Original Article

Serum Vitamin D Levels and Their Association with Periodontal Parameters in Periodontitis Patients from Northeastern Peninsular Malaysia

Hanim Afzan Ibrahim[1], Siti Aisyah Zulkipeli[2], Haslina Taib[3], Tuan Nadrah Naim Tuan Ismail[4], Nik Aloesnisa Nik Mohd Alwi[5], Nurul Huda Abdullah[6], Nur Karyatee Kassim[7]

ABSTRACT

Background

Vitamin D plays a significant role in modulating immune system, inflammatory processes, and bone metabolism. Vitamin D deficiency has been proposed as a risk factor for periodontitis. However, the association between vitamin D levels and periodontitis remains inconclusive. ***Objective:*** The aim of this study was to determine the serum vitamin D levels in periodontitis patients from the northeastern Peninsular Malaysia and to assess its association with periodontal parameters.

Methodology

A cross-sectional study was conducted among 43 participants, comprising 20 periodontitis patients and 23 healthy controls. Periodontal parameters were assessed, including plaque score, gingivitis score and periodontal pocket depth. Blood samples were collected and analyzed for serum vitamin D levels using an automated COBAS 6000 analyser.

Results

The mean age of periodontitis patients and healthy controls was 41 ± 13 and 35 ± 14 years, respectively. Both groups exhibited low mean serum vitamin D levels: 20.48 ± 4.1 ng/mL in the periodontitis group and 20.99 ± 3.9 ng/mL in the healthy group, with no statistically significant difference ($p > 0.05$). Approximately 50% of periodontitis patients were vitamin D deficient (<20 ng/mL). No significant correlations were found between serum vitamin D levels and any of the periodontal parameters.

Conclusion

This study found that periodontitis patients had low serum vitamin D levels; however, there was no statistically significant association with periodontal parameters. These findings underscore the need for further multicentre studies with larger sample sizes to elucidate the potential role of vitamin D in periodontal health.

Keywords

Vitamin D level, periodontitis, Vitamin D deficiency, periodontal parameters, risk factors, Malaysia

INTRODUCTION

Periodontitis is a chronic, multifactorial inflammatory disease characterised by the progressive and pathological destruction of the tooth-supporting apparatus, specifically involving the irreversible loss of periodontal ligament and alveolar bone[1,2]. In 2022, The World Health Organisation[3] reported that approximately 14% of adults suffered from severe periodontal disease, including periodontitis, which involved more than one billion cases globally. According

1. Medical and Basic Dental Sciences Unit, School of Dental Sciences, Universiti Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
2. School of Dental Sciences, University Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
3. Associate Professor, School of Dental Sciences, University Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
4. Medical and Basic Dental Sciences Unit, School of Dental Sciences, University Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
5. Medical and Basic Dental Sciences Unit, School of Dental Sciences, University Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
6. School of Medical Sciences, University Sains Malaysia Health Campus,16150 Kubang Kerian, Kelantan. Malaysia.
7. Associate Professor, Medical and Basic Dental Sciences Unit, School of Dental Sciences, University Sains Malaysia Health Campus, 16150 Kota Bharu, Kelantan, Malaysia.

Correspondence

Dr Nur Karyatee Kassim, Associate Professor, Medical and Basic Dental Sciences Unit, School of Dental Sciences, University Sains Malaysia Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia E-mail: karyatee@usm.my / drkaryatee@gmail.com

Bangladesh Journal of Medical Science Vol. 25 No. 01 January'26

DOI: https://doi.org/10.3329/bjms.v25i1.86410

to the Ministry of Health Malaysia, the National Oral Health Survey of Adults (NOHSA)[4] in 2010, revealed that 94% of dentate adults were affected by periodontal disease, with 18.2% classified as having a severe form. A study has demonstrated that lower serum levels of vitamin D are significantly associated with increased periodontal tissue destruction and more severe stages of periodontitis, suggesting a potential role of vitamin D in modulating periodontal inflammation and bone loss[5]. Furthermore, a systematic review and meta-analysis support the hypothesis that individuals with higher vitamin D levels tend to exhibit less gingival bleeding and improved periodontal parameters compared to those with lower levels, indicating a protective, anti-inflammatory role of vitamin D in maintaining periodontal health[6].

Vitamin D is a secosteroid hormone synthesised in the skin from 7-dehydrocholesterol through a photochemical reaction induced by ultraviolet (UV) radiation. It can also be acquired exogenously through dietary intake and nutritional supplementation[7]. According to Jagelavičienė and colleagues[8], vitamin D has been demonstrated to have immunomodulatory, anti-inflammatory, antiproliferative, and cell apoptotic properties, potentially lowering the risk of periodontitis and gingivitis. Furthermore, 25(OH)D, a form of vitamin D found in serum, has been recognised to have a strong correlation with periodontitis and is essential for maintaining dental homeostasis[9]. Consequently, insufficient serum vitamin D levels may adversely affect the periodontium and contribute to periodontal disease progression[10]. In Malaysia, a cross-sectional study involving various ethnic groups reported that approximately 67.4% of participants exhibited vitamin D deficiency, defined as serum levels below 20 ng/ml[11]. Another study by Syafii et al.[12] reported that 76.7% of healthy Malay adults were vitamin D deficient, with the majority being female. In this study, a serum vitamin D level of less than 30 nmol/L was classified as indicative of vitamin D deficiency.

Vitamin D plays a critical role in modulating the immune response and has been implicated in the pathogenesis and progression of periodontitis[9]. Several studies have demonstrated a significant association between serum vitamin D levels and periodontal health, suggesting that vitamin D deficiency may contribute to increased susceptibility to periodontal disease. The incidence of periodontitis may therefore be positively influenced by the prevention and correction of vitamin D deficiency. In this context, vitamin D supplementation has been proposed as an effective, affordable, and safe adjunct in the prevention and management of periodontal disease[9,13].

Despite growing global evidence supporting this association, data on the relationship between serum vitamin D levels and periodontitis remain limited in Malaysia. The lack of local data highlights a critical gap in the literature, particularly given the high prevalence of periodontal disease in the region. Addressing this gap is essential for informing targeted public health interventions and improving periodontal outcomes at the population level. This study was therefore designed to investigate the association between serum vitamin D levels and clinical periodontal parameters in individuals with periodontitis. We hypothesised that insufficient serum vitamin D may play a contributory role in the pathogenesis of periodontitis.

The findings from this study may support the use of vitamin D supplementation as an adjunctive strategy to optimise periodontal treatment outcomes. Additionally, serum vitamin D levels may serve as valuable biomarkers for diagnosis, monitoring, and prognostic assessment of periodontitis. This research also aims to strengthen collaboration between medical and dental professionals, providing a foundation for future interdisciplinary studies. Ultimately, the study holds significant potential benefits for both patients and the broader community, particularly in enhancing understanding of the bidirectional relationship between vitamin D status and periodontal health.

MATERIALS AND METHODS

This was a cross-sectional study, comprising 43 participants recruited within the compound of Hospital Pakar Universiti Sains Malaysia (HPUSM), a tertiary referral center situated within the northeast region of Peninsular Malaysia. A convenience sampling method was carried out to select patients among individuals attending the Dental Clinic at the HPUSM. The sample size was calculated using PS Software v3.1.2 [12]. The acquired sample size of 33 for the periodontitis group and 33 for the healthy subjects, giving a sample size of 66[14].

The study population was divided into two groups: Group 1 included 20 patients diagnosed with periodontitis, and Group 2 consisted of 23 healthy controls. The inclusion

criteria for both groups included patients aged 18 years and above who had at least 12 teeth in the oral cavity and had no scaling and or root planning within the last six months. For periodontitis, only patients with moderate to severe disease, characterised by a periodontal pocket depth (PPD) greater than 3 mm, were included[15]. The exclusion criteria included in the following: pregnant women, patients taking vitamin D altering drugs such as antiepileptic drugs, cancer and HIV patients, patients with reduced vitamin D intake due to chronic diseases that could influence result interpretation and patients who face another risk of hypovitaminosis, such as gastric or bowel resection and malabsorption. This study was conducted within 3 months, between July and September 2024, to decrease the seasonal variability.

Data collection was done by filling out the proforma and consent forms to provide information on participants' socio-demographic characteristics, such as gender, ethnicity, and age. Next, the periodontal parameters such as plaque score (PS), gingivitis score (GS) and periodontal pocket depth (PPD) were assessed and recorded. About five mls of blood was withdrawn from each participant in both groups and sent to the laboratory to assess their serum vitamin D levels.

We assessed the periodontal status of the participants using periodontal parameters such as PS, GS, and PPD. For the PS, four surfaces (mesial, distal, buccal, and lingual) of each tooth, excluding all third molars, were evaluated for the presence of plaque, and any surface with plaque was recorded on the chart, divided by the total number of surfaces checked, and multiplied by 100. The patient's PS was expressed as a percentage, following the method proposed by O'Leary. Following the method established by Ainamo and Bay in 1975, the Gingival Score (GS) was determined by gently probing the gingival crevice at four sites per tooth, with third molars excluded from the assessment[17]. Positive sites were identified when bleeding occurred within 10 seconds. The bleeding score was calculated by dividing the number of positive sites by the total number of sites examined, then multiplying by 100, and expressed as a percentage. PPD was measured from the free gingival margin to the base of the pocket on six sites per tooth (facial, mesiofacial, distofacial, lingual, mesiolingual, and distolingual) by using a periodontal probe. A PPD greater than 3 mm was considered indicative of periodontal disease in the corresponding tooth[14]. The periodontal assessments were conducted by a trained examiner who had been calibrated by a senior specialist. Inter- and intra-examiner reliability assessments indicated that approximately 90% of the measurements were reproducible within ±1.0 mm.

Five mls of blood was withdrawn from each participant in both groups by authorised medical practitioners while following standard infection control and universal venepuncture procedures. The blood samples collected were then sent to the Endocrine Laboratory, Department of Chemical Pathology, HPUSM. The samples were centrifuged at 3500 RPM for 10 minutes and stored at -80°C. An automated COBAS 6000 analyser (Roche Diagnostics) was used to measure the serum vitamin D levels using competitive protein binding assay, with CVs for repeatability between 1.7% and 7.8% and the CVs for intermediate precision were between 2.2% and 10.7%. The method used is traceable to a Joint Committee for Traceability in Laboratory Medicine (JCTLM)-approved isotope dilution liquid chromatography mass spectrometry (ID-LC-MS/MS). All samples were discarded after study completion.

According to the Endocrine Society Clinical Practice Guidelines (ESCPG) and the Institute of Medicine, serum 25(OH)D levels for overall health are categorised as follows: deficient (<20 ng/mL), insufficient (20–29.9 ng/mL), and sufficient (≥30 ng/mL)[9,18,19].

Data were entered and analysed with the Statistical Package for Social Science (SPSS) version 28.0. Descriptive statistics, such as mean, standard deviation (SD), frequency, and percentages, were calculated. An Independent T-test was used to compare the mean and standard deviation of serum vitamin D levels between Group 1 and Group 2. Since the data distribution was not normally distributed, the Spearman correlation test was used to assess the correlation between periodontal parameters (PS, GS, PPD/T and PPD/M) and serum vitamin D levels. P-value less than 0.05 were considered statistically significant.

Ethical Clearance:

This study was submitted, reviewed and approved by the Human Research Ethics Committee of USM (JEPeM USM code: USM JEPeM/18020160). The study was conducted according to the guidelines described by the Helsinki Declaration of 1975, as revised in 2013. Informed consent was obtained from all patients prior to the dental examination.

RESULTS

A total of 43 participants were recruited in this study as shown in Table 1, comprising 20 patients diagnosed with periodontitis and 23 healthy in controls. The mean age of periodontitis patients was 41 ± 13 years, while the mean age among healthy controls was 35 ± 14 years. In terms of gender distribution, males constituted the majority in both groups: 70% (n=14) in the periodontitis group and 73.9% (n=17) among healthy controls. All study participants were of Malay ethnicity.

Table 1. Sociodemographic characteristics of study subjects.

Variables	Periodontitis	Healthy participants
Age in years	41 ± 13	35 ± 14
Gender		
Female: n (%)	6 (13.9)	5 (17.2)
Male: n (%)	14 (32.6)	17 (23.3)
Race		
Malay: n (%)	20 (100)	23 (100)

Periodontitis (n=20), Healthy controls (n=23)

Table 2 represents the comparison of serum vitamin D levels between periodontitis patients and healthy controls. Both groups demonstrated low mean serum vitamin D concentrations, with the periodontitis group recording 20.48 ± 4.1 ng/mL and the healthy controls showing a slightly higher mean of 20.99 ± 3.9 ng/mL. Despite this numerical difference, the variation was not statistically significant (p = 0.173).

Table 2. Comparison of serum vitamin D levels between periodontitis patients and healthy subjects.

Categories	Serum Vitamin D Level (ng/ml) (Mean ± SD)	t-statistic (df)	p-value
Periodontitis	20.48 ± 4.1	-0.4 (41)	0.173
Healthy subjects	20.99 ± 3.9		

Statistical test: Independent T test for comparison between two groups

p-value with a significance level of <0.05

Table 3 shows the distribution of serum vitamin D levels among periodontitis patients. Half of the subjects (50%) had serum vitamin D levels below 20 ng/mL, indicating deficiency, while the remaining 50% had levels ranging from 20–29.9 ng/mL, classified as insufficient. None of the periodontitis patients exhibited sufficient vitamin D levels (≥30 ng/mL).

Table 3. Measurement of serum vitamin D level among periodontitis patients.

Serum Vitamin D Levels	
Mean ± SD	20.48 ± 4.1 ng/mL
<20ng/ml (deficient) n (%)	10 (50%)
20-29.9 ng/ml (insufficient) n (%)	10 (50%)
≥30 ng/ml (sufficient) n (%)	-

Correlation analysis between serum vitamin D levels and periodontal clinical parameters in the periodontitis group is presented in Table 4. No statistically significant associations were observed between serum vitamin D levels and plaque score (r = 0.154, p = 0.262), gingivitis score (r = 0.071, p = 0.605), probing pocket depth per tooth (r = 0.078, p = 0.704), or probing pocket depth per mouth (r = 0.071, p = 0.729).

Table 4. Association of serum vitamin D levels and periodontal parameters in periodontitis patients

Variable	Serum Vitamin D Level	
	r_s [a]	p-value
Plaque score	0.154	0.262
Gingivitis score	0.071	0.605
Probing pocket depth/tooth	0.078	0.704
Probing pocket depth/mouth	0.071	0.729

[a]spearman's rank correlation (r_s), *p*-value with a significance level of <0.05

DISCUSSION

Periodontal diseases are prevalent among the Malaysian population[4]. Vitamin D plays an important role in the immune response and may play a key role in the pathogenesis of periodontitis[8]. The present investigation discovered that periodontitis patients

had low mean serum vitamin D levels. It was revealed that approximately 50% of them suffered from vitamin D deficiency, another half suffered from vitamin D insufficiency, and none of them achieved vitamin D sufficiency. Serum 25(OH)D levels are categorised as deficient (>20 ng/ml), insufficient (20– 29.9 ng/ml), and sufficient (30 ng/ml) for general health by the Endocrine Society Clinical Practice Guidelines (ESCPG) and the Institute of Medicine[9,18,19]. Despite receiving approximately 6 hours of sunlight each day, as reported by the Ministry of Science, Technology, and Innovation (MOSTI) in 2011, few studies have found that Malaysians have a high prevalence of vitamin D deficiency[4]. Another study conducted in Dhaka reported that 43.8% of participants had vitamin D deficiency, 37.2% had insufficient levels, while only 18% had sufficient vitamin D levels[20].

A study by Anbarcioglu et al.[21] revealed that those with vitamin D deficiency had a higher prevalence of developing aggressive periodontitis. Thus, this study suggested vitamin D deficiency as one of the risk factors for periodontitis. A previous study by Alshouibi et al.[8] in 2013 found that those who received more than 800 IU of vitamin D daily had a lower risk of suffering from a severe case of chronic periodontitis, while those who received less than 400 IU of vitamin D experienced more advanced alveolar bone resorption. This may be explained by the capability of vitamin D to reduce the quantity of *Porphyromonas gingivalis* via active autophagy[6,24]. *Porphyromonas gingivalis* has been strongly associated with the development of periodontal disease [25]. Furthermore, vitamin D deficiency results in osteoporosis, decreased bone mineral density, the advancement of periodontal diseases, and jawbone resorption.

This present study showed that periodontitis patients had lower mean serum vitamin D levels than healthy controls. Despite a lower mean serum vitamin D level in the periodontitis group, the difference was not statistically significant when compared to healthy controls. This observation was consistent with previous research, which reported that periodontitis patients had significantly lower serum vitamin D levels than a control group of Puerto Rican adults[18]. Another study also discovered that the prevalence of deficient and insufficient serum vitamin D levels in periodontitis patients was significantly higher compared to healthy subjects[9]. Thus, earlier findings and the current investigation revealed a relationship between low serum vitamin D levels and periodontal disease. However, the lack of significant difference in serum vitamin D levels between periodontitis patients and healthy subjects in the present study highlighted the complexities of the relationship between vitamin D status and periodontal health. The lack of significant difference could be attributed to various factors, including small sample size, heterogeneity within the study population, and potential confounding factors other than serum vitamin D levels that could contribute to the development and progression of periodontitis. A larger sample size than required produces more accurate results because it is more representative of the population[19]. Other than serum vitamin D levels, confounding factors influencing the development and progression of periodontal disease include smoking status, systemic diseases such as diabetes, medication such as corticosteroids, dietary habits, and oral hygiene practices[26,27]. Smoking and diabetes Type 1 and 2, are considered to be the well-established risk factors for periodontal disease[5].

This study also found no significant association between serum vitamin D levels and periodontal parameters. The study's findings were consistent with previous research, which found no significant link between serum vitamin D deficiency and periodontitis. However, several studies discovered contradictory results as they postulated a strong association between low serum vitamin D levels and periodontitis[6,9,22,28]. Therefore, this discrepancy and contradicting findings could be related to the heterogeneity of the study's demographics, methodology, and potential confounding factors across different studies. The lack of significant association observed in the current study is possibly explained by the influence of genetic, environmental, and lifestyle factors that may confound the relationship between serum vitamin D levels and periodontal parameters.

The discrepancy between the current study and previous findings raises the importance of acknowledging the limitations of this study to be highlighted for reference in future research. The limitations of this study included the study design, small sample size, and confounding factors not accounted for in the analysis. The current study was a cross-sectional study that precluded the establishment of causality and confounding factors such as smoking status, systemic diseases such as diabetes, medication, dietary intake, and oral hygiene practices, which can influence the observed association between

serum vitamin D level and periodontal parameters, should be considered in the study analysis. Furthermore, the participation of all Malays for both groups in this study may need to provide a better representation of the population, particularly in Malaysia, a multi-ethnic populated country. A survey in 2016 among 858 Malaysians disclosed that Indian participants possessed the highest proportion of vitamin D deficiency, followed by Malays, other races, and Chinese[11].

CONCLUSION

Vitamin D is essential for periodontal health since it modulates bone metabolism and calcium absorption, regulates immune response, and exhibits anti-inflammatory effects[29]. This study demonstrated low mean serum vitamin D among periodontitis patients; however, there was no significant association between serum vitamin D levels and the periodontal parameters. Thus, despite the conflicting findings of this study in comparison to the other studies, which showed a strong association between low serum vitamin D levels and periodontitis, further research is warranted to elucidate the underlying mechanism linking serum vitamin D and periodontal health. Addressing the current study's limitations requires a future longitudinal study design with larger, more diverse populations and a comprehensive assessment of confounding variables to understand the relationship between serum vitamin D and periodontal parameters.

Source of Fund: This research was funded by the Fundamental Research Grant Scheme [FRGS/1/2019 203/PPSG/6171232], Ministry of Education, Malaysia.

Conflict of Interest

The authors declared no conflict.

Acknowledgement

The authors would like to express appreciation to the staff from the Dental Clinic and the Chemical Pathology Laboratory, HPUSM, who provided help and assistance in the research.

Authors' contribution

Siti Aisyah Zulkipeli, Hanim Afzan Ibrahim, Haslina Taib, Tuan Nadrah Naim Tuan Ismail ,Nurul Huda Abdullah, Nik Aloesnisa Nik Mohd Alwi, Nur Karyatee Kassim contributed equally to this work. Hanim Afzan Ibrahim , Nur Karyatee Kassim and Tuan Nadrah Naim Tuan Ismail supervised and designed the study objective and methodology, Nik Aloesnisa Nik Mohd Alwi, Nurul Huda Abdullah and Haslina Taib recruited the subjects of the study, Siti Aisyah Zulkipeli and Hanim Afzan Ibrahim analysed the sample and Nik Aloesnisa Nik Mohd Alwi led the data analysis. Haslina Taib and Nurul Huda Abdullah performed the literature review, and Siti Aisyah Zulkipeli and Haslina Taib wrote the manuscript. Nur Karyatee Kassim performed the major work in structuring and harmonising the overall content. All authors have read and approved the final version of the manuscript.

REFERENCES

1. Slots J. Periodontitis: facts, fallacies and the future. *Periodontology 2000*. 2017;**75**(1):7-23.
2. Abd Rahman NA, Hanafi MH, Ibrahim HA, Omar J, Chaudhry A, Sirajudeen K, Kassim NK. Nexus between Periodontal Disease and Chronic Kidney Disease: A NarrativeReview. *Bangladesh Journal of Medical Science*. 2023;**22**(2):260-271.
3. World Health Organization (2022). *Oral health*. Available at: https://www.who.int/news-room/fact-sheets/detail/oral-health.
4. Syakimah W. Periodontal Disease Amongst Malaysian. 2013;
5. Lu EMC. The role of vitamin D in periodontal health and disease. *Journal of Periodontal Research*. 2023;**58**(2):213-224.
6. Machado V, Lobo S, Proença L, Mendes JJ, Botelho J. Vitamin D and Periodontitis: A Systematic Review and Meta-Analysis. *Nutrients*. 2020;**12**(8):2177.
7. Botelho J, Machado V, Proença L, Delgado AS, Mendes JJ. Vitamin D Deficiency and Oral Health: A Comprehensive Review. *Nutrients*. 2020;**12**(5)
8. Jagelavičienė E, Vaitkevičienė I, Šilingaitė D, Šinkūnaitė E, Daugėlaitė G. The Relationship between Vitamin D and Periodontal Pathology. *Medicina*. 2018;54(3):45.
9. Alzahrani AAH, Alharbi RA, Alzahrani MSA, Sindi MA, Shamlan G, Alzahrani FA. Association between periodontitis and vitamin D status: A case-control study. *Saudi Journal of Biological Sciences*. 2021;**28**(7):4016-4021.
10. Bashutski JD, Eber RM, Kinney JS, Benavides E, Maitra S, Braun TM. The Impact of Vitamin D Status on Periodontal Surgery Outcomes. *Journal of Dental Research*. 2011;**90**(8):1007-1012.
11. Shafinaz IS, Moy FM. Vitamin D level and its association with adiposity among multi-ethnic adults in Kuala Lumpur, Malaysia: a cross sectional study. *BMC Public Health*. 2016;**16**(1)
12. Syafii N, Ismail TST, Rosdi RAM, Muda BC, Azman WNW, Ibrahim HA, Badruridzwanullah A. The Association between Vitamin D and Lipid Profile among Healthy Malay Adults in K ota Bharu, Malaysia. *Bangladesh Journal of Medical Science*. 2024;**23**(1):214-220.
13. Bhargava P, Fitzgerald KC, Calabresi PA, Mowry EM. Metabolic alterations in multiple sclerosis and the impact of vitamin D supplementation. *JCI Insight*. 2017;**2**(19)
14. Dupont WD, Plummer WD. Power and sample size calculations. *Controlled Clinical Trials*. 1990;**11**(2):116-128.
15. American Academy of Periodontology. American Academy of Periodontology Task Force report on the update to the 1999 classification of periodontal diseases and conditions. *J Periodontol*. 2015;**86**(7):835-838.
16. O'Leary T. The Periodontal Screening Examination. *The Journal of Periodontology*. 1967;**38**(6P2):617-624.
17. Ainamo J, Bay I. Problems and proposals for recording gingivitis and plaque. *International dental journal*. 1975;**25**(4):229-235.
18. Del Valle HB, Yaktine AL, Taylor CL, Ross AC. Dietary reference intakes for calcium and vitamin D. 2011;
19. Holick MF, Binkley NC, Bischoff-Ferrari HA, Gordon CM, Hanley DA, Heaney RP. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *The Journal of Clinical Endocrinology and Metabolism*. 2011;**96**(7):1911-1930.
20. Afrin SF, Rahman MH, Bari KF, Al Mahmood AK. Status of Vitamin D by measuring 25-Hydroxyvitamin D level in Dhaka City. *Bangladesh Journal of Medical Science*. 2019;**18**(3):624-627.
21. Madi M, Pavlic V, Alammar MS, Alsulaimi M, Alotaibi S, AlOtaibi M. The association between vitamin D level and periodontal disease in Saudi population, a preliminary study. *The Saudi Dental Journal*. 2021;**33**(7):595-600.
22. Anbarcioglu E, Kirtiloglu T, Öztürk A, Kolbakir F, Acıkgöz G, Colak R. Vitamin D deficiency in patients with aggressive periodontitis. *Oral Diseases*. 2018;**25**(1):242-249.
23. Alshouibi EN, Kaye EK, Cabral HJ, Leone CW, Garcia RI. Vitamin D and Periodontal Health in Older Men. *Journal of Dental Research*. 2013;**92**(8):689-693.
24. Hu X, Niu L, Ma C, Huang Y, Yang X, Shi Y. Calcitriol decreases live Porphyromonas gingivalis internalized into epithelial cells and monocytes by promoting autophagy. *Journal of Periodontology*. 2019;**91**(7):956-966.
25. Rafiei M, Kiani F, Sayehmiri F, Sayehmiri K, Sheikhi A, Zamanian Azodi M. Study of Porphyromonas gingivalis in periodontal diseases: A systematic review and meta-analysis. *Medical Journal of the Islamic Republic of Iran*. 2017;**31**:62.
26. Andrade C. Sample size and its importance in research. *Indian Journal of Psychological Medicine*. 2020;**42**(1):102-103.
27. Darby I. Risk factors for periodontitis & peri-implantitis. *Periodontology 2000*. 2022;**90**(1):9-12.
28. Yussif N, Selim K. Deficient serum vitamin D level is not a risk factor for periodontitis. A cross-sectional clinical study. *Journal of Osseointegration*. 2021;13(2):70-74.
29. Antonoglou GN, Knuuttila M, Niemelä O, Raunio T, Karttunen R, Vainio O. Low serum level of $1,25(OH)_2$ D is associated with chronic periodontitis. *Journal of Periodontal Research*. 2014;**50**(2):274-280.