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ORIGINAL ARTICLE

The impact of vitamin D on clinical parameters and bone turnover biomarkers in ligature-induced periodontitis: An experimental study in rats

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KEYWORDS

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Abstract *Objectives and Background:* Vitamin D has been associated with an increased risk of tooth loss and the severity of periodontal diseases. This study aimed to evaluate the effect of vitamin D on the clinical, radiographic, and serum level changes of bone turnover biomarkers in ligature-induced periodontitis.

Methods: A total of 28 rats were included in this study and divided into test groups: Vitamin D supplement (VS), Vitamin D deficient (VD), and control (CG). Ligature-induced periodontal tissue destruction was performed and kept for 21 days. Clinical attachment and radiographic changes were recorded, and serum samples were tested for Osteoprotegerin (OPG), Dickkopf-1 (DKK1), Sclerostin (SOST), and Fibroblast growth factor 23 (FGF23) on the initial and final day of the study.

Results: Groups that were made VD exhibited a more significant amount of clinical attachment loss (1.05 ± 0.50 mm) compared to the CG (0.83 ± 0.14 mm) and VS group (0.60 ± 0.13 mm), showing significant differences ($p < 0.05$). The radiographic alveolar bone loss amount was greater in the VD group compared to the other groups. For serum level assessment, the VD groups also exhibited a statistically significant reduction in the levels of OPG. They showed higher concentrations of DKK1, SOST, and FGF23 than other groups, with significant differences ($p < 0.05$).

Conclusion: The results revealed that Vitamin D may play a role in the progression of periodontal disease. It was found to affect both clinical parameters and bone turnover biomarkers, suggesting its potential impact on the disease process.

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1. Introduction

Periodontitis is a prevalent oral health problem that affects approximately half of the adult population globally. The disease is caused by bacterial plaque, which triggers a chronic inflammatory response in the periodontium. This inflammation leads to the destruction of the bone and connective tissue supporting the teeth, resulting in tooth loss (Kinane et al., 2017). Additionally, periodontitis has been linked to several systemic health conditions, including cardiovascular disease, diabetes, and osteoporosis (Winning and Linden, 2015) Vitamin D is a crucial nutrient that plays a significant role in maintaining overall body health. It has multiple functions, such as regulating calcium and phosphorus levels, promoting bone health, and supporting the immune system (Lee et al., 2017). In recent years, several studies have investigated the potential relationship between vitamin D and periodontitis, which is a chronic inflammatory disease affecting the gingiva and supporting tissues of the teeth. These studies have found that low levels of vitamin D are associated with an increased risk of periodontal disease and subsequent tooth loss. Individuals with low vitamin D levels were more likely to have periodontitis and a higher risk of tooth loss (Kim et al., 2020). Similarly, another study reported that vitamin D supplementation was linked to improved periodontal health and a reduced incidence of tooth loss (Jimenez et al., 2014).

It is believed that vitamin D may help prevent tooth loss by regulating the immune system and reducing periodontal inflammation. Vitamin D plays a role in modulating the immune response by suppressing the production of pro-inflammatory cytokines and promoting the production of anti-inflammatory cytokines. This modulation helps to reduce periodontal inflammation and improve periodontal health, potentially lowering the risk of tooth loss. Furthermore, vitamin D has been shown to enhance the ability of immune cells to eliminate bacteria, which may also contribute to its potential role in reducing the severity of periodontitis and the risk of tooth loss (Costantini et al., 2020). However, it is important to note that the relationship between vitamin D and periodontitis is still an ongoing subject of research, and more studies are needed to establish a clear cause-and-effect relationship. The aim of this study was to evaluate the effect of vitamin D on clinical, radiographic, and serum level changes of bone turnover biomarkers in ligature-induced periodontitis.

2. Materials and methods

2.1. Animals

The study was conducted from September 2017 to August 2019. Prior to the commencement of the study, ethical approval for the use of animals was obtained from the Universiti Institute Teknologi MARA (UiTM) Animal Ethics Committee (ref- 600 FF.PS.17/2/1). Animal study is in this research is conducted and reported according to ARRIVE guideline in compliance to Malaysian code of practice for the care and use of animals for scientific purposes, Animal Welfare Act 2015. Considering the attrition rate during the study, a total of thirty male Sprague-Dawley rats were utilized in this study, weighing between 100 and 150 g and aged 4 weeks. The rats were then randomly divided into three groups, with 10 rats were allocated into each group. The rats underwent a period of acclimatization for 3 days and were then placed in individually housed plastic cages with free access to food and water in the Bioscience Laboratory of UiTM for the experimental period of 32 days.

2.2. Experimental design

The rats were then randomly divided into three groups, with 10 rats in each group (considering potential dropouts due to death). The control group (CG) rats were exposed to adequate sunlight for 6 h, provided with free access to water and a standard diet (gold chain rodent diet), and received a daily gastric gavage of saline as a control. The Vitamin D supplemented group (VS) rats were housed in a similar setting with free access to water and food, but they received Vitamin D3 in liquid form through daily gastric gavage using a mini syringe (10,000 I.U./kg of vitamin D) as well as adequate sunlight exposure for 6 h. In the Vitamin D deficient group (VD), low vitamin D serum levels were simulated by limiting sunlight exposure to only 2 h. This was achieved by blocking the cage circumference with a black block card. The VD rats were provided with free access to water, a vitamin D deficient food (Sigma Aldrich vitamin D deficient custom diet) and received a daily gastric gavage of saline as a control. All living conditions were maintained for a period of 32 days, with a relative humidity of 30–70% and a temperature ranging from 18 to 26 °C (Fig. 1).

2.3. Ligature placement

One week after the start of the intervention, the rats were anesthetized by intraperitoneal administration of Zoletil 100 (250 mg tiletamine, 250 mg zolazepam, Virbac UK Veterinary) at a dosage of 50 mg/kg. Following anaesthesia, the rats underwent periodontal probing in the lower anterior incisor mid-interdental region using a Williams probe with millimetre markings, which were verified with a micro calliper. Measurements were recorded from the gingival margin to the deepest point of the periodontal pocket, known as probing pocket depth. A specialized holding apparatus was used to obtain periapical radiographic records of the lower anterior incisors. The radiographic measurements were taken from the incisal tip to the base of the alveolar bone. After recording the baseline measurements, an experimental periodontitis model was induced by placing a 4.0 silk ligature in a figure of "8" configuration around the mandibular central incisors and securing it on the buccal surface (Ionel, 2015).

2.4. Clinical, radiological and immunological procedures

After three weeks of ligature placement and continuous monitoring, the rats were placed under general anaesthesia. The ligature was removed, and clinical records were repeated. Changes in attachment level were measured in millimetres (mm), and values indicating attachment loss were determined by comparing the measurements from day 1 to day 21 of ligature placement. In ensuring consistency in data collection, an interrater reliability analysis was conducted on clinical and radiological assessments using the Kappa statistic, with data recorded at a reliability rate of 92%. Radiographic data

were also repeated. Changes in periodontal alveolar bone were measured in millimetres (mm), and values indicating bone loss were assessed using digital radiographic software (EasyDent V4 Viewer, Software version 4.1.4.5). The measurements were taken by comparing the radiographic images from day 1 to day 21.

2.5. Blood sampling record and animal euthanasia

The rats were placed under anesthesia on day 21, blood samples were collected using an intravenous method from the tail. A total of 0.2 ml blood is collected for Miliplex testing.

The collected blood sample was allowed to clot, then placed in a centrifuge machine for 10 min at 1000 Xg. After centrifugation, the serum was separated from the clot. Prior to initiating the immunoassay procedure, all reagents were prepared and made to reach room temperature (20–25 °C).

The plate was prepared following the MILIPLEX MAP Rat Bone Magnetic Bead Panel 1 protocol to test serum levels of OPG, DKK1, SOST, and FGF23. The plate was then analyzed using Luminex® 200™, HTS, FLEXMAP 3D®, or MAGPIX® with xPONENT® software. At 32 days, the rats were sacrificed via cardiac puncture.

2.6. Statistical analysis

The statistical analysis of the study was conducted using IBM SPSS Statistics 26. Kruskal-Wallis H tests were utilized to determine the significance between groups in terms of clinical attachment loss, radiographic alveolar bone loss, and serum levels of OPG, DKK1, SOST, and FGF23.

3. Result

All rats showed weight gain at the end of study. The weights of the rats were standardized, except for the CG group, which had a slightly higher weight of 120.50 ± 1.26 g. In terms of clinical attachment level, the data showed no statistical difference between group (0.42 ± 0.03 mm) at day 1. The results indicated that rats in the VD group exhibited greater clinical attachment loss (1.05 ± 0.50 mm) compared to the CG (0.83 ± 0.14 mm) and the group receiving vitamin D supplementation (VS) (0.60 ± 0.13 mm) (Table 1). Moreover, the study found that the amount of radiographic alveolar bone loss was more significant in the VD group (0.61 ± 0.06 mm) compared to the CG (0.45 ± 0.04 mm) and VS (0.26 ± 0.03 mm) groups. These findings support the results of clinical attachment loss and suggest that vitamin D deficiency may increase the risk of bone loss in the jaw.

The serum levels assesstment results (Table 2) showed that the VD group had a statistically significant reduction in levels of OPG (421.65 ± 29.49 pg/ml) compared to the CG (584.84 ± 137.96 pg/ml) and VS group (715.68 ± 71.37 pg/ml). The study also found that the VD group had higher concentrations of DKK1 (1336.56 ± 272.71 pg/ml) compared to the CG (1243.86 ± 207.40 pg/ml) and VS group (909.42 ± 149.06 pg/ml).

Additionally, the study found that the VD group had higher concentrations of SOST (1684.12 ± 41.92 pg/ml) compared to the CG (1682.14 ± 41.92 pg/ml) and VS (1284.22 ± 34.20 pg/ml). The results also showed that the VD group had lower concentrations of FGF23 (531.09 ± 12.84 pg/ml) compared to the CG (627.11 ± 18.58 pg/ml) and the VS group (717.79 ± 20.45 pg/ml).

4. Discussion

Numerous studies have investigated the potential impact of vitamin D on periodontitis, with some indicating that insufficient vitamin D levels could contribute to the development and progression of the disease (Dietrich et al., 2005). The findings of this study support the notion that low vitamin D levels can have a significant effect on periodontal health. The group with vitamin D deficiency demonstrated a more substantial decrease in clinical attachment levels compared to the other two groups. Osteoprotegerin (OPG) is a protein that plays a critical role in regulating bone resorption by inhibiting the activity of osteoclasts, the cells responsible for breaking down bone tissue. Low vitamin D levels have been associated with reduced OPG expression in serum. In healthy individuals, elevated OPG levels were observed in gingival tissues, and higher OPG expression was correlated with decreased disease severity (Hassan et al., 2015). This suggests that OPG may be involved in the regulation of bone remodelling in periodontitis. Another study reported that inadequate vitamin D levels were linked to decreased OPG expression in human bone marrow stromal cells, potentially contributing to increased bone resorption and reduced bone density (Takahashi et al., 2014).

DKK1, short for Dickkopf-1, is a protein that plays a crucial role in regulating the Wnt signalling pathway. Elevated levels of DKK1 have been observed in the gingival tissues and serum of patients with periodontitis. It has been proposed that DKK1 contributes to the destruction of periodontal tissues by inhibiting the Wnt signalling pathway and promoting osteoclastogenesis (Samiei et al., 2019). Increased levels of DKK1 have been associated with greater severity of periodontitis and enhanced bone loss. Conversely, inhibition of DKK1 has been found to reduce bone loss and inflammation in periodontitis (Liu et al., 2017). The current study found that vitamin D supplementation was linked to a significant reduction in serum DKK1 levels, suggesting that vitamin D may regulate the expression of DKK1. Sclerostin (SOST) is a protein primarily produced by osteocytes and plays a crucial role in bone metabolism by inhibiting bone formation. This study indicates that vitamin D may regulate the expression of SOST, and this interaction may be relevant to various bone-related disorders. A study has shown that supplementation with vitamin D and calcium significantly lowers serum SOST levels, suggesting that these nutrients may help regulate SOST expression and improve bone metabolism (Cidem et al., 2015). Fibroblast growth factor 23 (FGF23) is a vital hormone involved in the regulation of phosphate and vitamin D metabolism. FGF23 is mainly produced by osteocytes and osteoblasts in the bone and acts by reducing phosphate reabsorption in the kidneys and decreasing the production of active vitamin D. There is a complex interplay between FGF23 and vitamin D. When vitamin D levels are low, FGF23 levels increase, which helps to decrease phosphate levels in the blood and maintain bone health (Goretti Penido and Alon, 2012). However, in certain conditions like hypophosphatemic rickets, FGF23 levels can become abnormally high. Some studies have suggested that vitamin D deficiency

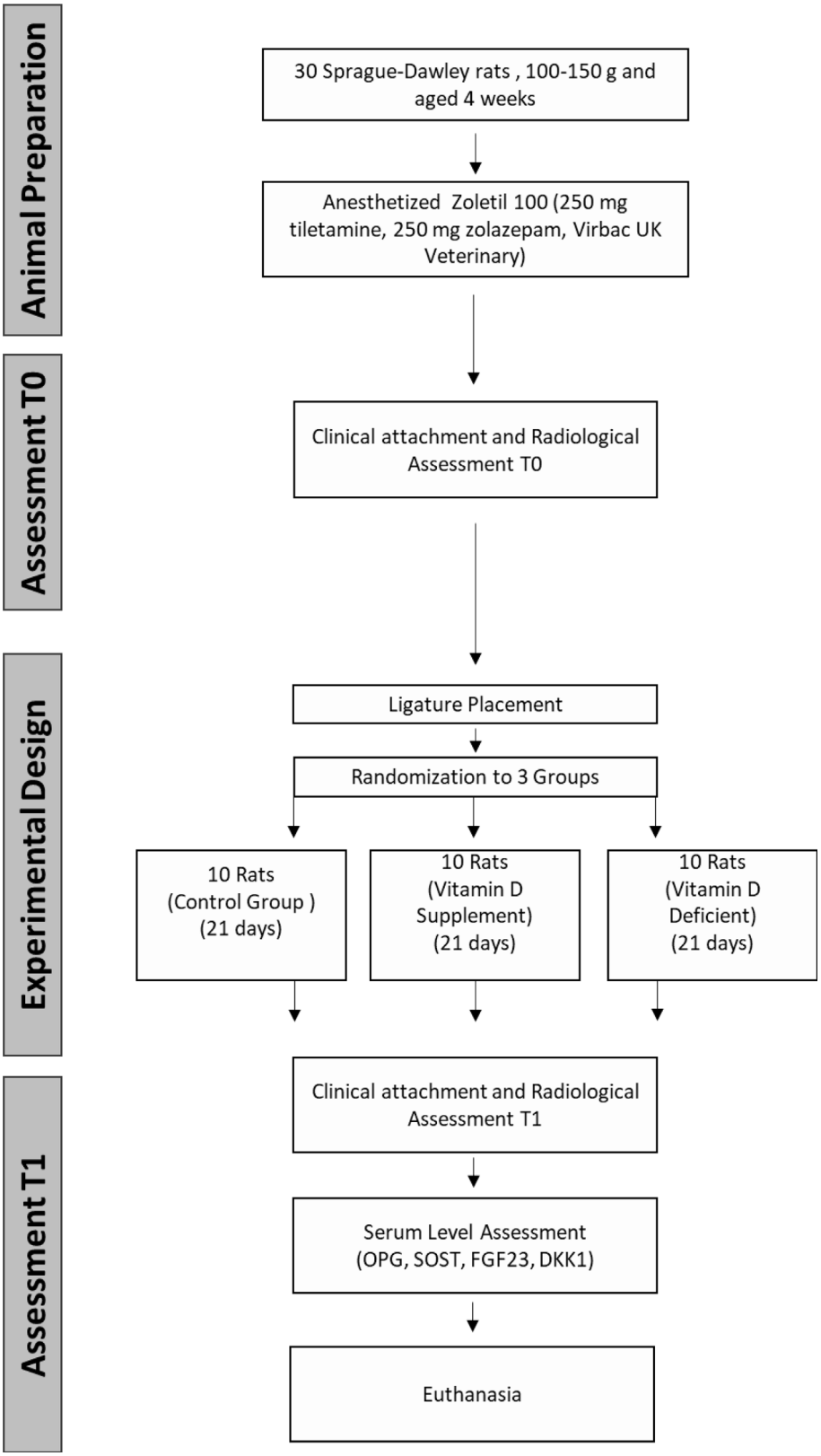

Fig. 1 Workflow.

may be associated with elevated FGF23 levels. The present study concluded that vitamin D supplementation decreases serum FGF23 levels, while decreased vitamin D intake increases serum FGF23 levels (Quarles, 2012).

Table 1 Kruskal- Wallis H test, SD standard deviation, significance level set at p value < 0.05.

Variables	Control Group (CG)	Vitamin D Supplement (VS)	Vitamin D Deficient (VD)	P Value
Attachment level (millimetres) Mean ± SD	0.83 ± 0.14	0.60 ± 0.13	1.05 ± 0.50	0.001
Radiographic bone loss (millimetres) Mean ± SD	0.45 ± 0.04	0.26 ± 0.03	0.61 ± 0.06	0.001

Clinical and radiographical result table.

Table 2 Kruskal- Wallis H test, SD standard deviation, significance level set at p value < 0.05.

Variables	Control Group (CG)	Vitamin D Supplement (VS)	Vitamin D Deficient (VD)	P Value
OPG (Pg/ml) Mean ± SD	584.84 ± 137.96	715.68 ± 71.37	421.65 ± 29.49	0.001
DKK1 (Pg/ml) Mean ± SD	1243.86 ± 207.40	909.42 ± 149.06	1336.56 ± 272.71	0.001
SOST (Pg/ml) Mean ± SD	1682.14 ± 41.92	1284.22 ± 34.20	1684.12 ± 41.92	0.001
FGF23 (Pg/ml) Mean ± SD	627.11 ± 18.58	531.09 ± 12.84	717.79 ± 20.45	0.001

Serum level assessment result table.

5. Conclusion

It is believed that the beneficial effects of vitamin D on periodontitis are attributed to its regulatory role in the immune system. However, further research is necessary to fully comprehend the intricate relationship between vitamin D and periodontitis, as well as to determine the optimal levels of vitamin D needed to prevent or treat the condition effectively.

Ethical approval

The study was conducted from September 2017 – August 2019, prior commencement of the study, ethics for the animals were obtained from the UiTM Animal Ethics Committee (ref- 600 FF.PS.17/2/1).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix. Fig. 1.

Clinical and radiographical result table
Table 1.
Serum level assessment result table
Table 2.

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