The effect of vitamin D supplementation on serum vitamin D and interleukin-6 levels in diabetes mellitus-induced rats

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Abstract. One of the hallmarks of DM-related inflammation is the elevated production of pro-inflammatory cytokines, such as interleukin-6 (IL-6). Recent evidence has suggested that vitamin D, a fat-soluble secosteroid hormone, may modulate immune responses and inflammatory processes. this study aimed to investigate the effects of different doses of vitamin D supplementation on serum vitamin D levels and IL-6 concentrations in DM-induced rats. An in vitro study was conducted on 15 DM-induced rats, divided into three groups: DM without treatment, DM treated with oral vitamin D at 415 IU, and DM treated with oral vitamin D at 1100 IU. Serum vitamin D and IL-6 levels were measured post-treatment. The Kruskal-Wallis test showed significant differences in serum vitamin D levels among the groups ($p = 0.016$). ANOVA indicated significant differences in IL-6 levels among groups ($p=0.017$). Spearman correlation analysis demonstrated a negative correlation between serum vitamin D and IL-6 levels ($r= -0.542$, $p=0.037$). The findings suggest that higher doses of vitamin D may have more pronounced anti-inflammatory effects, highlighting its potential role in managing inflammation in diabetes mellitus.
Keywords: diabetic rat, interleukin-6, Vitamin D

1 Introduction

Diabetes mellitus (DM) is a chronic metabolic condition marked by sustained hyperglycemia due to deficiencies in insulin production, insulin action, or both. The worldwide incidence of

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diabetes mellitus is escalating rapidly, with the International Diabetes Federation forecasting that 537 million adults were affected in 2021, estimated to climb to 783 million by 2045 [1]. Type 2 diabetes mellitus (T2DM) constitutes more than 90% of all instances and is closely linked to obesity, insulin resistance, and persistent low-grade systemic inflammation [2].

A characteristic feature of diabetes mellitus-related inflammation is the increased synthesis of pro-inflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and C-reactive protein (CRP) [3]. IL-6, in particular, performs a dual role as both a pro-inflammatory and anti-inflammatory cytokine. In the context of diabetes mellitus, interleukin-6 (IL-6) is mostly pro-inflammatory, exacerbating insulin resistance by disrupting insulin signaling pathways in skeletal muscle and adipose tissue [4]. Persistently high IL-6 levels are associated with the onset of microvascular and macrovascular problems, such as diabetic nephropathy and cardiovascular disease [5].

Recent data indicates that vitamin D, a fat-soluble steroid hormone, may regulate immunological responses and inflammatory processes. In addition to its traditional functions in calcium and phosphate regulation, vitamin D influences the immune system by activating the vitamin D receptor (VDR) found on many immune cells, including monocytes, macrophages, dendritic cells, and T lymphocytes [6]. The activation of VDR inhibits nuclear factor-kappa B (NF-κB) signaling pathways, leading to reduced transcription of pro-inflammatory cytokines, such as IL-6 [7]. Observational studies have indicated inverse correlations between vitamin D levels and inflammatory biomarkers in individuals with T2DM [8].

Given the pro-inflammatory state in DM and the proposed role of vitamin D as an immunomodulator, further research is needed to elucidate the effects of vitamin D supplementation on systemic inflammation, particularly IL-6 levels, in diabetic conditions. Animal models of DM provide a controlled environment to investigate these effects and to explore dose-dependent responses.

Therefore, this study aimed to investigate the effects of different doses of vitamin D supplementation on serum vitamin D levels and IL-6 concentrations in DM-induced rats. We hypothesized that higher doses of vitamin D would result in greater increases in serum vitamin D levels and more substantial reductions in IL-6 concentrations, providing experimental support for its potential therapeutic role in managing inflammation in diabetes mellitus

2 Method

This study was conducted at the Animal Laboratory and the Research and Innovation Laboratory, Faculty of Medicine, Universitas Baiturrahmah, Padang, using 15 male Sprague Dawley rats (*Rattus norvegicus*) aged 8–12 weeks with a body weight of 150–200 grams. The rats were divided into three groups, each consisting of five rats. Group A consisted of DM rats without vitamin D supplementation; Group B consisted of DM rats supplemented with vitamin D at a dose of 415 IU/kgBW/day; and Group C consisted of DM rats supplemented with vitamin D at a dose of 1100 IU/kgBW/day.

Type 2 DM induction was initiated by administering a high-fat diet at 30 grams/day for three weeks. After three weeks, each rat was intraperitoneally injected with Streptozotocin (STZ) at a dose of 50 mg/kgBW. Blood glucose levels were measured 10 days after STZ injection to confirm type 2 DM diagnosis (blood glucose >200 mg/dL). Rats confirmed to have type 2 DM were then treated according to their respective group interventions. Vitamin D supplementation was administered via oral gavage at the respective group doses for 30 days. On day 30, the rats were terminated, and blood samples were collected from the heart

for measurement of vitamin D and IL-6 levels. Serum vitamin D levels were measured using a competitive ELISA method (Cat. No. E-EL-0014, Elabscience®, USA), while IL-6 levels were measured using a sandwich ELISA method (Cat. No. E-EL-R0015, Elabscience®, USA).

Data analysis was performed using the Statistical Product and Service Solution (SPSS) software version 26. Data normality was assessed prior to analysis. Normally distributed data were analyzed using ANOVA with post hoc testing. The correlation between vitamin D levels and IL-6 levels was analyzed using Pearson's correlation test. Data are presented as mean ± standard deviation.

3 Results and Discussion

The Kruskal-Wallis test showed significant differences in serum vitamin D levels among the groups ($p = 0.016$). Post-hoc analysis revealed that the group receiving 1100 IU of vitamin D had significantly higher serum vitamin D levels compared to the untreated DM group ($p=0.014$). Similarly, ANOVA indicated significant differences in IL-6 levels among groups ($p=0.017$). Post-hoc tests revealed that vitamin D supplementation reduced IL-6 levels, with the 1100 IU dose showing a greater reduction compared to the untreated group ($p=0.007$). Spearman correlation analysis demonstrated a negative correlation between serum vitamin D and IL-6 levels ($r= -0.542$, $p=0.037$).

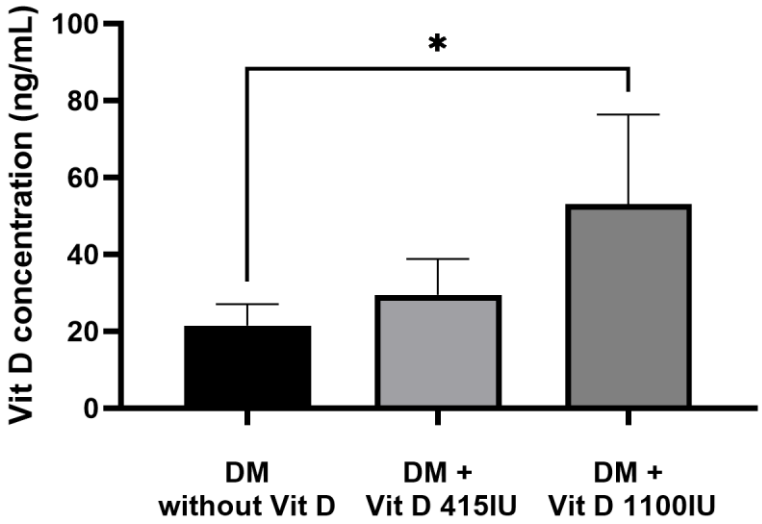

Fig 1. Vitamin D concentration (ng/mL) after 30 days of supplementation (Kruskal-wallis, $p<0.05$)

This study demonstrated that vitamin D supplementation in diabetes mellitus (DM)-induced rats significantly increased serum vitamin D levels and decreased interleukin-6 (IL-6) concentrations, particularly in the group receiving the higher dose (1100 IU). The greater reduction in IL-6 levels in the 1100 IU group suggests a dose-dependent relationship, potentially due to increased VDR activation leading to stronger suppression of pro-inflammatory pathways [9]. Our findings are consistent with Upreti et al. (2018), who reported that vitamin D supplementation reduced inflammatory markers, including IL-6, in patients T2DM with low vitamin D levels [10]. Similarly, Al-Sofiani et al. (2015)

demonstrated that vitamin D supplementation decreased IL-6 levels in individuals with T2DM [11].

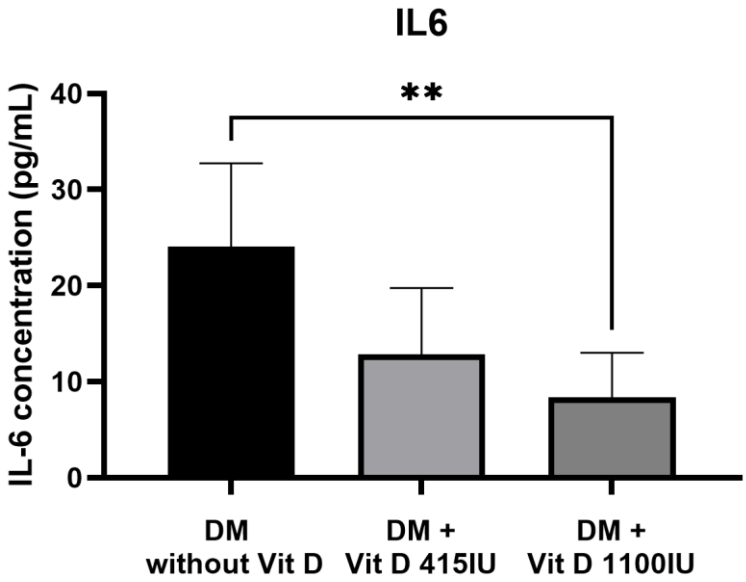

Fig 2. IL-6 concentrations (pg/mL) after 30 days of Vitamin D supplementation (one-way ANOVA, $p<0.05$)

These findings are consistent with the theory that DM triggers systemic inflammation through activation of inflammatory pathways, resulting in elevated levels of pro-inflammatory cytokines such as IL-6. In the context of chronic hyperglycemia, IL-6 contributes to worsening insulin resistance and plays a role in the development of microvascular complications in diabetes [12].

Vitamin D is known to act as an immunomodulator, primarily through the activation of vitamin D receptors (VDR) on immune cells, which suppress the expression of pro-inflammatory genes and inhibit the activation of the NF-κB signaling pathway, a key regulator of IL-6 and other cytokines. NF-κB is a central transcription factor that upregulates several pro-inflammatory cytokines, including IL-6. Vitamin D, through VDR activation, may interfere with the nuclear translocation of NF-κB, thereby suppressing its activity and reducing the downstream expression of inflammatory cytokines. The significant reduction of IL-6 observed in the 1100 IU group supports previous findings that the anti-inflammatory effects of vitamin D may be dose-dependent [5,13,14].

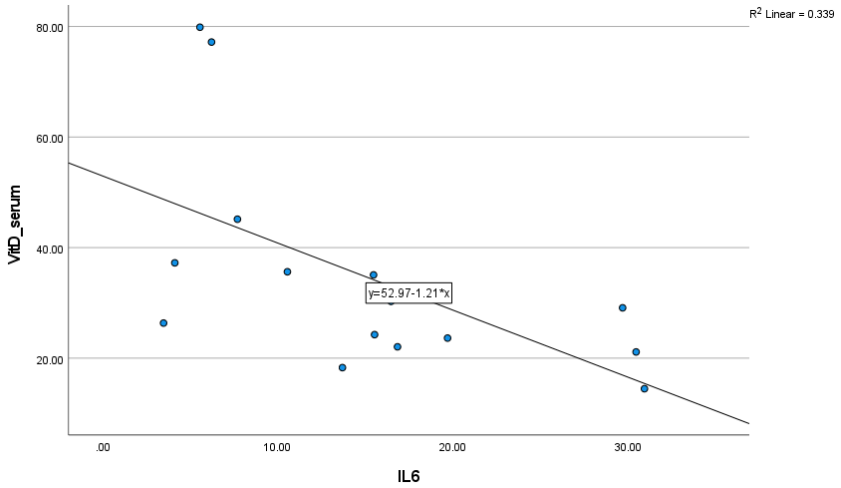

Fig 3. Correlation of Vitamin D concentration and IL-6 levels (Spearman rho, r = -0.542; p = 0.037)

The negative correlation between serum vitamin D and IL-6 levels (r = -0.542; p = 0.037) further supports the hypothesis that higher vitamin D levels directly contribute to the attenuation of inflammation in diabetic conditions. These results highlight the potential of vitamin D not only in calcium homeostasis but also as a therapeutic agent in reducing chronic inflammation associated with diabetes mellitus. Elevated IL-6 levels are consistently observed in patients and experimental models of T2DM and correlate with poor glycemic control and increased risk of microvascular complications [11,12].

Vitamin D influences the balance between pro-inflammatory and anti-inflammatory T cell subsets. By promoting the differentiation of regulatory T cells (Tregs) and favouring a shift towards a Th2 profile, vitamin D indirectly limits the generation of inflammatory cytokines, including IL-6, which are typically secreted by Th1 and Th17 cells [4,7,13].

4 Conclusion

The findings suggest that higher doses of vitamin D may have more pronounced anti-inflammatory effects, highlighting its potential role in managing inflammation in diabetes mellitus. However, it is important to note that while the higher dose resulted in a greater reduction in IL-6, the relationship between dose and effectiveness may not be linear or free from long-term side effects. Therefore, further studies with longer durations and broader dose ranges are necessary to evaluate the optimal safety and efficacy of vitamin D supplementation in diabetic individuals.

This study was funded by Ministry of Education, Culture, Research, and Higher Education of the Republic of Indonesia,

Kurnia Maidarmi Handayani: Methodology, Conceptualization, Writing-original draft. Widia Sari: Methodology, Investigation, Data curation, Writing-review & editing. Rendri Bayu Hansah: Funding acquisition, supervision. Rifkind Malik: Resources, Validation, Project administration, Data curation. Ghaniyyatul Khudri: Validation, Formal analysis.

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