18)	0.05 (0.04 - 0.06)		1.24†
[25(OH)D][a]					
• < 75 nmol/L	0.82 (0.77 - 0.87)	0.12 (0.09 - 0.15)	0.06 (0.04 - 0.08)	0.26	0.83*
• ≥ 75 nmol/L	0.82 (0.75 - 0.87)	0.14 (0.10 - 0.20)	0.04 (0.03 - 0.06)		1.33†
Mean [25(OH)D][b] (nmol/L)	86.9 (76.4 - 97.4)	82.9 (63.6 - 102.1)	77.1 (55.5 - 98.7)	0.55 0.18	1.00 (0.99 - 1.00) 1.00 (0.99 - 1.00)
Vitamin D supplement use[a]					
• Yes	0.74 (0.62 - 0.84)	0.19 (0.11 - 0.31)	0.07 (0.03 - 0.15)	0.10	1.79*
• No	0.84 (0.79 - 0.88)	0.12 (0.09 - 0.15)	0.04 (0.03 - 0.07)		1.75†
Multivitamin or vitamin D supplement use[a]					
• Yes	0.78 (0.70 - 0.85)	0.15 (0.10 - 0.22)	0.07 (0.04 - 0.11)	0.04	1.49*
• No	0.86 (0.82 - 0.89)	0.11 (0.08 - 0.14)	0.03 (0.02 - 0.05)		2.31†
Smoking[a]					
• Former or current	0.81 (0.74 - 0.86)	0.14 (0.10 - 0.20)	0.05 (0.04 - 0.07)	0.098	1.53*
• Never	0.86 (0.80 - 0.90)	0.10 (0.07 - 0.14)	0.04 (0.03 - 0.08)		1.23†
Mean pack years of smoking[b]	12.4 (11.1 - 13.7)	17.3 (11.4 - 23.3)	14.3 (6.4 - 22.2)	0.09 0.61	1.01 (1.00 - 1.03) 1.01 (0.98 - 1.04)
Diabetes[a]					
• Yes	0.78 (0.66 - 0.86)	0.13 (0.08 - 0.22)	0.09 (0.05 - 0.17)	0.10	1.10*
• No	0.82 (0.77 - 0.86)	0.13 (0.10 - 0.17)	0.05 (0.04 - 0.07)		1.97†
Mean glycosylated hemoglobin[b] (%)	5.6 (5.5 - 5.7)	5.7 (5.6 - 5.8)	6.0 (5.7 - 6.2)	0.005 0.007	Unable to calculate
Glycosylated hemoglobin[a]					
• ≤ 7%	0.83 (0.77 - 0.87)	0.12 (0.09 - 0.16)	0.05 (0.04 - 0.07)	0.0044	1.33* (0.77 - 2.31)
• > 7%	0.73 (0.60 - 0.83)	0.15 (0.09 - 0.24)	0.13 (0.07 - 0.23)		2.93† (1.36 - 6.33)
Mean body mass index[b] (kg/m^2)	26.4 (25.9 - 26.9)	26.5 (25.8 -27.2)	26.6 (25.0 - 28.1)	0.75 0.86	1.00 (0.99 - 1.02) 1.00 (0.95 - 1.06)
Body mass index[a]					
• < 25 kg/m^2	0.82 (0.75 – 0.88)	0.12 (0.09 – 0.16)	0.05 (0.03 – 0.09)	0.65	1.13 (0.87 – 1.49)*α
• 25 - < 30 kg/m^2	0.82 (0.76 – 0.86)	0.14 (0.10 – 0.19)	0.04 (0.03 – 0.06)		1.05 (0.80 – 1.39)*β
• ≥ 30 kg/m^2	0.81 (0.76 – 0.86)	0.13 (0.10 – 0.16)	0.06 (0.04 – 0.09)		0.82 (0.40 – 1.71)†α 1.13 (0.52 – 2.44)†β
Visits dental professional once/yr. or more[a]					
• Yes	0.81 (0.76 - 0.86)	0.13 (0.10 - 0.17)	0.06 (0.04 - 0.08)	0.20	1.08*
• No	0.84 (0.79 - 0.88)	0.12 (0.09 - 0.17)	0.04 (0.03 - 0.06)		1.49†
Brushes twice/d or more[a]					
• Yes	0.82 (0.76 - 0.86)	0.14 (0.10 - 0.18)	0.05 (0.03 - 0.07)	0.080	1.32*
• No	0.84 (0.79 - 0.87)	0.11 (0.08 - 0.13)	0.06 (0.04 - 0.09)		0.84†
Flosses once/d or more[a]					

• Yes • No	0.81 (0.76 - 0.86) 0.84 (0.79 - 0.88)	0.14 (0.11 - 0.18) 0.11 (0.08 - 0.16)	0.05 (0.03 - 0.07) 0.06 (0.04 - 0.08)	0.29	1.29* 0.90†
Plaque • 0 • 1 • 2 • 3	 0.82 (0.73 - 0.89) 0.82 (0.76 - 0.88) 0.77 (0.72 - 0.81) 0.74 (0.68 - 0.79)	 0.13 (0.08 - 0.20) 0.13 (0.08 - 0.19) 0.17 (0.15 - 0.21) 0.15 (0.10 - 0.22)	 0.05 (0.02 - 0.12) 0.05 (0.03 - 0.07) 0.06 (0.03 - 0.11) 0.11 (0.07 - 0.18)	0.18	0.99 (0.53 - 1.84)*[1] 1.46 (0.82 - 2.62)*[2] 1.29 (0.54 - 3.09)*[3] 1.04 (0.25 - 4.26)†[1] 1.26 (0.28 - 5.70)†[2] 2.60 (0.60 - 11.17)†[3]
Mean age[b] (yrs.)	43.7 (43.1 - 44.3)	53.2 (50.6 - 55.7)	54.4 (49.9 - 58.9)	P < 0.001	1.04 (1.03 - 1.06) 1.05 (1.02 - 1.07)
Sex[a] • Male • Female	 0.81 (0.76 - 0.85) 0.84 (0.78 - 0.88)	 0.14 (0.11 - 0.17) 0.12 (0.09 - 0.17)	 0.06 (0.04 - 0.08) 0.04 (0.03 - 0.06)	0.16	 0.86* 0.69†
Income[a] • < $20,000 • $20,000 - $60,000 • > $60,000	 0.81 (0.73 - 0.87) 0.80 (0.75 - 0.84) 0.84 (0.78 - 0.89)	 0.12 (0.08 - 0.20) 0.13 (0.11 - 0.16) 0.13 (0.09 - 0.19)	 0.06 (0.04 - 0.11) 0.07 (0.05 - 0.11) 0.03 (0.02 - 0.05)	0.04	 1.06*α 1.01*β 1.16†α 0.50†β

a - χ^2 test

b – General linear model

* - LOA = 4 – 5 mm versus LOA ≤ 3 mm

† - LOA > 5 mm versus LOA ≤ 3 mm

α - Income = $20,000 - $60,000 versus Income < $20,000 or BMI 25 - < 30 kg/m^2

β - Income = > $60,000 versus Income < $20,000 or BMI ≥ 30 kg/m^2

Slight LOA - LOA ≤ 3 mm

Moderate LOA - LOA = 4 – 5 mm

Severe LOA – LOA > 5 mm

Table 4: Multiple Logistic Regression for Loss of Attachment							
Independent Variables		Loss of Attachment					
		Moderate versus Slight			Severe versus Slight		
		Relative Risk Ratios	95% CI	p Value	Relative Risk Ratios	95% CI	p value
Model A: Vitamin D <50 nmol/L	Vitamin D < 50 nmol/L	0.65	0.33 – 1.29	0.20	1.24	0.56 – 2.72	0.56
	Age	1.06	1.04 – 1.08	0.000	1.04	0.99 – 1.08	0.11
	Mean glycosylated hemoglobin (%)	0.0002	$5.41e^{-21}$ – 5.35^{e12}	0.63	4.25^{e11}	2.05^{e-20} – 8.78^{e42}	0.43
	Multivitamin or vitamin D supplement use	1.11	0.65 – 1.92	0.68	2.34	0.84 – 6.51	0.095
	Smoking (former or current)	1.79	1.17 – 2.74	0.01	1.60	0.59 – 4.29	0.32
	Brushes twice/d or more	1.49	0.84 – 2.64	0.15	0.79	0.25 – 2.51	0.66
	Income • $20,000 - $60,000 • > $60,000	0.98 0.69	0.62 – 1.56 0.41 – 1.16	0.94 0.15	1.64 0.67	0.72 – 3.73 0.27 – 1.69	0.21 0.37
Model B: Vitamin D <75 nmol/L	Vitamin D < 75 nmol/L	0.91	0.63 – 1.31	0.58	2.09	1.02 – 4.30	0.05
	Age	1.06	1.04 – 1.08	0.000	1.04	0.99 – 1.09	0.09
	Mean glycosylated hemoglobin (%)	0.0001	4.18^{e-21} – 2.36^{e12}	0.60	4.44^{e10}	1.18^{e-20} – 1.67^{e41}	0.46
	Multivitamin or vitamin D supplement use	1.13	0.67 – 1.91	0.62	2.52	0.92 – 6.92	0.07
	Smoking (former or current)	1.76	1.16 – 2.67	0.01	1.59	0.59 – 4.31	0.33
	Brushes twice/d or more	1.50	0.85 – 2.64	0.14	0.80	0.25 – 2.59	0.69
	Income • $20,000 - $60,000 • > $60,000	1.01 0.74	0.64 – 1.58 0.46 – 1.19	0.97 0.19	1.70 0.71	0.78 – 3.68 0.30 – 1.67	0.16 0.39
Model C: Mean Vitamin D (nmol/L)	Mean Vitamin D nmol/L	1.00	1.00 – 1.00	0.67	1.00	0.98 – 1.02	0.76
	Age	1.06	1.04 – 1.08	0.000	1.04	0.99 – 1.08	0.11
	Glycosylated hemoglobin (%)	0.0001	4.55^{e-21} – 2.01^{e12}	0.60	3.03^{e11}	1.24^{e-20} – 7.39^{e42}	0.44
	Multivitamin or vitamin D supplement use	1.14	0.66 – 1.97	0.60	2.33	0.86 – 6.28	0.09
	Smoking (former or current)	1.75	1.16 – 2.65	0.01	1.61	0.60 – 4.30	0.31
	Brushes twice/d or more	1.50	0.85 – 2.65	0.14	0.79	0.25 – 2.50	0.66
	Income • $20,000 - $60,000 • > $60,000	1.02 0.75	0.65 – 1.58 0.48 – 1.18	0.94 0.19	1.62 0.66	0.74 – 3.51 0.27 – 1.62	0.20 0.33

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