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Vitamin D and periodontitis; is there an association between vitamin D deficiency and periodontitis severity? A cross-sectional study

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Abbreviations

BOP – Bleeding on probing

BW – Bite wing x-rays

CAL – Clinical attachment level

CEJ – Cemento-enamel junction

CEO – Chief executive officer

CI – Confidence interval

D_2 – Ergocalciferol

D_3 – Cholecalciferol

LCMS-MS – Liquid chromatography tandem mass-spectrometry

mm – Millimeters

µg - Micrograms

nmol/L – Nanomole per liter

NOK – Norwegian krone(r)

NSD – The Norwegian Centre for Research Data

NSSDS – The Norwegian Social Science Data Services

OPG – Orthopantomogram

OR – Odds ratio

PPD – Probing pocket depth

REK – The Regional Committees for Medical and Health Research Ethics

Tromsø 7 – The seventh survey of the Tromsø study (2015 – 2016)

UV – Ultraviolet (light)

VDR – Vitamin D receptor

Q1 – Questionnaire 1

Q2 – Questionnaire 2

Abstract

Background: Periodontitis is an oral disease which is highly prevalent in the adult population worldwide. Periodontitis is characterized by an excessive inflammatory reaction which leads to the destruction of supportive periodontal tissues and potentially tooth loss in the more severe stages of disease. Known risk factors for periodontitis include conditions and lifestyle factors which increase the person's overall inflammatory risk.

Vitamin D has recently been found to play an important role in the immune system and in regulating inflammatory processes. Over the recent decades, there has been an increase in studies investigating the potential influence of vitamin D status on inflammatory diseases and conditions. However, there is still little research to be found on the relationship between vitamin D and periodontitis.

The aim of this study was to examine the associations between levels of serum 25(OH)D and periodontitis stage and periodontal pocket depth in Norwegian adults.

Methods: The study design was cross-sectional. The data used was obtained from the population-based Tromsø 7 study conducted in 2015 – 2016. From the original Tromsø 7 study sample, only those who had both a valid periodontal diagnosis and data on serum 25(OH)D levels were included in the present study, giving a total study sample of 3 693 participants.

Using descriptive statistics and bivariate logistic regression analysis, associations were tested. Periodontitis stage and number of periodontal pockets of ≥ 5 mm were used as dependent variables for their respective separate analyses, and serum 25(OH)D was used as the independent variable for both. An additional analysis of vitamin D and periodontitis stage was performed in which participants were split into two groups according to whether blood samples had been collected between March and September or between October and February. All results were adjusted for selected covariates included in the analyses.

Results: Most participants had periodontitis (89.2%) and 19.8% were classified as stage III-IV. 3.8% of the participants were at deficient levels of serum 25(OH)D. The adjusted regression analyses showed that persons with periodontitis stage II-III/IV and persons with periodontal pockets of ≥ 5 mm had higher odds of being vitamin D deficient when adjusting

for age, sex, smoking, oral hygiene, and socioeconomic factors. The month when blood samples had been collected was a significant predictor for serum 25(OH)D levels in this sample.

Conclusion: The study results suggest that vitamin D status was associated with both periodontitis stage and probing pocket depth. In order to prove causality or direction of the relationship, more prospective research is needed. Nevertheless, these findings would indicate that periodontitis patients might benefit from striving to uphold an intake of vitamin D at the recommended amounts in order to maintain sufficient vitamin D levels throughout the year, even during seasons of restricted sun exposure.

Keywords: Periodontitis, Periodontitis stage, Periodontal disease, Inflammation, Vitamin D, 25(OH)D, Epidemiology, The Tromsø study, Tromsø 7, Northern Norway

1 Background

1.1 The periodontium

The periodontium (peri = around, odontos = tooth) is the attachment apparatus of the teeth and comprises the different structures and tissues that surround and support each tooth. The periodontium consists of four tissues (Figure 1). The (1.) *alveolar bone* is the ridge of jawbone that holds the tooth sockets. The (2.) *root cementum* is a thin layer of mineralized tissue which covers the root of the tooth and functions as an anchor for the (3.) *periodontal ligament*, a fibrous collagenous tissue which connects the root cementum to the alveolar bone. The (4.) *gingival epithelium*, also referred to as gingiva or gums, is the soft outermost and protective layer of the attachment apparatus, and the only visible part of the periodontium (1-4).

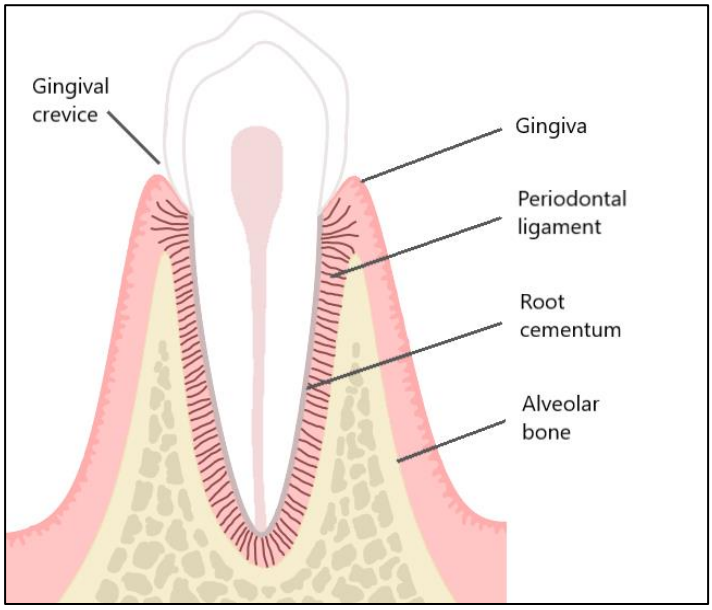

Figure 1, The periodontium. Figure illustrated by author February 2022 based on illustrations from Luis et al. and periodontal-health.com (4, 5).

In a healthy periodontium, the gingival tissue firmly surrounds the teeth, forming only a shallow gingival crevice of 1-3 mm in depth around the tooth (Figure 1). On gentle probing there should be absence of bleeding from the crevice (1, 2, 4).

1.1.1 Periodontal inflammation

There are many forms of bacteria naturally present in the mouth which cause a mild but constant inflammation in the softer periodontal tissues. To prevent bacteria from invading the body through the gingival crevice, white blood cells (leukocytes) are consistently transported to the connective tissues and gingiva by blood vessels throughout the periodontium. In active inflammation, the number of leukocytes is drastically increased, but even in a clinically healthy periodontium without visible signs of inflammation, small amounts of bacterial buildup is enough to trigger an initial inflammatory process. The cardinal signs of inflammation are swelling, redness, pain, and heat in and/or around the afflicted area (1, 2, 4).

The inflammatory reaction is a necessary and important part of the body's defense against microbial invasion or other harmful factors. Cells in the adjacent tissues will detect signs of such potentially harmful factors and trigger an acute inflammatory *host response*, to protect the local tissues from pathological infiltration. Leukocytes are the most important cells of the body's inner defense. There are several types of leukocytes, including lymphocytes, granulocytes, monocytes, and their subtypes, which regulate the inflammatory process and eliminate pathogens through each of their specific functions. Some leukocytes also produce cytokines, which play an important role in the initiation and cessation of inflammation (1, 2, 4).

Although the purpose of the host response reaction is to protect the body from pathogens, the inflammatory process can nevertheless cause some destruction to the cells of the surrounding tissues. While some kinds of bacteria, such as *polyhormonas gingivalis,* are in themselves able to release harmful substances, most of the tissue destructive substances found in periodontal inflammation are released by the body's own cells. Some leukocytes release proteases which break down proteins in the gingiva, and oxygen free radicals that cause destruction of collagen in the periodontal ligament. To protect the body's own structures, protease inhibitors and antioxidants are released simultaneously to counteract the harmful effects of inflammation (1, 2, 4).

In instances where bacterial biofilm (dental plaque) is allowed to accumulate on the tooth surface and along the gingival margin over extended periods of time, the bacterial biofilm

triggers a host response reaction of the gingival soft tissues, a condition known as gingivitis (Figure 2). The most notable signs of periodontal inflammation are redness and swelling of the gingiva, often in combination with bleeding on probing (BOP), and sometimes pain on gentle probing or flossing. In gingivitis, the destructive and rebuilding processes are balanced so that the inflammation is strong enough to keep bacteria from penetrating the deeper structures without causing irreversible damage to the surrounding tissues. Gingivitis is a reversible condition limited to the gingival margin and in most cases, thorough brushing and flossing is sufficient as treatment (1, 2, 4).

If left untreated, and most often in combination with other accelerating risk factors, gingivitis might, however, over time trigger an uncontrolled inflammatory response in the deeper-lying periodontal tissues. If the inflammatory balance is disrupted, destructive pro-inflammatory substances are released faster and in larger quantities than the body's counteractive mechanisms can handle. This leads to an increased destruction of periodontal tissues and the development of periodontitis (1-3, 6).

1.2 Periodontitis

Periodontitis is a more advanced inflammatory form of periodontal disease. Periodontitis may initially present itself visually much the same way as gingivitis, with swollen, reddened, and bleeding gums, but is distinctly characterized by the destruction of the supporting tissues around the affected teeth (Figure 2), which may ultimately lead to tooth loss. Periodontitis is an irreversible condition, but in most cases its progression can be halted and managed through adequately performed oral hygiene practices and professional periodontal treatment (1, 2, 4, 6, 7).

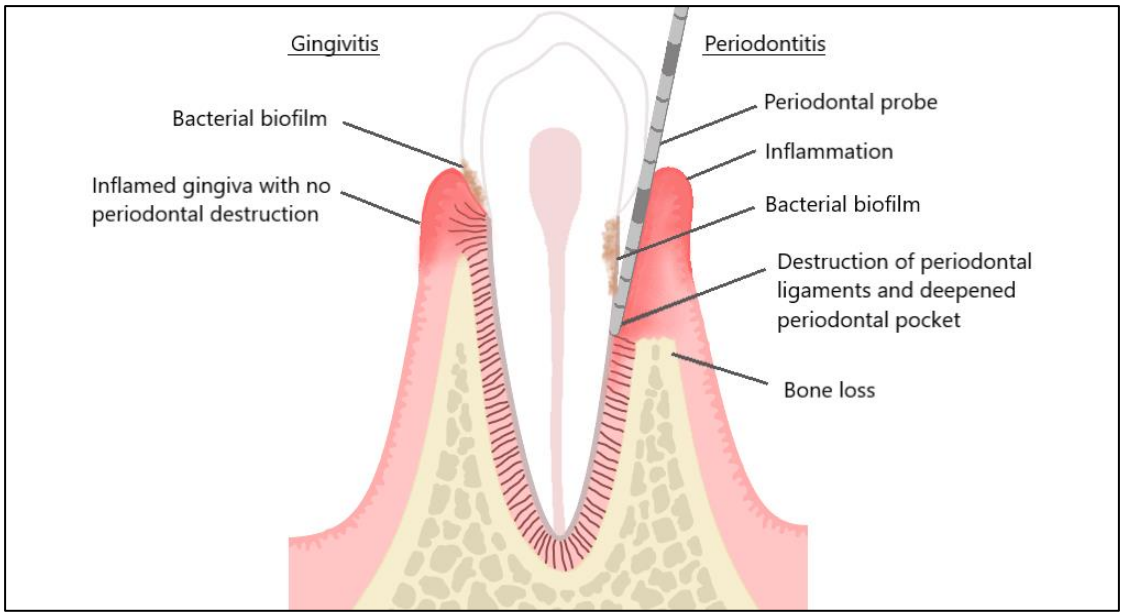

Figure 2, Gingivitis and periodontitis. Figure illustrated by author February 2022 based on illustrations from Luis et al. and periodontal-health.com (4, 5).

Researchers have found somewhat heightened concentrations of pro-inflammatory cytokines and less of anti-inflammatory cytokines in cases of more severe periodontitis. The imbalance between inflammatory- and anti-inflammatory processes could be a possible reason for the excessive inflammatory reaction seen in periodontitis and could further explain why the acute inflammatory reaction progresses into chronic inflammation and does not subside in the same way as gingivitis. Additionally, some types of cytokines such as IL-1β and TNF-α activate osteoclasts, which is a type of bone cell that breaks down bone tissue. An increased release of collagen-destructive enzymes (collagenases) is also considered typical for periodontitis (1, 4).

1.2.1 Diagnosis and classification

Periodontitis diagnosis is mainly based on the measurement of detectable periodontal breakdown at the time of examination. At present, there is no certain way of detecting periodontitis before destruction of tissue has occurred. The standardized periodontal probe is the primary diagnostic tool and can be used to measure probing pocket depth (PPD) and

clinical attachment loss (CAL) (Figure 3). PPD is measured in millimeters (mm) from the base of the crevice to the gingival margin at multiple sites for each tooth by gentle probing of the crevice. CAL is registered similarly to PPD but is measured from the crevice base to the cemento-enamel junction (CEJ). Both measurements reflect attachment loss, but in practice, PPD is generally easier to determine and more commonly used. In order to correctly detect marginal bone loss, radiographic images are needed (8-10).

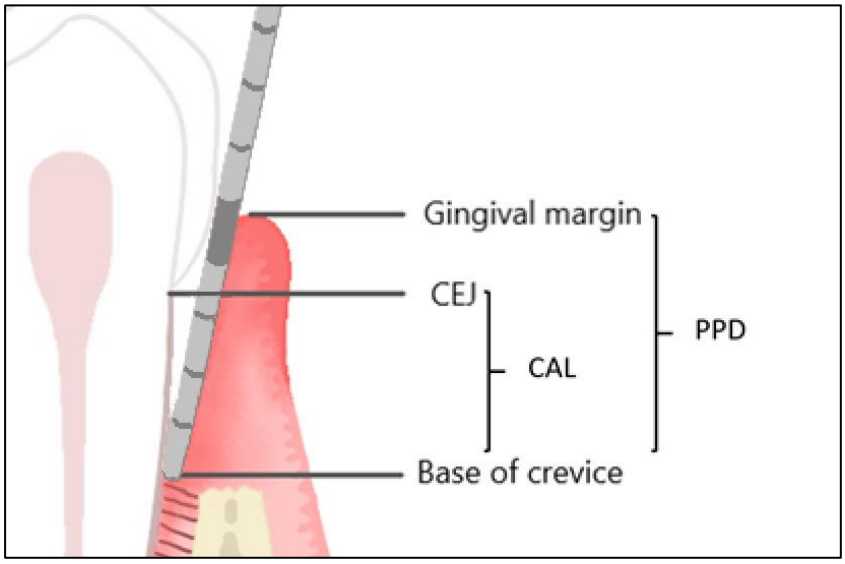

Figure 3, Clinical attachment loss and probing pocket depth. Figure illustrated by author February 2022 based on illustrations from Luis et al. and periodontal-health.com (4, 5).

The periodontitis classification systems and case definitions have differed and evolved over the years. One of the most recent systems of classification and case definition, established in 2017, assesses periodontitis severity through staging and grading. Stages I to IV of periodontitis (Table 1) describe the standard dimensions of severity, complexity, and extent of periodontitis. Stage of periodontitis is determined based on a summary of clinical measurements, primarily radiographic bone loss, CAL, PPD, and tooth loss, with added complexity factors such as furcation involvement, mobility, as well as the number and distribution of teeth affected. Grade of periodontitis is a supplemental assessment of the direct and indirect evidence of estimated future risk of progression, responsiveness to treatment and potential health impact (8, 10).

Table 1, "Periodontitis stage" (8).

Periodontitis stage		Stage I	Stage II	Stage III	Stage IV
Severity	Interdental CAL at site of greatest loss	1 to 2 mm	3 to 4 mm	≥5 mm	≥5 mm
	Radiographic bone loss	Coronal third (<15%)	Coronal third (15% to 33%)	Extending to mid-third of root and beyond	Extending to mid-third of root and beyond
	Tooth loss	No tooth loss due to periodontitis		Tooth loss due to periodontitis of ≤4 teeth	Tooth loss due to periodontitis of ≥5 teeth
Complexity	Local	Maximum probing depth ≤4 mm Mostly horizontal bone loss	Maximum probing depth ≤5 mm Mostly horizontal bone loss	In addition to stage II complexity: Probing depth ≥6 mm Vertical bone loss ≥3 mm Furcation involvement Class II or III Moderate ridge defect	In addition to stage III complexity: Need for complex rehabilitation due to: Masticatory dysfunction Secondary occlusal trauma (tooth mobility degree ≥2) Severe ridge defect Bite collapse, drifting, flaring Less than 20 remaining teeth (10 opposing pairs)
Extent and distribution	Add to stage as descriptor	For each stage, describe extent as localized (<30% of teeth involved), generalized, or molar/incisor pattern			

1.2.2 Risk factors

There is still uncertainty as to why some develop periodontitis and others do not. At present, the common theory is that an imbalance in the microflora (i.e., the accumulation of bacterial biofilm) and a susceptible host are the two major etiologic factors underlying the initiation and perhaps also progression of periodontitis (11, 12). Studies have suggested several protective factors and risk factors associated with susceptibility to developing periodontitis, both local and systemic. It is therefore crucial to identify such potential factors and their management in the care for periodontal patients. Strong risk factors include smoking and diabetes. Researchers have further indicated that periodontitis seems to be somewhat more prevalent among males, persons with lower socioeconomic status, and for those with conditions or illnesses that are immunocompromising or cause deviations in the inflammatory process (11, 13, 14). Severity of periodontitis is highly correlated with old age, as the healing capacity of cells and tissues change, and the inflammatory risk is heightened with increasing age (15, 16).

1.2.3 Prevalence in Norway

Periodontitis is highly prevalent in the adult population but varies both between countries and within populations. Severe periodontitis has been estimated to affect approximately 10% of the global population. Globally, the prevalence and incidence of periodontitis has risen over the last decades and is estimated to continue rising with the increasing population growth and aging (17-19). In the Nordic countries, there has been a decrease in the prevalence of more severe periodontitis (20-22). However, results from recent studies have shown that the burden of periodontitis remains high among adults in Norway in general, and in Northern Norway in particular (23-27).

1.3 Vitamin D

Vitamin D is an essential micronutrient primarily known for its important role in calcium absorption and homeostasis for proper bone mineral matrix formation and skeletal health. Vitamin D functions as a promotive factor for both bone formation and bone regeneration and is an important element in the prevention and treatment of disease-related bone loss (28, 29).

The two major forms of vitamin D are *cholecalciferol* (D_3) and *ergocalciferol* (D_2). Vitamin D is primarily obtained from exposure to ultraviolet (UV) B radiation from sunlight (in the form of D_3), and additionally through nutritional supplements or in small quantities in the diet (as D_2 and D_3). However, only a few foods are good sources to vitamin D. Food items containing D_3 include fatty fish or fish products rich in fish fats, along with fortified dairy products and margarine. In addition, some mushrooms grown in UV-light, such as chantarelles, contain some vitamin D_2 (30).

In the liver, vitamin D is metabolized into serum 25-hydroxyvitamin-D (25(OH)D), and then hydroxylated into serum 1,25-hydroxyvitamin-D (1,25(OH)D) in the kidneys. The 1,25(OH)D is the active vitamin D metabolite; however, serum 25(OH)D is the acknowledged biomarker for the body's vitamin D stores. Serum 25(OH)D can be measured in blood samples in order to determine a person's vitamin D status (28, 30-32).

1.3.1 Thresholds and recommendations

The recommended intake of supplemental vitamin D for adults is 10 µg/day. Generally, people are considered at risk of vitamin D deficiency at serum 25(OH)D concentrations less than 30 nmol/L, and potentially at risk of vitamin D inadequacy at 30 to 50 nmol/L (Table 2). A concentration of 25(OH)D at more than 50 nmol/L has been suggested as optimal (30, 33-35).

Table 2, Serum 25(OH)D thresholds.

25(OH)D (nmol/L)	**Status description**
< 30	Vitamin D deficiency
30–50	Insufficient vitamin D status
> 50	Sufficient vitamin D status

In the last two decades, prevalence rates of vitamin D deficiency (< 30 nmol/L) in Europe has been estimated as 13%, and 40% for vitamin D insufficiency (< 50 nmol/L) (33). Deficiency can be developed due to dietary intake lower than the recommended levels, limited sun exposure, inadequate function of the liver or kidneys, lowered ability for absorption, or lacking functional vitamin D receptors (VDR). Individuals with dark skin, and persons with limited sun exposure due to e.g., working indoors during sun hours or frequently wearing long robes and veils, are among the known risk groups. Nevertheless, vitamin D deficiency can be found in any part of the general population (30, 33, 36-39).

Vitamin D status is often found to vary between different age groups within the population. While many sources have reported higher risk of deficiency in elderly adults due to both changes in the skin's ability to synthesize vitamin D (40) or lifestyle factors, other studies have instead found that deficiency was instead more prevalent among younger adults (38, 39).

1.3.2 Vitamin D intake and sunlight exposure in Norway

Results from the Norwegian population survey NORKOST 3, conducted between 2010 and 2011, showed that the average daily intake of vitamin D among Norwegian adults was about two thirds of the recommended amount, although with higher intake in the older age groups (41). Even though other studies have shown that the Norwegian population in general has had more vitamin D sufficiency compared to the other Nordic countries, there are still relatively large population groups in which 25(OH)D levels are insufficient. There has also been reports of a general drop in 25(OH)D concentrations during winter months (42-46).

The dermal (skin) vitamin D synthesis is estimated to produce approximately 25 µg of vitamin D per day within only one minute of whole-body UV-exposure and optimal sunlight conditions (47). During summer months at latitudes around 60° N, exposing the face, arms, and hands to direct sunlight is estimated to provide approximately 5-10 µg of D_3 in light-skinned persons after about 6-8 minutes a day two or three times a week, and after about 10-15 minutes per day for persons with darker skin (30).

Being located above the arctic circle at 69° N, the city of Tromsø experiences extreme variations in duration of daylight and sun exposure between the different seasons. Between mid-May and mid-July, the midnight sun season exhibits endless daylight where the sun does not set below the horizon at any point during the day or night. Contrastingly, throughout the polar night season from mid-November until mid-January, the sun does not rise above the horizon at all (Figure 4). Furthermore, the mountainous terrain and average cloud cover of 78% throughout the year means that sunlight is often reduced (48-51).

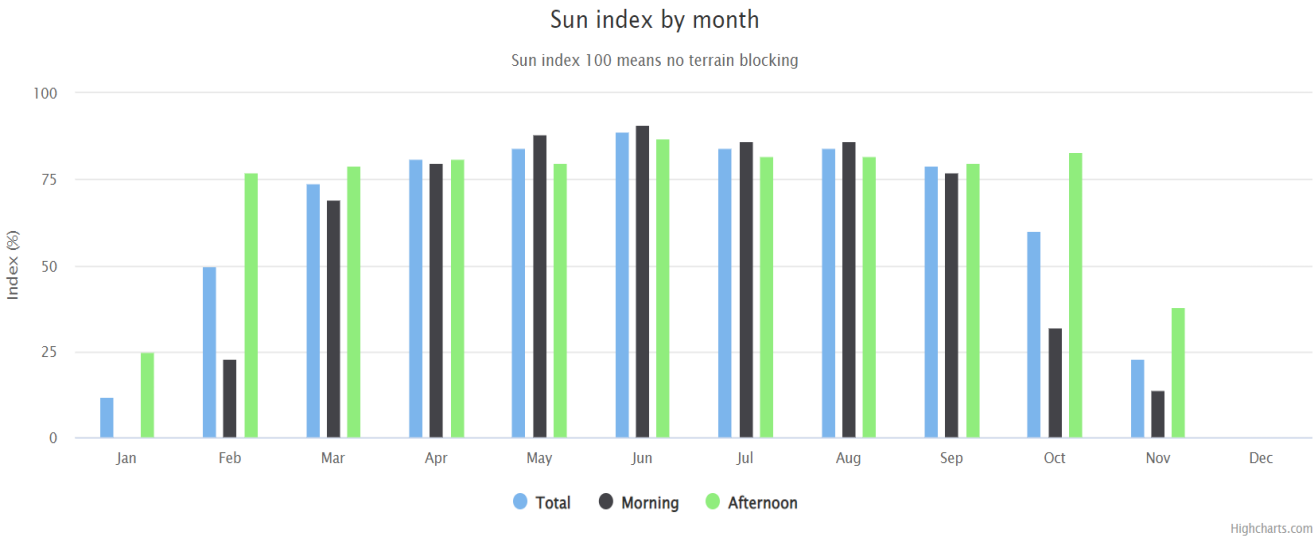

Figure 4, Monthly sun index for Tromsø (49).

1.3.3 Vitamin D as a factor in the immune system

Vitamin D deficiency has long been recognized as a public health concern in relation to its potential consequences for skeletal health. In recent decades, however, vitamin D has become a hot topic of research and discussion as its potential additional functions in the immune system is being unraveled (33, 52). Researchers have found correlations between autoimmune diseases and 25(OH)D serum levels, vitamin D intake, UV-exposure, and polymorphisms in the VDRs (52, 53). Studies have further indicated that vitamin D has an important function in the regulation of specific leukocytes and cell functions in both the innate and adaptive immune responses. Vitamin D can influence and adjust the inflammatory process by regulating the dendritic cell function and the production of inflammatory cytokines, and by inhibiting the reproduction of pro-inflammatory cells (Figure 5) (31, 32, 54-56). Additionally, VDR expression and activity has been found to affect the development, differentiation, and effector function of T-lymphocytes, which in turn play an essential role in the adaptive immune system (53, 54).

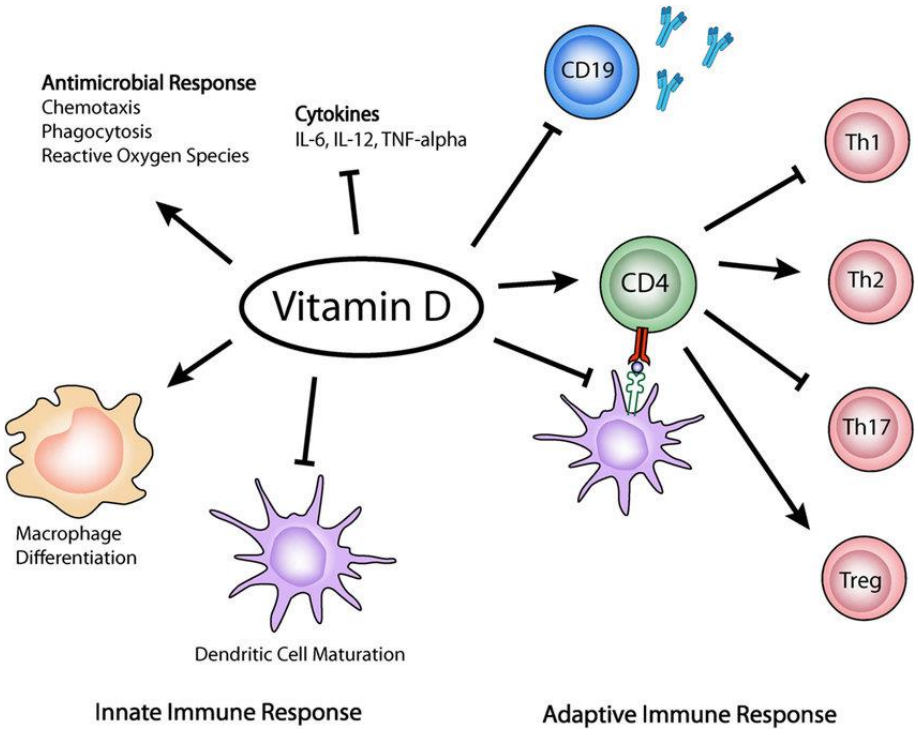

Figure 5, "Vitamin D effects on the innate and adaptive immune response". Vitamin D enhances macrophage differentiation, antimicrobial peptide transcription (Treg and Th2), and chemotaxis and phagocytosis of innate immune cells, while inhibiting pro-inflammatory cytokines, Th1 and Th17 differentiation, immunoglobulin production, and dendritic cell maturation (56).

However, there is still some uncertainty about the exact relationship of serum vitamin D levels and inflammatory diseases, and a causal relationship has not yet been established. As hypothesized by Autier et al., low vitamin D status might not be a risk factor, but rather a consequence of ill health (57). Thus, vitamin D supplementation might prove to be beneficial in decreasing both risk and unfavorable outcomes of inflammatory disease in deficient patients, but the relevance and benefits for specific diseases remain to be unearthed in future research (31-33, 52, 57).

1.4 Justification for the study

Although there has been an upsurge in studies examining vitamin D's involvement in autoimmune disease, there is still fairly little research to be found on the relationship between vitamin D and periodontitis. It is nevertheless clear that periodontitis progression is often

more rapid in patients with other simultaneous systemic inflammation or weakened immune systems (13, 58-60). It is therefore plausible that vitamin D's ability to regulate immune responses (31, 32, 52, 53, 55) might influence the individual's susceptibility to developing more severe periodontitis.

Antonoglou et al. suggested that low levels of vitamin D may affect a person's susceptibility to develop periodontitis, while Andrukhov et al. proposed that vitamin D might also play a role in the progression of periodontal disease via regulation of cytokine production (61, 62). Furthermore, it has been suggested that vitamin D supplementation in patients prone to vitamin D deficiency might improve the effects of treatment of periodontitis, reduce systemic inflammation and promote anti-microbial functions (63, 64). As an additional aspect, it is plausible that the individual's vitamin D levels would affect the destruction and regeneration of alveolar bone cells in the more severe periodontitis cases. In an experimental study on mice with induced periodontitis, Li et al. found that mice that had received vitamin D supplementation experienced less alveolar bone loss compared to mice who did not receive supplementation (65).

Periodontitis is highly prevalent in the adult Norwegian population, and an important cause of tooth loss (23-27). Studies have shown that periodontal disease has a significant negative impact on the individual's overall health and oral health related quality of life, with stronger impact at greater severity of disease (66-68). As a population living in a geographical area where sun exposure is limited for parts of the year (49), the population of Tromsø is naturally at risk of vitamin D deficiency if the intake is not compensative. Vitamin D is a relatively cheap and easily accessible supplement, and as such, examining its full potential might be highly profitable in terms of population health.

1.4.1 Aim and hypothesis

The aim of this study was to examine the relationship between levels of serum 25(OH)D and periodontitis in Norwegian adults by compiling and comparing data from the Tromsø 7 study. The study hypothesis was that individuals with more severe periodontitis would have more

vitamin D deficiency and -insufficiency compared to those with less severe- or no periodontitis.

2 Material and methods

2.1 Study design

The study design was cross-sectional. The data used was pre-collected from an adult Norwegian population participating in the seventh survey of the population-based Tromsø Study, which was conducted in Tromsø, northern Norway between March of 2015 and November of 2016.

2.2 The Tromsø study

The Tromsø study is an ongoing population-based cohort study comprising seven surveys, the first completed in 1974 and the seventh and most recent (Tromsø 7) in 2015 – 2016. The study is considered to be Norway's longest-lasting, most participated health-study, and consists of a comprehensive collection of health data from questionnaires and measurements, biological samples, and clinical surveys. All surveys of the Tromsø study were conducted in the Tromsø municipality in Northern Norway (69-72).

2.2.1 The seventh survey of the Tromsø Study

In Tromsø 7, all inhabitants of Tromsø municipality from the age of 40 years and up were invited to participate. Of the total 32 591 invited, 21 083 persons (64%) aged 40-99 years from both urban and rural areas agreed to attend the survey. 52,5% of the participants were female and 47,5% male (69, 70, 72). The study population was representative of the general 2016 Tromsø population in distribution of sex and age (72, 73). The study was conducted

through a total of two main sets of questionnaires (Q1 and Q2), one additional food frequency questionnaire, one main on-site visit and a second on-site visit for some (69-72).

2.2.2 Data collection

All participants were invited to the first on-site visit, during which a basic health examination and biological sampling were conducted. Levels of serum 25(OH)D (nmol/L) were measured through blood samples in 20 922 individuals. At every hour, the first two patients to attend the first health examination were invited to further participate in an oral examination, to which only three refused. The clinical dental examination included a total of 3 943 persons (69-72).

The dental examination was performed by specially trained dental hygienists. The periodontal measurements included PPD measured to the closest millimeter using a periodontal probe with single-millimeter graduations (WHO-probe LM555B) at four sites per tooth for all teeth, and registration of BOP. An orthopantomogram was used to assess radiographic marginal bone loss (72). Bone loss of interproximal surfaces of all teeth, excluding third molars, was measured linearly with a transparent plastic ruler on the orthopantomogram as described by Holde et al. (24).

Blood sampling was performed by specially trained research technicians. All collected blood samples were analyzed within 24 hours at the UNN laboratory in Tromsø and serum samples were processed after 30–60 minutes in room temperature (72). Serum 25(OH)D levels were measured using liquid chromatography tandem mass-spectrometry (LCMS-MS), which measured both the separate and the collective sum of serum 25(OH)D_2 and serum 25(OH)D_3 in each blood sample. In a liquid-liquid extraction using methanol and isopropanol, D_2 and D_3 were separated and measured quantitatively.

The Q1 was sent to each invitee in paper form along with the invitation to participate in the study. The invitation also included a username and password for accessing the Q1 and Q2 and

in electronic form. Questions covered socio-demographics, mental and somatic health, lifestyle, and wellbeing. Both questionnaires had high submission and completion (69-72).

2.2.3 Inclusion and exclusion criteria

The present study sample consisted of participants from Tromsø 7 aged between 40-94 years old who had attended both the first health examination and the oral examination. Included participants had to have both a valid periodontal diagnosis and registered data on 25(OH)D-levels obtained from blood samples (Figure 6). Participants with registered vitamin D levels but no registered periodontal diagnosis, and participants with registered periodontal diagnosis but no registered vitamin D levels, were excluded from this study.

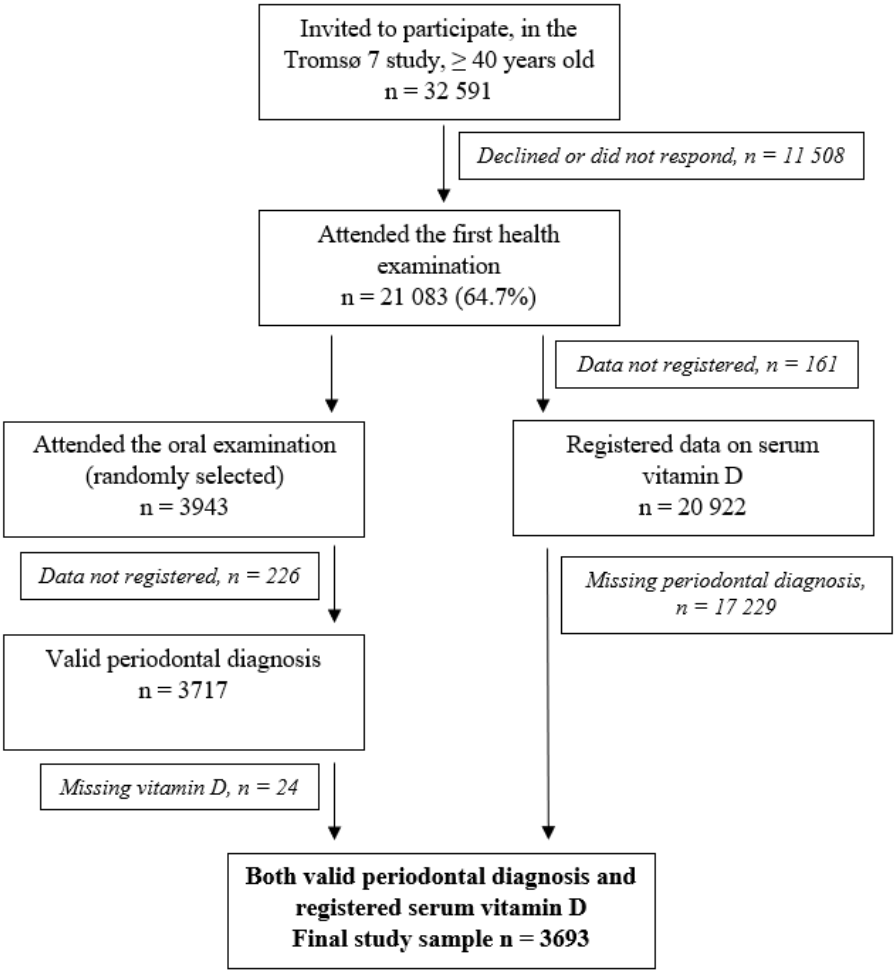

Figure 6, flowchart of included participants.

2.3 Description of variables

Table 3 describes the variables included in the analyses, how they were measured or determined in the Tromsø 7 data collection process, and their original categories. The variables serum 25(OH)D and number of pockets ≥ 4 mm and ≥ 5 mm were continuous.

Table 3, Description of variables included in the analyses and their original categories.

Variable	**Measured as**	**Original categories**
Periodontal diagnosis	Diagnosis as stages (8), determined based on the collective findings from the oral examination, including radiographic images and PPD.	Periodontally healthy Gingivitis case Periodontal stability Periodontitis stage I Periodontitis stage II Periodontitis stage III-IV
Serum 25(OH)D	Nmol/L as the collective quantitative sum of vitamin D_2 and D_3 measured through blood samples.	-
Number of pockets ≥ 4mm	Total number of teeth with periodontal pockets of 4 mm or more.	-
Number of pockets ≥ 5mm	Total number of teeth with periodontal pockets of 5 mm or more.	-
Sex	Reported through questionnaire.	Female Male
Age	Ages as per 31.12.2015, divided into 10-year age groups.	40 – 49 50 – 59 60 – 69 70 – 79 80+

Date of attendance	Date of attending the first health examination and blood sampling (as date/month/year).	-
Smoking status	Reported through questionnaire. .("*Do you/did you smoke daily?*")	Current (daily) smoker Previous (daily) smoker Never smoked
Oral hygiene	Frequency of tooth brushing, self-reported. ("*How often do you usually brush your teeth?*")	Once a week or less often A couple of times every week Once a day Twice a day or more often
Education	Highest completed level of education, self-reported. ("*What is the highest levels of education you have completed?*")	Primary education 1-10 years Upper secondary education College or university < 4 years College or university ≥ 4 years
Income	Total household (taxable) income as of the previous year in Norwegian kroner (NOK), self-reported. ("*What was the households total taxable income last year? Include income from work, social benefits and similar*")	Less than 150 000 150 000 – 250 000 251 000 – 350 000 351 000 – 450 000 451 000 – 550 000 551 000 – 750 000 751 000 – 1 000 000 More than 1 000 000

2.3.1 Variables for analysis

The periodontal diagnosis variable originally comprised 6 categories; periodontal health, gingivitis case, periodontal stability, periodontitis stage I, periodontitis stage II, and periodontitis stage III-IV. In the preparation for the analysis, periodontal health and gingivitis case were merged into one group labelled "non-periodontitis cases". The periodontal stability group, although having no periodontal pockets of 4 mm or more at the time of examination, included persons in periodontal disease remission and were therefore not categorized as non-

periodontitis cases in this study, but were instead merged with the periodontitis stage I group. Serum 25(OH)D measurements were split into three groups of < 30 nmol/L, 30–50 nmol/L and > 50 nmol/L, in accordance with the suggested thresholds for deficiency, insufficiency and sufficiency (Table 2) (30).

The two included PPD variables were originally measured as total number of periodontal pockets of 4 mm or more, and total number of periodontal pockets of 5 mm or more. These measurements were analyzed as quartiles and fit into categories of 0, 1-2, and 3 or more, and further into a second variable of yes (at least one) and no (none), for both variables separately. Participants were sorted into 10-year age groups of 40-49, 50-59, 60-69, and 70+. As there were few participants over the age of 80 (3,1%), all participants aged 70 and up were grouped together.

Date of attendance refers to the date when blood samples were taken. Attendance dates were split into two groups; sunnier months from March 1st to September 30th, and less sunny months from October 1st to February 29th based on the midnight sun and polar night seasons and average monthly sun exposure as exhibited in Figure 4 (49). For the oral hygiene variable, all groups that had reported brushing their teeth less than twice a day were merged into one group of "once a day or less", indicating insufficient oral hygiene in comparison to the "twice a day or more" group (74), which was also the largest. For simplicity, the two college/university level education groups in the education variable were merged into one category labelled "Tertiary" (education). The household income variable categories were collapsed into four income groups with split points at 350 000 NOK, 551 000 NOK, and 751 000 NOK.

2.4 Statistical analyses

2.4.1 Descriptive statistics

Descriptive statistics were used to describe the frequencies and proportions of each variable within the respective periodontal groups or vitamin D status groups. Higher presence of deep

PPD indicate a higher inflammatory activity and was therefore examined against vitamin D status. Associations were checked for each variable using Pearson's chi-square test.

2.4.2 Regression analysis of vitamin D status across periodontitis stages

All statistical analyses was done using the statistical program IBM SPSS software version 28. To examine the associations between levels of vitamin D and periodontitis severity, three sets of binary multiple logistic regression analyses were conducted. Statistical significance was examined at the 5% level.

First, periodontitis classification was used as dependent variable in the analysis. In order to perform a binary analysis, the periodontitis diagnosis variable was dichotomized into two groups by merging periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I in one group, versus periodontitis stages II-IV in the other. The association between periodontitis and vitamin D status was tested through binary logistic regression analyses. Odds ratio (OR), confidence intervals (CI 95%) and *p*-values were extracted from all regression analyses. Wald statistic was also examined for each variable in order to assess and compare their individual importance for the model, although this was only presented as an addition to some of the *p*-values.

The independent variables in the analysis were vitamin D status, sex, 10-year age groups, smoking status, tooth brushing frequency, education, and income. All independent variables were categorical. Reference categories were chosen as those that were presumed to be of least risk of more severe periodontitis, according to the study hypothesis and previously stated risk factors (11, 13, 14), so as to obtain a positive OR and simpler interpretation of the output. Missing cases for smoking, tooth brushing, education, and income (n = 254) were excluded in the respective analyses. Assumptions for the regression analyses were checked by investigating correlations and collinearity for all variables included in the models.

2.4.3 Subgroup analysis of participants attending in October-February

In the second analysis, the dataset was split according to the attendance seasons (March-September and October-February), and the regression analysis was performed separately for the two groups. This complementary analysis was done in order to examine whether the seasonal sun exposure might influence the participants' measured vitamin D status and its effects. The dependent and independent variables were otherwise introduced in the same way as in the main analysis. Missing cases for smoking, tooth brushing, education, and income (n = 80) were excluded in the analysis.

2.4.4 Regression analysis of vitamin D status association with presence of periodontal pockets

The third binary logistic regression analysis was performed using the number of periodontal pockets of 5 mm or more-variable as dependent. Although pocket depth measurement is included in the periodontal diagnosis, the additional analysis was performed in order to further examine the effect of vitamin D status on periodontal inflammation. Here, the PPD variable was dichotomized as; at least one periodontal pocket of 5 mm or more, versus none. Vitamin D status, sex, age groups, and smoking were used as independent variables. The PPD ≥ 5 mm or more-variable was used in the regression analysis in order to distinguish between the more severe cases of periodontitis (stages II-III/IV) and those who had PPD 4 mm or less (stage I, periodontal stability, and non-periodontitis cases) (Table 1). Missing cases for smoking (n = 70) were excluded in the analysis.

2.5 Ethical perspectives and data safety

All data collection in Tromsø 7 was performed in accordance with the guidelines from The Norwegian Social Science Data Services (NSSDS) and The Regional committee for medical and health research ethics (REK). Written consent of participation and consent to data being used for medical research was retrieved from all participants before any data collection.

Dental examinations were free of charge and all patients were informed about their own treatment needs related to findings and were given further recommendations about dental health care where needed. None of the variables included in the present study included information that could be used to identify any specific participant. By incorporating only few measurements and by using the 10-year age group variable instead of precise ages, the dataset was regarded as being anonymous, and the hypothetic possibility of backwards identification of a participant through the combining of variables was considered very low.

Thus, as all data variables used for this master's thesis were considered anonymous at baseline, and included only few and grouped variables, special REK-approval was not required and the project was not required to be presented to the The Norwegian Centre for Research Data (NSD), as confirmed by the CEO (daglig leder) of The Tromsø Study. The dataset was exclusively stored as a password-protected file on a two-step verification protected cloud service on a personal password-protected computer and was handled solely by the author. Immediately after the completion of this study, the dataset in its entirety will be deleted from all personal storage.

3 Results

3.1 Study sample characteristics

As displayed in Table 4, the study sample of 3 693 participants consisted of 51.4% females, with a mean age of 53 years. 10.8% of the participants had no periodontitis, the majority of which were in the 40-49 age group (61.2%). Most participants had periodontitis (89.2%) and 19.8% were classified as stage III-IV. Most participants had sufficient levels of serum 25(OH)D (73.7%) whereas 3.8% were at deficient levels. The majority (67.6%) of the blood samples were collected during the period of March to September, when the population is most exposed to sunlight. The variables "Serum 25(OH)D" and "Month of attendance" did not show a statistically significant association with periodontitis in the descriptive analyses (p =

0,537 and $p = 0.692$, respectively). All other variables were statistically significant at the 1% level ($p < 0.01$).

There was a statistically significant difference in periodontitis stage distribution between the different groups of the categorical variables. Periodontitis stage III-IV seemed to be most prevalent among males, in the older age groups, among those who brushed their teeth once a day or less often, and among current- and previous smokers. There also seemed to be more non-periodontitis cases in the youngest age group, among those who brushed their teeth twice a day or more often, those with higher education and those with higher income (Table 4).

Table 4, Characteristics of the study sample by periodontal diagnosis.

		Non-periodontitis case[a]	**Periodontal stability or periodontitis stage I**	**Periodontitis stage II**	**Periodontitis stage III-IV**	
		n (%)	**n (%)**	**n (%)**	**n (%)**	
Variables	**Total n (%)**	397 (10.8)	1174 (31.8)	1389 (37.6)	733 (19.8)	***p*-value**
Serum 25(OH)D						
<30 nmol/L	139 (3.8)	20 (5.0)	38 (3.2)	52 (3.7)	29 (4.0)	0.537
30-50 nmol/L	832 (22.5)	100 (25.2)	264 (22.5)	307 (22.1)	161 (22.0)	
>50 nmol/L	2722 (73.7)	277 (69.8)	872 (74.3)	1030 (74.2)	543 (74.1)	
Sex						
Female	1898 (51.4)	222 (55.9)	685 (58.3)	672 (48.4)	319 (43.5)	<0.001
Male	1795 (48.6)	175 (44.1)	489 (41.7)	717 (51.6)	414 (56.5)	
Age group						
40-49	1059 (28.7)	243 (61.2)	415 (35.3)	313 (22.5)	88 (12.0)	<0.001
50-59	1062 (28.8)	98 (24.7)	371 (31.6)	403 (29.0)	190 (25.9)	
60-69	979 (26.5)	29 (7.3)	249 (21.2)	430 (31.0)	271 (37.0)	
70+	593 (16.0)	27 (6.8)	139 (11.8)	243 (17.5)	184 (25.1)	

Month of attendance[b]						
March-September	2498 (67.6)	270 (68.0)	788 (67.1)	954 (68.7)	486 (66.3)	0.692
October-February	1195 (32.4)	127 (32.0)	386 (32.9)	435 (31.3)	247 (33.7)	
Tooth brushing frequency						
Once a day or less	726 (20.0)	73 (18.6)	183 (15.8)	286 (21.0)	184 (25.9)	<0.001
Twice a day or more	2898 (80.0)	320 (81.4)	972 (84.2)	1079 (79.0)	527 (74.1)	
Smoking status						
Current	478 (13.2)	23 (5.9)	107 (9.3)	175 (12.8)	173 (24.3)	<0.001
Previous	1587 (43.8)	119 (30.4)	423 (36.7)	671 (49.2)	374 (52.5)	
Never	1558 (43.0)	250 (63.8)	623 (54.0)	519 (38.0)	166 (23.3)	
Education[c]						
Primary	849 (23.4)	51 (13.0)	226 (19.6)	340 (25.0)	232 (32.1)	<0.001
Secondary	1085 (29.9)	104 (26.6)	327 (28.3)	408 (30.0)	246 (34.1)	
Tertiary	1696 (46.7)	236 (60.4)	602 (52.1)	614 (45.1)	244 (33.8)	
Total household income[d]						
<351 000	439 (12.4)	33 (8.6)	117 (10.4)	173 (13.0)	116 (16.5)	<0.001
351 000–551 000	764 (21.6)	51 (13.4)	211 (18.7)	317 (23.8)	185 (26.3)	
551 000–1000 000	1457 (41.1)	159 (41.6)	451 (40.1)	541 (40.6)	306 (43.5)	
>1000 000	885 (25.0)	139 (36.4)	347 (30.8)	302 (22.7)	97 (13.8)	

Results are presented as frequencies and proportions within the respective periodontitis diagnosis group. P-values were calculated as Pearson's Chi-square.
[a] Periodontal health and gingivitis cases.
[b] Derived from date of attendance for the first health examination.
[c] Primary: Up to 10 years of primary education, Secondary: 1-3 years of upper secondary education, Tertiary: University or college education.
[d] In NOK

3.1.1 Distribution of serum vitamin D levels

As presented in Table 5, persons with serum 25(OH)D levels < 30 nmol/L had more and deeper periodontal pockets than those in the > 50 nmol/L group, with similar results for both PPD variables. Deficiency was most prevalent in males, the 40-49 age group, those who reported brushing their teeth once a day or less often, and among both current-and never

smokers. Deficiency was also slightly more prevalent in those with lower education and lower income. Those with most vitamin D sufficiency were females, the oldest age groups of 60 years and older, those brushing their teeth twice a day or more, and previous smokers. Table 5 further showed a higher proportion of vitamin D deficiency in persons having attended the health examination and given blood samples during the months of October-February, compared to those having attended during March-September. All included variables were significant at the 5% level.

Table 5, Characteristics of the study sample by serum vitamin D status.

		Serum 25(OH)D			
		<30 nmol/L	**30-50 nmol/L**	**>50 nmol/L**	
		n (%)	**n (%)**	**n (%)**	
Variables	**Total n (%)**	139 (3.8)	832 (22.5)	2722 (73.7)	***p*-value**
Number of pockets ≥ 4mm					
0	778 (21.1)	15 (10.8)	162 (19.5)	601 (22.1)	0.005
1-2	953 (25.8)	41 (29.5)	201 (24.2)	711 (26.1)	
3 or more	1962 (53.1)	83 (59.7)	469 (56.4)	1410 (51.8)	
Number of pockets ≥ 5mm					
0	2363 (64.0)	80 (57.6)	520 (62.5)	1763 (64.8)	0.039
1-2	783 (21.2)	32 (23.0)	167 (20.1)	584 (21.5)	
3 or more	547 (14.8)	27 (19.4)	145 (17.4)	375 (13.8)	
Sex					
Female	1898 (51.4)	52 (37.4)	368 (44.2)	1478 (54.3)	<0.001
Male	1795 (48.6)	87 (62.6)	464 (55.8)	1244 (45.7)	
Age group					
40-49	1059 (2.7)	75 (54.0)	355 (42.7)	629 (23.1)	<0.001
50-59	1062 (28.8)	39 (28.1)	257 (30.9)	766 (28.1)	
60-69	979 (26.5)	15 (10.8)	164 (19.7)	800 (29.4)	
70+	593 (16.0)	10 (7.2)	56 (6.7)	527 (19.4)	

Month of attendance[b]					
March-September	2498 (67.6)	85 (61.2)	538 (64.7)	1875 (68.9)	0.019
October-February	1195 (32.4)	54 (38.8)	294 (35.3)	847 (31.1)	
Tooth brushing frequency					
Once a day or less	726 (20.0)	53 (40.5)	177 (21.8)	496 (18.5)	<0.001
Twice a day or more	2898 (80.0)	78 (59.5)	634 (78.2)	2186 (81.5)	
Smoking status					
Current	478 (13.2)	27 (20.5)	115 (14.1)	336 (12.5)	0.039
Previous	1587 (43.8)	45 (34.1)	351 (43.2)	1191 (44.5)	
Never	1558 (43.0)	60 (45.5)	347 (42.7)	1151 (43.0)	
Education[c]					
Primary	849 (23.4)	41 (29.7)	145 (17.6)	663 (24.9)	<0.001
Secondary	1085 (29.9)	41 (29.7)	253 (30.6)	791 (29.7)	
College/university	1696 (46.7)	56 (40.6)	428 (51.8)	1212 (45.5)	
Total household income[d]					
<351 000	439 (12.4)	18 (13.4)	78 (9.6)	343 (13.2)	0.024
351 000–551 000	764 (21.5)	35 (26.1)	159 (19.5)	570 (22.0)	
551 000–1000 000	1457 (41.1)	48 (35.8)	357 (43.8)	1052 (40.5)	
>1000 000	885 (25.0)	33 (24.6)	222 (27.2)	630 (24.3)	

Results are presented as frequencies and proportions within the respective periodontitis diagnosis group. P-values were calculated as Pearson's Chi-square.
[b] Derived from date of attendance for the first health examination.
[c] Primary: Up to 10 years of primary education, Secondary: 1-3 years of upper secondary education, Tertiary: University or college education.
[d] In NOK

3.2 Regression analyses

3.2.1 Assumptions

The collinearity analysis gave a condition index of < 30 for all dimensions, although with a slight correlation between the two variables education and outcome (values > 0,50) at the fifth dimension. However, the Pearson correlation coefficient for these two variables was 0,390,

indicating that the correlation was acceptable (value < 0,7), and thus both were kept in the analyses. The variance inflation factor (< 10) and tolerance (> 0,1) indicated no collinearity, and thus no objection to the regression analyses.

3.2.2 Associations between periodontitis and vitamin D

In Table 4, the *p*-value of 0.537 for Pearson´s chi-square test of association indicated that periodontitis and vitamin D status were not significantly associated in this sample. However, in the adjusted regression analysis, periodontitis was significantly associated with vitamin D status ($p = 0.036$ and 0.038). Tooth brushing frequency and education were not statistically significant ($p > 0.05$), and neither was the income category of <351 000 NOK (Table 6). The Wald-test values indicated that age was the most important predictor for severe periodontitis in this study sample with the largest value (198.8), followed by smoking (Wald statistic = 135.5) and sex (Wald statistic = 47.9). For vitamin D status, the Wald statistic was 7.6.

Table 6, Odds for selected characteristics by periodontitis stage, divided into two groups (Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I vs. Periodontitis stages II-IV).

		Periodontitis[a] Odds ratio (95% CI)	***p*-value**
Vitamin D status	<30	1.53 (1.03-2.28)	0.036
	30-50	1.21 (1.01-1.45)	0.038
	>50 (ref.)	1	
Sex	Female (ref.)	1	
	Male	1.73 (1.48-2.02)	<0.001
Age group	40-49 (ref.)	1	
	50-59	2.00 (1.66-2.42)	<0.001
	60-69	3.88 (3.15-4.77)	<0.001
	70+	4.47 (3.40-5.88)	<0.001
Smoking status	Current	3.36 (2.63-4.31)	<0.001
	Previous	2.12 (1.81-2.48)	<0.001
	Never (ref.)	1	
Tooth brushing frequency	Once a day or less	1.12 (0.92-1.36)	0.253
	Twice a day or more (ref.)	1	
Education	Primary	1.05 (0.85-1.30)	0.653
	Secondary	1.00 (0.84-1.20)	0.998
	Tertiary (ref.)	1	
Total household income	<351 000	1.32 (0.98-1.79)	0.068
	351 000–551 000	1.50 (1.19-1.90)	<0.001
	551 000–1 000 000	1.28 (1.06-1.55)	0.011
	>1 000 000 (ref.)	1	

[a] Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I (n = 1 571) vs. Periodontitis stages II-IV (n = 2 122)

Ref.: Reference category

Adjusted for 10-year age groups (ref.: 40-49 years), sex (ref.: female), smoking status (ref.: never), tooth brushing frequency (ref.: twice a day or more), education (ref.: tertiary), income (ref.: >1 000 000)

3.2.3 Associations between periodontitis and vitamin D in participants attending in October-February

The results presented in Table 7 display participants having given blood samples during October-February. The results showed no statistical significance between periodontitis and vitamin D status when adjusting for sex, age, smoking, toothbrushing frequency, education, and income. However, in the respective analysis for the March-September group (see supplementary table 8), OR for vitamin D status < 30 was 1.63 (1.00-2.68), $p = 0.052$, and for vitamin D status 30–50, OR was 1.31 (1.05-1.63), $p = 0.018$, indicating a stronger association between periodontitis and vitamin D status in this group.

Table 7, Odds for selected characteristics by periodontitis stage, divided into two groups (Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I vs. Periodontitis stages II-IV), for participants having attended during the months of October-February.

		Periodontitis[a] Odds ratio (95% CI)	***p*-value**
Vitamin D status	<30	1.43 (0.72-2.87)	0.309
	30-50	1.09 (0.79-1.50)	0.603
	>50 (ref.)	1	
Sex	Female (ref.)	1	
	Male	2.21 (1.65-2.94)	<0.001
Age group	40-49 (ref.)	1	
	50-59	2.48 (1.75-3.50)	<0.001
	60-69	4.90 (3.33-7.22)	<0.001
	70+	6.84 (4.09-11.43)	<0.001
Smoking status	Current	4.43 (2.78-7.06)	<0.001
	Previous	2.65 (1.99-3.53)	<0.001
	Never (ref.)	1	
Tooth brushing frequency	Once a day or less	1.19 (0.84-1.69)	0.325
	Twice a day or more (ref.)	1	
Education	Primary	1.01 (0.68-1.50)	0.960
	Secondary	0.87 (0.63-1.50)	0.386
	Tertiary (ref.)	1	
Total household income	<351 000	1.54 (0.87-2.73)	0.137
	351 000–551 000	2.02 (1.31-3.09)	0.001
	551 000–1 000 000	1.69 (1.20-2.37)	0.003
	>1 000 000 (ref.)	1	

[a] Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I (n = 513) vs. Periodontitis stages II-IV (n = 682)

Ref.: Reference category

Adjusted for 10-year age groups (ref.: 40-49 years), sex (ref.: female), smoking status (ref.: never), tooth brushing frequency (ref.: twice a day or more), education (ref.: tertiary), income (ref.: >1 000 000)

3.2.4 Associations between periodontal pockets and vitamin D

In the regression models, vitamin D deficiency and insufficiency were associated with PPD ≥ 5 mm when adjusting for sex, age, and smoking. The odds of having serum vitamin D levels of < 30 compared to having serum vitamin D levels of > 50 was 1.78 (95% CI 1.22-2.60) for those with one or more PPD ≥ 5 mm (Table 8). In this analysis, smoking was the most important predictor (Wald statistic = 131.2). Wald statistic for vitamin D status was 12.6.

Table 8, Odds for selected characteristics across number of periodontal pockets of ≥ 5 mm, divided into two groups (no teeth with PPD ≥ 5 mm versus at least one tooth with PPD ≥ 5 mm).

		PPD ≥ 5mm[c] Odds ratio (95% CI)	***p*-value**
Vitamin D status	<30	1.78 (1.22-2.60)	0.003
	30-50	1.23 (1.03-1.47)	0.020
	>50 (ref.)	1	
Sex	Female (ref.)	1	
	Male	1.60 (1.39-1.85)	<0.001
Age group	40-49 (ref.)	1	
	50-59	1.57 (1.29-1.91)	0.002
	60-69	2.61 (2.14-3.20)	<0.001
	70+	2.32 (1.84-2.93)	<0.001
Smoking status	Current	3.37 (2.70-4.20)	<0.001
	Previous	1.83 (1.57-2.14)	<0.001
	Never (ref.)	1	

[c] No teeth with PPD ≥ 5 mm (n = 2 363) vs. one or more PPD ≥ 5 mm (n = 1 330)

Ref.: Reference category

Adjusted for 10-year age groups (ref.: 40-49 years), sex (ref.: female) and smoking status (ref.: never)

4 Discussion

4.1 Associations between periodontitis and vitamin D

The findings from the regression analyses indicate that vitamin D status was associated with periodontitis. Persons with periodontitis stage II-III/IV had 53% higher odds of having deficient vitamin D levels compared with having sufficient levels. Periodontitis stage II-III/IV was most significantly associated with those aged 70+, current daily smokers, and males. Income and education were not significantly associated with periodontitis stage II-III/IV (tables 4 and 5). These findings are similar to those of previous cross-sectional studies of periodontitis and serum 25(OH)D measured through blood sampling (75-77).

In the descriptive frequency distribution analyses, there was an equal proportion of vitamin D deficiency across the different periodontitis groups (Table 4) which might initially seem to conflict with the study hypothesis that there was a difference in periodontitis stage distribution across vitamin D levels. On the other hand, the adjusted regression analysis displayed a statistically significant increase in odds of being vitamin D deficient for those diagnosed with periodontitis stage II-III/IV. It is therefore likely that the apparent absence of difference in the proportions of vitamin D deficiency and insufficiency across the periodontitis stages can be explained by differences in the age distribution. The Vitamin D deficiency was most prevalent in the 40-49 age group, who also made up the majority of the non-periodontitis cases.

Similar to the findings of Parva et al. (38), vitamin D deficiency was most prevalent in the youngest study participants, while sufficiency was most prevalent in the oldest (Table 5). The most likely explanation for this is differences in diet. It has been shown that consumption of fish, which is an important source of dietary vitamin D, is low in the younger parts of the of the Norwegian population (41, 43, 78). In addition, as reasoned by Parva et al. (38), spending more time in indoor working spaces during the sunniest hours of day, and the technological development of televisions, computers, smartphones and games, might compel younger adults to spend more time indoors than the older generations, who, after retirement might find more leisure time for outdoor activities. The frequent reports of elderly adults having a heightened

risk of vitamin D deficiency is most likely to be found in the very old age groups for which physical activity and dermal synthesis of vitamin D are reduced (40, 79, 80)

The males in this study sample had more prevalence of stage III-IV periodontitis, and more vitamin D deficiency and insufficiency. The females, on the other hand, made up the largest portion of the sufficient group and had slightly less severe periodontitis. These findings correspond with earlier studies that have shown differences in nutrition between men and women (41).

Systematic reviews have concluded that results from previous clinical- and case-control studies on periodontitis and vitamin D often are inconclusive and heterogenous, indicating a persistent need for more well-designed longitudinal studies (81-83). Interestingly, a study by Ketharanathan et al. (84) found significant differences in vitamin D status among ethnic Norwegian patients with periodontitis, but not among the Tamil patients with periodontitis, indicating that associations may vary between different populations.

4.2 Associations between periodontitis and vitamin D in participants attending in October-February

There was a higher proportion of vitamin D deficiency in persons who had attended the blood sampling during the months of October-February, and a respective lower proportion for those having attended during March-September (Table 5). This was presumably a result of the seasonal variation in sun-induced vitamin D production, as seasonal variation in vitamin D status in the population in northern Norway has been previously demonstrated (43, 44).

The reason for performing separate analyses for persons having attended the health examination during the months of October-February and those having attended in March-September was that the high number of measurements during sunnier months (Table 4) might result in a skewed proportion of vitamin D sufficiency. Thus, the separate analysis was done in order to remove the effect of seasonal sun-exposure on vitamin D levels and eliminate the cases where vitamin D-sufficiency might only have been temporary.

In the regression analysis, the distinct increase in significance in the March-September group compared to the October-February group, can be understood to mean that those who display low levels of vitamin D in the summer months are likely to have very low levels during winter months (Table 7, Supplementary table S1). Thus, if vitamin D plays a role in those with deficient levels, this should be observed among those with the lowest vitamin D status, i.e., those low during summer. However, this interpretation requires confirmation in further studies with longitudinal designs.

4.3 Associations between pocket depth and vitamin D

Table 5 showed that when vitamin D levels were higher (i.e., sufficient), there were less periodontal pockets. The regression analyses further showed that odds of having at least one periodontal pocket of 5 mm or more was 78% higher for persons with vitamin D deficiency compared with persons who were vitamin D sufficient even when adjusting for age and smoking, which are known to be some of the most important risk factors for developing periodontal pockets (11, 14). These findings would indicate that either periodontal inflammation was in fact increased in those groups where serum 25(OH)D was low, or alternatively, that vitamin D might be a marker of disease activity in this sample. Similar to these findings, Alshouibi et al. and Miley et al. found that persons who took vitamin D supplements had fewer and shallower periodontal pockets, although serum 25(OH)D levels were not measured in these studies (85, 86).

4.4 Methodological discussion

This is the first study to explore the relationship between periodontitis and vitamin D using data from the Tromsø 7 (2015 – 2016) study. The available data on measured pocket depths and serum 25(OH)D derived from blood samples meant that the possible correlations between individual vitamin D status and indicators of periodontal inflammation could be analyzed without relying on self-reported data or estimations.

4.4.1 Included and excluded variables

The associations between the included covariates and periodontitis were quite similar to the findings of previous studies (24, 25). The date of participation was the only included variable available for estimating the sun-induced vitamin D in this analysis. Dietary intake data are available in the Tromsø 7 dataset, but not included in this present analysis. Although data on intake of vitamin D supplements, intake of fatty fish or fish oils as reported in the food frequency questionnaire could have contributed additional aspects in this analysis, it was nevertheless beyond the scope of this master's thesis. Participants in Tromsø 7 were also asked whether they had recently been on sun destination holiday. This information was however not available in the dataset used. The 25(OH)D biomarker is a satisfactory measure of the sum of both diet and sun-induced vitamin D and is considered a valid indication for vitamin D status (28, 30). It is, however, important to remember that any future recommendations will have to focus on dietary intake and nutritional supplements, as the prospects of obtaining adequate amounts of dermal vitamin D in the Northern Norwegian winter are unrealistic (30, 47).

There is much research pointing towards a strong two-way relationship between periodontitis and diabetes (11, 13, 67). Still, diabetes was not included as a confounding variable in the current study due to the fact that an earlier study found no statistically significant association between diabetes and periodontitis among the participants of Tromsø 7 (87). Data on BOP was also available for the Tromsø 7 study population, but as bleeding can be affected by factors such as smoking (14), it was considered too unreliable to reflect periodontal inflammation by itself and was thus not included.

4.4.2 Strengths and limitations

Strengths of this study includes the reliability of the collected Tromsø 7 data. The Tromsø study included a large number of participants from all population groups over 40 years of age, with a high response rate (72). The sample of included participants in this study was

representative of the whole Tromsø population in 2016, regarding distribution of sex and 10-year age groups (70, 73). Attendees of the oral health examination were selected randomly from the total study sample, and there was very low rate of refusal to participate in the oral examination, minimizing the risk of selection bias. However, it is not unusual that health study responders are of generally better health than the non-responders, a phenomenon well-examined in previous studies (88-90). Thus, it is possible that there may be some degree of response bias in the original sample population, and that responders of the Tromsø study had better general health and nutrition and higher serum 25(OH)D levels than the non-responders, leading to skewed representation.

The oral examination included full-mouth periodontal measurement by specially trained dental hygienists in order to facilitate the best most accurate measurements possible and avoid information bias. There is always a risk of PPD measurements being influenced by personal variations in the amount of pressure used on probing or by local swelling or retractions of the gingiva, although the calibration and training of the dental hygienists would have minimized the risk of such occurrences.

The most immediate limitation of this study is undoubtedly that a cross-sectional study design is not sufficient to determine causality. It is therefore not possible to determine the direction of the relationship between exposure and outcome even where associations were found. In other words, there will be no telling whether persons with low vitamin D levels at onset are likely to develop more severe periodontitis, or if persons with more severe periodontitis will be prone to display lower vitamin D levels. One might lead to the other, or both might appear simultaneously, but in order to investigate such matters, prospective clinical trials are needed. Furthermore, it is not possible to determine whether vitamin D levels influenced the alveolar bone quality of the periodontitis patients.

Some of the included covariates such as smoking and frequency of tooth brushing were self-reported, which means that there was a risk of both recall- and reporting bias which might have influenced the results of the adjusted analyses. However, as the main variables serum 25(OH)D, periodontitis stage and PPD ≥ 5 were all assessed and measured by professionals, the risk of recall- and reporting bias in some of the covariates was not considered to pose a

meaningful threat to the internal validity of the study. An analytic challenge of the current study was the small proportions of vitamin D deficiency and -insufficiency (3,8% and 22,5% of the total sample, respectively). In order to fully capture the characteristics of the vitamin D deficient patient groups, further studies might aim to incorporate more deficient persons.

5 Conclusions

The results of this study showed a significant association between vitamin D status and periodontitis stage II-III/IV, as well as for vitamin D status and PPD. The month of attending the health examination and giving blood samples was a significant predictor for serum 25(OH)D levels in this study population. Although these findings do not prove a causal relationship between vitamin D deficiency and periodontitis, the associations suggest that vitamin D deficiency might increase the individual inflammatory risk. Thus, periodontitis patients might be advised to strive to comply with the recommendations on intake of vitamin D, especially throughout the seasons of less sun exposure, so as to maintain sufficient serum 25(OH)D levels.

When interpreting the results of this study, it is important to remember that even where associations were found, the direction of the association is not known. It is possible that vitamin D supplementation might have an effect in promoting periodontal health, for instance as a factor for preventing the onset or progression of more severe periodontitis in the younger and middle-aged adult population. However, in order to determine whether this relationship is true in other populations and the direction of that relationship, more prospective research is needed.

As suggested by Holvik et al. (35), vitamin D should not be recommended in excess or unnecessarily, but its potential benefits for oral health are nevertheless interesting and might well prove an important subject of examination in relation to periodontal health in population groups at risk of vitamin D deficiency. Understanding and examining the connections between oral health and general health, instead of viewing periodontitis as simply a local

condition, will continue to give us a deeper understanding of the etiology and progression of periodontitis for the specific individual.

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Supplementary table

Table S 1, Odds for selected characteristics across periodontal stage, divided into two groups (Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I vs. Periodontitis stages II-IV) for participants having attended during the months of March-September.

		Periodontal diagnosis[c] Odds ratio (95% CI)	**p-value**
Vitamin D status	<30	1,63 (1,00-2,68)	0,052
	30-50	1,31 (1,05-1,63)	0,018
	>50 (ref.)	1	
Sex	Female (ref.)	1	
	Male	1,58 (1,32-1,90)	<0,001
Age group	40-49 (ref.)	1	
	50-59	1,87 (1,49-2,34)	<0,001
	60-69	3,61 (2,81-4,63)	<0,001
	70+	3,88 (2,80-5,38)	<0,001
Smoking status	Current	3,01 (2,25-4,04)	<0,001
	Previous	1,95 (1,61-2,36)	<0,001
	Never (ref.)		
Tooth brushing frequency	Once a day or less	1,07 (0,85-1,36)	0,557
	Twice a day or more (ref.)	1	
Education	Primary	1,06 (0,82-1,36)	0,664
	Secondary	1,07 (0,86-1,33)	0,531
	Tertiary (ref.)	1	
Total household income	<351 000	1,21 (0,85-1,73)	0,284
	351 000–551 000	1,31 (0,99-1,73)	0,064
	551 000–1 000 000	1,12 (0,89-1,41)	0,347
	>1 000 000 (ref.)	1	

[c] Periodontally healthy, gingivitis case, periodontal stability, and periodontitis stage I (n = 1 058) vs. Periodontitis stages II-IV (n = 1 440)

Ref.: Reference category

Adjusted for 10-year age groups (ref.: 40-49 years), sex (ref.: female), smoking status (ref.: never), tooth brushing frequency (ref.: twice a day or more), education (ref.: tertiary), income (ref.: >1 000 000)