ISSN: 1607-8322, e-ISSN: 2220-5799 Anaesthesia, Pain & Intensive Care
Vol 27(4); August 2023 DOI: 10.35975/apic.v27i4.2259

ORIGINAL RESEARCH PAIN MANAGEMENT

The effect of vitamin D supplementation on pain due to periodontitis in Wistar rats with hypovitaminosis

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ABSTRACT

Background & Objective: Vitamin D (vit D) plays an important role in inflammation and pain as a result of inflammatory processes, probably through its neuroprotective function. But the association of dietary supplementation and pain improvement remains unclear. We conducted this experimental study on to evaluate the effect of vit D supplementation in Wistar rats with hypovitaminosis D due to periodontitis.

Methodology: We used 20 samples of Wistar rats with induced hypovitaminosis D due to periodontitis during 7 weeks; the first week allocated for cage adaptation, in next 2 weeks, frontal teeth ligation done aimed for vit D deficiency, and during next 4 weeks vit D supplementation was done. The rats were divided into four groups; P1 (controls without ligation), P2 (ligated without vit D supplementation), P3 (vit D 4000 IU/kg supplementation), and P4 (vit D 2000 IU/kg supplementation). Pain intensity was observed by rat grimace score (RGS) at one week of cage adaptation and at four weeks after periodontitis, as well as vit D levels.

Results: Vit D deficiency was observed at 6 weeks, either the RGS worsened. The improvement noticed in P2 (49.95%), P3 (34.30%), and P4 (59.23%) supplemented groups without statistically significant differences (P = 0.236; P = 0.280). The RGS showed decrease on P1 (P = 0.009), P2 (P = 0.011), and P4 (P = 0.049), but without significance in accordance to vit D improvement (P = 0.528).

Conclusion: Vit D supplementation is beneficial for chronic pain when its level in the serum is low, but the effect is not statistically significant.

Abbreviations: MSK: Musculoskeletal; RGS: Rat Grimace Score; CWP: Chronic Widespread Pain

Keywords: Vitamin D; Supplementation; Wistar rat, Periodontitis; Chronic pain; Perception

Citation: Budisulistyo T, Yuwono E, Muhartomo H, Pramukarso DT. The effect of vitamin D supplementation on pain due to periodontitis in Wistar rats with hypovitaminosis. Anaesth. pain intensive care 2023;27(4):502−507; **DOI:** 10.35975/apic.v27i4.2259

Received: December 18, 2021; **Reviewed:** March 28, 2023; **Accepted:** April 04, 2023

1. INTRODUCTION

Musculoskeletal pain syndrome (MPS), or commonly recognized as chronic widespread pain (CWP), might cause comorbidity in otherwise healthy-looking persons. The pathogenesis of CWP is difficult to understand, due to the involvement of multiple physical and psychological factors.[1] MPS is either found in pediatric patients with a prevalence estimated to be around 4% to

40%. It can be due to some orthopedic problems such are: muscles spasm, inappropriate body posture and gait; and reluctance to mobilize. Thus MPS might lead to impaired hypersensitivity, thermoregulation, or autonomic dysfunction, so can lead to disequilibrium, and developmental or growth problems.[2] The lack of vitamin D (vit D) or 25-hydroxyvitamin D (25(OH)D) level might cause MPS, fibromyalgia, poor muscle strength, as well as lower bone marrow density (BMD). Thus, some studies pointed out that MPS and fatigue may be a result of significant vit D deficiency (48%), and supplementation treatment leads to significant reduction of pain and muscle weakness.[3,4]

Vit D or (25(OH)D) is a fat-soluble vit with an important role in the systemic diseases, which may be a cause of pain,[5] but its exact role is still not clearly understood. It is synthesized in the skin tissues, approximately 90% in association of sun rays and 10% through meals.[6] Very low serum vit D levels have been observed to lead to significant CWP development.[1] Vit D is a mineral that is available in inactive form called pro-vit D, that requires sunlight exposure (290 to 315 mm) to be in its active form by the receptors located within the keratinocytes on skin. Thus, ultraviolet B (UVB) rays are the most intense and vit D synthesis is possible in the tropical zones extending between latitudes 23.5°N and 23.5°S to the equator. In Indonesia sunlight exposure is a routine, so it leads 7-dehydrocholesterol in the skin to be converted to pre-vit D3, which is then converted into active form.[4] The prevalence of CWP observed higher among non-Caucasians, such as South Asian people is related to a lower level of vit D.[1] A survey that involved more than 2,500 people of South and Southeast Asian countries close to the equator, showed that Singapore and Thailand were slightly frequent of vit D deficiency, and it was more common among females, teenagers, living in an urban area and being less physically active.[7]

Ninety-three percent among vit D deficient people have persistent non-specific pain, 86% suffered from rheumatoid arthritis, and 26% of CWP. It might play a role in protective mechanisms, against modulation, and chronic pain development.[8] Thermal, mechanical, or chemical stimuli are detected by nociceptors,[9] and the cell bodies are located in the dorsal root ganglion (DRG) or the trigeminal ganglion with a cell shape *pseudounipolar*.[10] The primary sensory afferent transmits the nociceptive impulses via the dorsal horn of the spinal cord.[11] Central transmission by spinothalamic tract (STT) and spinoreticular tract (SRT) goes to the lateral nucleus of the thalamus. The SRT continues transmission to the medial thalamus and nucleus raphe magnus (NRM) and periaqueductal gray (PAG). Those nuclei lie in the brainstem and are involved in the modulation of descending pathway of pain.[11,12] Then the impulses are projected to the primary and secondary somatosensory cortex, insula, anterior cingulate cortex, and prefrontal cortex.[11]

Vit D can influence the anatomy, hormonal, neurological, and immunological systems on pain manifestations. Thus, a low level of vit D might be an indicator of ongoing inflammation, so the long-termed state could interfere with the immune system.[12,13] The anti-inflammatory mechanism might interfere to produce cytokines or prostaglandin E2 inhibition,[14] so the pain is developed.[15] Even though recent studies suggest vit D deficiency could lead to CWP, but there remain conflicting pieces of evidence. In this study, we analyzed different doses of vit D supplementation among Wistar rats, then analyzed relationship of dosage to improvement in the serum levels and the association of pain intensity.

2. METHODOLOGY

This experimental research was based on 'Guide for the Care and Use of Laboratory Animals' regulations,[16] and was conducted in the Biology Laboratory of State University of Semarang (UNNES). The ethical clearance was received from the Ethics Commission of the Faculty of Medicine UNDIP (61/EC/H/FK UNDIP/VI/ 2020) on 30 June 2020, and permission from the Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, the State University of Semarang No. 282/ UN.37.1.4.5./KM/2020 was obtained.

The samples consisted of 20 male Wistar rats aged 10 weeks, with 200 grams average weight, grouped into four cages with the dimension of 60 cm x 50 cm x 50 cm. The 'Guide for the Care and Use of Laboratory Animals', 200 grams Wistar rat needed of 450 cm^2 space each, so we grouped them in fours and left them for a week to get adapted to new cages. All cages containing the animals were put inside a room with a spacious space of 50 to 60 m^2 and 20-30 lux of lighting for 12 h (6 pm to 6 am). For monitoring the serum vit D levels, we worked with the GAKI laboratory of the Faculty of Medicine, Diponegoro University, Semarang, in August to September 2020. A glass box 30 cm long, 10 cm wide and 25 cm high was prepared to monitor rat grimace scale (RGS) by putting them inside, with camera recording for a duration of 2 min each, and recorded at 2 weeks after teeth ligation. Thus, based on rat's habit expression, such as orbital tightening, nose or cheek flattening, ears or whisker changes were recorded while pain stimulation was given.[17]

All samples were placed in the cages with the same sunlight exposure, and grouping as P1 (control), P2 (chronic pain without vit D supplementation), P3 (chronic pain with 4000 IU/kg vit D supplementation), and P4 (chronic pain with 2000 IU/kg vit D supplementation). Hypovitaminosis D states was

Table 1: Vitamin D serum levels and RGS assessment in 4 groups during the study

No	Code	Group	Vitamin D Level (ng/mL)			RGS		
			Pre	Post	Delta	Pre	Post	Delta
1	K1	P1 (K1)	1.5183	1.9541	0.4358	0.25	0.25	0
2	K2	P1 (K2)	0.9873	1.299	0.3117	0	0	0
3	K3	P1 (K3)	1.1664	1.7363	0.5669	0	0	0
4	K4	P1 (K4)	1.1664	1.562	0.3956	0	0.25	0.25
5	K5	P1 (K5)	1.6506	2.1724	0.5128	0	0	0
6	1.1	P2 (1.1)	0.8965	1.6056	0.7091	0.5	0.25	0.25
7	1.2	P2 (1.2)	0.6638	0.8507	0.1869	0.75	1	0.25
8	1.3	P2 (1.3)	0.9873	1.2549	0.2676	0.75	1.25	0.5
9	1.4	P2 (1.4)	0.7581	1.299	0.5409	1	1	0
10	1.5	P2 (1.5)	1.0772	1.562	0.4848	1.25	1.25	0
11	2.1	P3 (2.1)	0.942	1.299	0.357	0.75	0.25	0.5
12	2.2	P3 (2.2)	0.7581	0.8507	0.0926	1.25	0	1.25
13	2.3	P3 (2.3)	0.8046	1.1664	0.3618	1	0.25	0.75
14	2.4	P3 (2.4)	0.8046	1.0772	0.2726	1	0	1
15	2.5	P3 (2.5)	1.2549	1.7363	0.4814	0.75	0	0.75
16	3.1	P4 (3.1)	1.1664	1.9105	0.7441	0.75	0.5	0.25
17	3.2	P4 (3.2)	0.7112	1.0772	0.366	0.75	0.25	0.5
18	3.3	P4 (3.3)	0.7581	1.2107	0.4526	0.75	0	0.75
19	3.4	P4 (3.4)	0.8507	1.3431	0.4924	0.75	0.25	0.5
20	3.5	P4 (3.5)	1.1664	1.867	0.7006	0.75	0.25	0.5

developed by 4.0 silk ligature at the inferior frontal teeth of the rats, under mild sedation of intraperitoneal ketamine 10% diluted with xylazine 2% injection in doses of 0.12 mL/100 gram.

Vit D serum levels and RGS were observed at week 2, as regards to the previous study with occurrences of hypovitaminosis, and being a baseline data. Except for the P1 group, the other groups were supplemented with

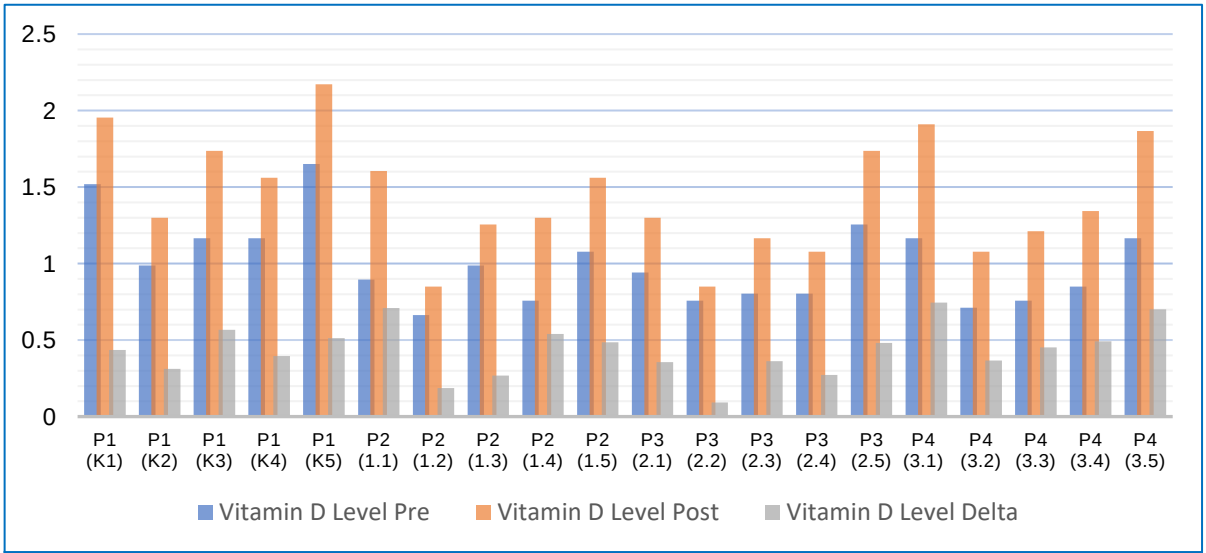

Figure 1: Vitamin D serum levels (ng/mL) in four groups during the study

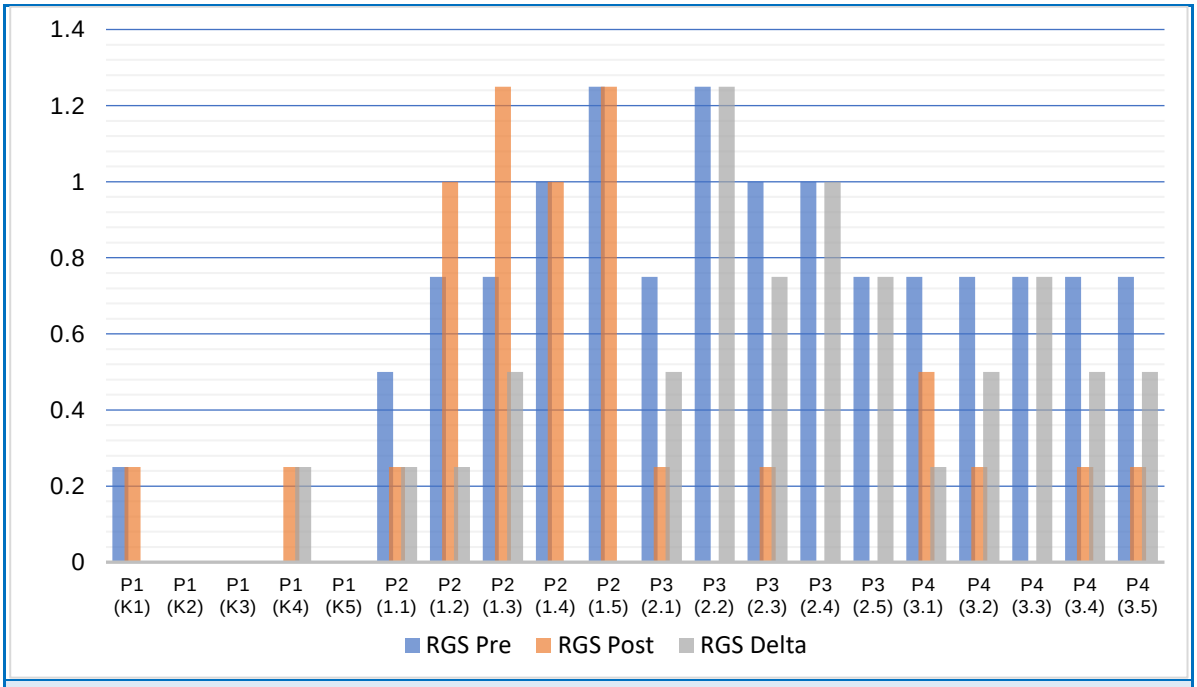

Figure 2. The RGS assessment monitor of 4 groups during the study.

vit D3 (calciferol) for 4 weeks as stated before. After 4 weeks, the intensity of pain and serum vit D levels (week 6) were re-checked.

The data were processed to be analyzed by using SPSS for Windows version 20 software. Data analysis included descriptive statistics and Mann-Whitney hypothesis testing, Bivariate correlation analysis (t-test), the association between vit D deficiency and chronic pain intensity (Pearson analysis), while $P < 0.05$ was considered to be significant.

3. RESULTS

Vit D deficiency was observed in chronic periodontitis groups (P2, P3, and P4) at 6 weeks. RGS assessment among them worsened than control during follow up (Table 1). Among chronic pain samples without vit D supplementation, the serum levels increased around 49.95% as compared to pre-treatment level (mean: 0.8766 ng/mL) to post-treatment level (mean: 1.3144 ng/mL) in group P2. While in group P3 (chronic pain with 4000 IU/kg vit D supplement) the serum levels increased by 34.30% (pre-treatment mean: 0.91284 ng/mL vs. post-treatment mean: 1.22592 ng/mL). In group P4 (chronic pain with 2000 IU/kg of vit D supplement) the serum levels increased around 59.23% (pre-treatment mean: 0.93056 vs. post-treatment mean: 1.4817). Statistical analysis showed significant improvement in serum vit D level in group P2 ($P = 0.008$), P3 ($P = 0.14$), and P4 ($P = 0.018$) compared to group P1 (control) (Figure 1). Whereas, enhancement of serum vit D level between supplemented Wistar rats did not show a significant difference, when we compared group P2 with P3 ($P = 0.236$), and P2 with P4 ($P = 0.280$).

The Mann Whitney analysis of RGS scores of each group found the significant differences. Decrease in RGS was significant between P2 and P3 ($P = 0.009$), P2 and P4 ($P = 0.011$), and P3 and P4 ($P = 0.049$) (Table 1). Vit D supplementation did not show a significant pain improvement as observed in the RGS examination ($P = 0.528$) (Figure 2).

4. DISCUSSION

The two groups of chronic pain did not have significant differences in serum vit D levels, whether supplemented or not. It can be interpreted that the dose of vit D supplementation given to the hypovitaminosis sample was not adequate to increase the serum levels. Recently nonspecific MPS patients underwent vit D supplementation with various doses and forms of vit D, the authors reported pain improvement after one week of administration of 50,000 IU vit D2, or either 6 weeks after administration of 1,200 IU vit D2, or 4 to 13 weeks after a single dose of intramuscular 300,000 IU vit D, or 4 weeks after injection of 600,000 IU vit D3, or 8 weeks after the oral supplementation with 50,000 IU/wk, or weeks to months of treatment with 2,000 to 3,000 IU vit D daily. This study, similar to others that used different doses of vit D supplementation, with no pain improvement, underwent 6 months 800 IU/d and 10,000

IU vit D3 among subjects aged 18 to 65 y, who had fibromyalgia. The authors observed no differences of pain improvement after 4 weeks administration of 300,000 IU vit D (orally or intramuscularly) in comparison with the placebo.[18]

In our study, Group P4, supplemented with 2000 IU/kg of vit D, observed a higher increase (59.23%) in vit D levels than group P3 that received 4000 IU/kg (49.95%). This could be possibly explained by the short dietary duration time of 6 weeks or it might be due to rats' poor compliance, so the rise in vit D serum level was not in proportion. Regarding the RGS examination, the pain intensity might change with improvements in movement and fatigue, hypersensitivity may be decreased, or by improvement of muscle physiology, even though the vit D serum level remains deficient or not.[2]

Vit D has been known as the 'sunshine vitamin' too, due to sunlight radiation being the primary source. The analyzed data could be associate with the geographic circumstances which this study underwent in Indonesia - a tropical country. A recent study mentioned that whole body exposure to sunlight of 290–370 nm for 15–30 min, from 11 am–3 pm daily, might maintain adequate serum vit D level.[19] In contrast to the four-season countries, such as the United States or Europe, the sunlight exposure of at least 50 nm radiation in the summer months might be a source of vit D, so higher serum hydroxyvitamin D [25(OH)D] levels are found.[16] Most of the vit D sources are obtained from the sunlight, which is exposed to at least 50% of the skin within 12 min is the same as a dose of 3000 IU of vit D3. Whereas only about 60% of oral vit D supplementation might change into active metabolites after many processes in the body tissues.[20] The Mann Whitney test observed pain intensity differences between P2 and P3 ($P = 0.009$), P2 and P4 ($P = 0.011$), and P3 and P4 ($P = 0.049$). Those results show similarity to few earlier studies observing of significant improvement of chronic pain after vit D supplementation,[20–24] sleep or quality of life improvement with vit D supplementation,[25] and vit D correlation to musculoskeletal pain or fibromyalgia syndromes.[26] Thus higher doses of vit D supplementation might fast improve the levels in serum near to normal, so the alteration of RGS response might not be observed in detail. It means by improving vit D serum level, the chronic pain might improve too.[20,27–29] Aside from that, the Cochrane Reviews mentioned the lesser support of the hypothesis of vit D supplementation being beneficial for chronic pain treatment.[21] Vit D deficiency has a role in the central sensitization of chronic pain rather than mechanical stimulation, and its addition in dietary supplementation might improve the pain intensity.[29] Vit D supplementation might show effectiveness for improving pain when the serum levels are obviously low.[20,29]

5. LIMITATIONS

We realized that serial monitoring of serum vit D levels need to be done. The principal limitation was that we did not monitor vit D levels and RGS before and one day after ligation. It could represent samples at the time of acute pain as the baseline, in which vit D plays a role in the inflammation pathway. Then it could be followed for the next 1, 2, 3, and 4 weeks. The second limitation is that we did not analyze histopathology of dorsal root ganglion (DRG), that plays role in chronic pain mechanism.

5. CONCLUSION

Vitamin D significantly improves pain intensity, if there is a noticeable obvious deficiency of serum vitamin D. For us who are living in equator's area, should be aware of our vitamin or micronutrient daily intake.

6. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

The authors express their sincere gratitude to Professor Yos Johan Utama, PhD, Rector of Diponegoro University Semarang, Dwi Pudjonarko, MD, PhD, Dean, Faculty of Medicine, Diponegoro University, Yovi Nitawardani, MD, a histopathologist, Anatomical Pathology Laboratory, Diponegoro National Hospital, Head of Animal Laboratory FMIPA Semarang State University, Head of GAKI Laboratory Faculty of Medicine Diponegoro University, and Mrs. Farida as analysts who helped our research.

9. Conflict of interest

The study utilized the hospital resources only, and no external or industry funding was involved.

10. Authors' contribution

TB: concept, conduction of the study work and manuscript editing

EY: conduction of the study and laboratory work

HM, DT: conduction of the study work and internal reviewer

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