



Effect of Vitamin D Supplementation on Regulatory T Cells of Systemic Lupus Erythematosus: A Systematic Review

Yuniza^{1*}, Radiyati Umi Partan², Krisna Murti³, Zen Hafy⁴

¹Division of Allergy and Clinical Immunology, Department of Internal Medicine,

²Division of Rheumatology, Department of Internal Medicine,

³Department of Anatomical Pathology,

⁴Department of Histology, Faculty of Medicine, Universitas Sriwijaya/Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

ARTICLE INFO

Keywords:

Vitamin D,
Regulatory T Cells,
Systemic Lupus Erythematosus

*Corresponding author:

Yuniza Yuniza

E-mail address:

yuniza@fk.unsri.ac.id

All authors have reviewed and approved the final version of the manuscript.

<https://doi.org/10.37275/IJR.v15i2.251>

ABSTRACT

Background. Systemic lupus erythematosus (SLE) is associated with a global reduction in regulatory T cells (Tregs). Vitamin D supplementation that is efficacious, safe, and reasonably priced has the potential to reduce morbidity in SLE. This study was aimed to systematically assess the efficacy of vitamin D supplementation in enhancing Tregs concentration or Tregs-related marker. **Methods.** A systematic review of the literature for clinical studies was performed on Pubmed, Proquest, and Google Scholar. Studies were assessed for risk of bias using Newcastle Ottawa Scale. All clinical studies that investigated the effects of vitamin D supplementation on Tregs in SLE patients were included. Key evidence was analyzed and qualitatively synthesized. **Results.** Three relevant studies were included. All studies were of high-quality. Vitamin D supplementation consistently increased Tregs concentration across all included studies. **Conclusion.** The current study supports the evidence that vitamin D supplementation enhanced Tregs concentration in SLE patients.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that affects the skin, joints, haematological system, nervous system, serous membranes, and kidneys.¹ Depending on the type and severity of organ involvement, therapeutic options include corticosteroids, nonsteroidal anti-inflammatory drugs, immunosuppressive agents, and/or targeted monoclonal antibodies. However, long-term use of corticosteroids and/or immunosuppressive agents is associated with a high incidence of adverse events and complication, thus increasing morbidity and mortality.² SLE is a T and B cell-dependent disease marked by the presence of

pathogenic autoantibodies, secreted by B cells.³ T cells play a pivotal role in initiating and maintaining B cell function for antibody secretion, particularly against double-stranded DNA.

SLE is associated with a global reduction in regulatory T cells (Tregs).⁴ Tregs have been proposed to control immune homeostasis and autoimmune reactions by attenuating tolerance to self-antigens. A reduction in Treg number or function is thought to favour SLE pathology. According to meta-analysis by Li et al, the percentages of Tregs in active SLE were significantly lower compared to controls when the enrolled SLE patients were diagnosed using the 1997 modified criteria.⁵ Thus, correction of abnormalities in

the number or function of Tregs may thus have significant therapeutic effects in SLE.

According to recent research, SLE disease activity has a significant inverse correlation with serum vitamin D concentration.^{6,7} As a result, it was hypothesized that vitamin D concentration correction may be beneficial in SLE. According to evidence from human observational studies, higher vitamin D levels are associated with greater Tregs and a more immunosuppressive trait.^{8,9} A few preliminary studies involving 30 to 60 participants have demonstrated that oral vitamin D supplementation at doses ranging from 12,000 to 30,000 IU per week enhances absolute Tregs concentrations in peripheral blood as well as Tregs' functional capacity to suppress effector cells in both healthy individuals and patients with autoimmune disease.^{10,11}

Given its potential efficacy, safety, and affordability, vitamin D supplementation has the potential to reduce morbidity in SLE. To date, no published study has reviewed the beneficial effects of vitamin D supplementation on Tregs in SLE. Therefore, the current study was aimed to systematically assess the efficacy of vitamin D supplementation in enhancing Tregs concentration or Tregs-related marker.

2. Methods

This study is a systematic review conducted in accordance to Preferred Reporting Items for Systematic Review and Meta-analysis (PRISMA) guideline. Clinical question was formulated using the PICO framework as follows: population (P), patients with SLE; intervention (I), vitamin D supplementation; comparison (C), any comparator; and outcomes (O), regulatory T cells and disease activity.

The literature search was conducted on July 1st to September 1st, 2022 on PubMed, ScienceDirect, and Google Scholar, using the following keywords: "Systemic Lupus Erythematosus" OR "Lupus" AND "Vitamin D" OR "cholecalciferol" AND "Regulatory T Cells" OR "Tregs" OR "Disease Activity". A bibliography search was also carried out from the studies obtained

by hand searching and snowballing.

According to the inclusion and exclusion criteria, the studies included in this systematic review were those involving individuals with SLE. There was no restriction on publication years. The inclusion criteria included: 1) human studies or in vitro studies of human cells, 2) subjects aged >18 years, and 3) studies including quantification of Tregs or associated markers and related disease activity. In vivo studies on experimental animals, studies not published in English or Indonesian and studies without interventions were excluded from the study selection.

To reduce the possibility of assessment bias, two independent reviewers, YY and KM, evaluated the studies separately. Any disparities were discussed together, and if no agreement was reached, a third reviewer (RU) was consulted. The risk of bias was also minimized by using the Newcastle Ottawa scale (NOS) which was adjusted for each research form.

All gathered studies were thoroughly examined. The suitability of the studies was ensured from the start by screening the title of the study, and then the study's characteristics were presented in tabular form. Extraction was done by evaluating the study abstracts and assessing the importance of the overall quality of the studies obtained. In cases of disagreement between two reviewers, a scoring system would be formulated based on their agreement.

3. Results

Description and Characteristics of Studies

The initial search yielded 2294 s-tudies, and 78 of them were excluded due to duplication in the database. After title and abstract screening, 2191 studies were excluded for the following reasons: 590 literature reviews, 80 books, 22 paediatrics patients, 54 studies in non-SLE autoimmune disease, 447 animal studies and 998 irrelevant original articles (in vitro studies). Subsequently, 20 remaining studies were assessed for full-text eligibility. Furthermore, 17 studies were excluded because they did not include regulatory T cells, leaving three studies for qualitative synthesis. The study selection flow is presented in

Figure 1.

Result of Bias Risk Assessment

Three studies included in the qualitative synthesis consisted of two clinical trials without comparator and one clinical trial. A risk of bias assessment was carried out for the three studies using the Newcastle Ottawa Scale (NOS). The NOS used in this study was adapted to clinical trial, the research design in three studies included. All studies were rated as good based on the NOS assessment and was tabulated in Table 1.

Results of Data Extraction

Of the three studies included in the systematic review, two studies evaluated the change of Tregs after various regimens of vitamin D supplementation and one study compared the dynamics of Treg after two different doses of vitamin D supplementation. All studies were conducted among European SLE patients. The studies included patients diagnosed with SLE according to ACR 1997 or 2012 criteria, had inactive or mild-moderate disease, had a stable dosage of corticosteroids or immunosuppressants, and had

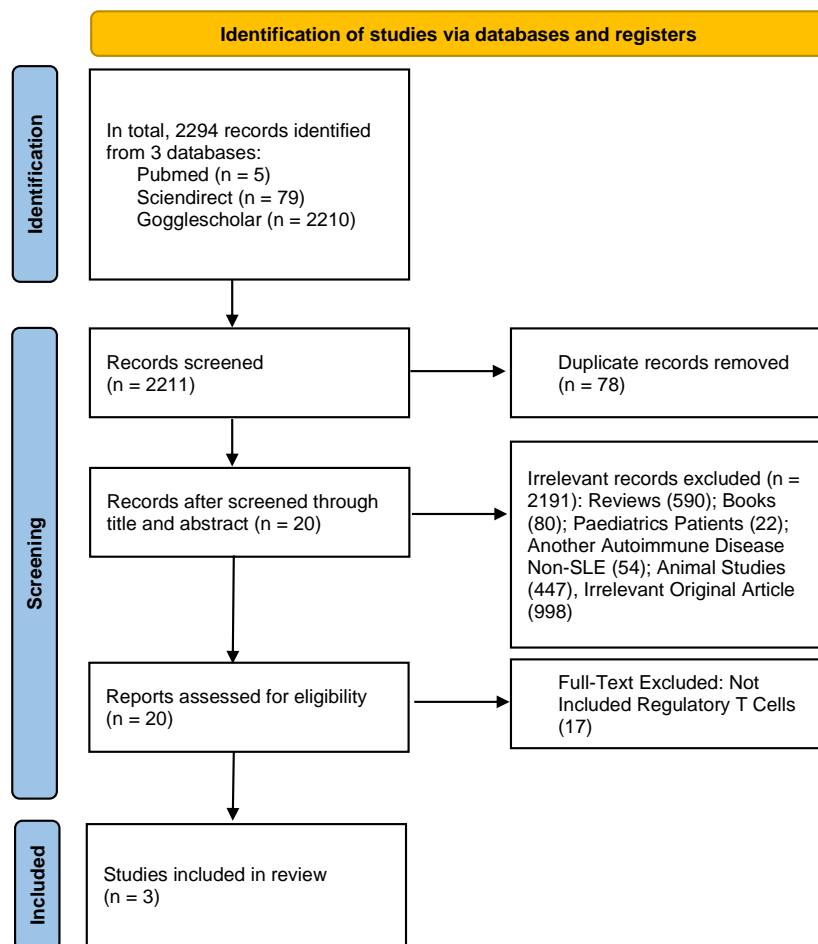


Figure 1. Prisma Flow of Systematic Searching

hypovitaminosis D. Hypovitaminosis D was diagnosed with different cut-off serum vitamin D levels. Meanwhile, the identification of Tregs was assessed using varying markers. The results of data extraction for each study are summarized in Table 1.

4. Discussion

Vitamin D deficiency is now identified as a pandemic¹², and there is compelling corroboration that it plays a role in increasing risk of autoimmune disorders.^{13,14} Vitamin D insufficiency (25-50 nmol/L)

and deficiency (less than 25 nmol/L) were defined differently across studies, and the extent of hypovitaminosis D varied by region. While 25(OH)D levels below 75 nmol/L are not uncommon throughout the world, levels below 25 nmol/L, which represent unequivocal vitamin D deficiency, were most seen in at-risk groups, particularly those with autoimmune diseases such as SLE.^{15,16}

Vitamin D deficiency is common in SLE patients, and it is linked to an increased risk of disease activity, flare-ups, and damage formation.¹⁵ Low vitamin D levels have been linked to increased pro-inflammatory cytokine production, such as IL-6 and TNF- α , and decreased anti-inflammatory cytokine production, such as IL-10.¹⁶ This imbalance can result in chronic inflammation, which is a defining feature of SLE. Vitamin D has immunomodulatory properties that may be useful in SLE. It has the ability to inhibit the production of autoantibodies as well as modulate the activity of T and B lymphocytes,¹⁷ both of which are important in the pathogenesis of SLE. Furthermore, vitamin D can promote the differentiation of regulatory T cells, which play an important role in immune tolerance and the prevention of autoimmunity.¹⁸

Some studies in SLE have found an inverse correlation between SLE disease activity and serum vitamin D levels, but the findings are debatable.¹⁹⁻²¹ Despite this, vitamin D supplementation is now recommended as a management strategy for certain patient populations. However, there was still a lack of data on the effects of its administration on immune regulation in SLE. T cells are well known for initiating and maintaining antibody secretion by B cells. The

higher degree of hypermutation in patients with SLE correlated with autoantibodies development.²² Pathologically altered immune responses are also associated with SLE, with hyperactive B cells playing an important role in its pathogenesis. SLE is a T and B cell-dependent disease associated with a rather functional, not absolute, deficiency of regulatory T cells.²³

Vitamin D has been shown to influence Treg differentiation and function. Vitamin D can help Treg differentiation from naive T cells by increasing the expression of Foxp3, a transcription factor required for Treg development and function.²⁴ Vitamin D can also improve Treg suppressive function by increasing IL-10 and TGF- β production.²⁵ Tregs are known to be deficient in number and function in SLE, contributing to immune tolerance breakdown and the development of autoimmunity.²⁶ In SLE patients, vitamin D supplementation has been shown to restore Treg function.

This review is the first study to summarize the effect of vitamin D supplementation on Tregs among SLE patients. Vitamin D supplementation consistently increased Tregs concentration in SLE patients in all studies, and with a significant increase observed in two studies. In the study by Piantoni et al., a significant increase was shown only for peripheral induced T-regs.²⁷ Further studies are required to evaluate the effects of vitamin D supplementation on Tregs and disease activity in placebo-controlled clinical trials with more representative sample. In an attempt to retain the accessibility and adherence, oral vitamin D supplementation is recommended.³⁰

Table 1. Data Extraction Results

Study, Year	Country	Study Type	Patients Characteristics	Sample Size (n)	Vitamin D Dosage	Comparator	Regulatory T Cells	Results	NOS
Terrier et al, 2012 ²⁸	France	<i>In Vivo</i> , Clinical Trials without Comparator	Diagnosed with ACR 1997 criteria, all women, inactive or mild-moderate disease, stable dosage of CS (for 1 mo) and IS (for 3 mo) with baseline serum 25(OH)D < 30 ng/mL.	20	100 000 IU of cholecalciferol per week for 4 weeks, followed by 100 000 IU of cholecalciferol per month for 6 months.	-	CD3 ⁺ CD4 ⁺ CD25 ^{hi} CD127 ⁻ FoxP3 ⁺ Tregs	The percentage and absolute count of Tregs was significantly increased at 2 nd and 6 th months of follow up. The expression of CD45RA and CD25 on CD4 ⁺ T cells revealed that the increase involved both resting and activated memory Tregs.	7
Piantoni et al, 2015 ²⁷	Italy	<i>In Vivo</i> , Cross-over Clinical Trials	Diagnosed with ACR 1997 criteria, all pre-menopausal women, inactive or mild-moderate disease, absent of flare in previous 6 years.	1 st year: 16 vs 18 2 nd year: 18 vs 16	Intensive Regimen: 300 000 IU of cholecalciferol on first month followed by 50 000 IU of cholecalciferol per month for 11 months (total 850 000 IU annually)	Standard Regimen: 25 000 IU of cholecalciferol per month for 12 months (300 000 IU annually)	CD4 ⁺ CD25 ^{high} CD127 ^{low} Tregs CD4 ⁺ CD25 ^{high} CD127 ^{low} CCR7 ⁻ Peripheral Induced Tregs CD4 ⁺ CD25 ^{high} CD127 ^{low} CD31 ⁺ Thymic Induced Tregs	The percentage and absolute count of Tregs was insignificantly increased at 12 th month in both groups, but peripheral induced Tregs was significantly increased in both groups.	8
Marinho et al, 2016 ²⁹	Portugal	<i>In Vivo</i> , Clinical Trials without Comparator	Diagnosed with ACR 1997 criteria and 2012 revised criteria, most are pre-menopausal women, inactive or mild-moderate disease, absent of flare in previous 1 years.	24 (1 male)	Dosage based on baseline Vitamin D level VitD<50 nmol/L: 50,000 IU/week/8 weeks, then 2000 U/day. Vitamin D 50 -75 nmol/L 4000 UI/day/8 weeks, then 2000 IU/day. Vitamin D >75 nmol/L 2000 IU/day	-	CD4 ⁺ FoxP3 ⁺ Tregs	The percentage and absolute count of Tregs was significantly increased at 6 th month	7

5. Conclusion

The present study supports the evidence that vitamin D supplementation enhanced Tregs concentration in SLE patients. The varied dosages of vitamin D and different methods employed to identify Tregs in SLE patients thus highlight a research gap, prompting further exploration of the effects of certain dosages of vitamin D supplementation on the immunologic parameters of SLE.

6. References

- Abida R, Yeoh SA, Isenberg DA. Advances in systemic lupus erythematosus. *Medicine (Baltimore)*. 2022;50(1):7-17. doi:10.1016/j.mpmed.2021.10.003
- Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. *The Lancet*. 2019;393(10188):2332-2343. doi:10.1016/S0140-6736(19)30237-5
- Bonasia CG, Abdulahad WH, Rutgers A, Heeringa P, Bos NA. B Cell Activation and Escape of Tolerance Checkpoints: Recent Insights from Studying Autoreactive B Cells. *Cells*. 2021;10(5):1190. doi:10.3390/cells10051190
- Miyara M, Amoura Z, Parizot C, et al. Global Natural Regulatory T Cell Depletion in Active Systemic Lupus Erythematosus. *J Immunol*. 2005;175(12):8392-8400. doi:10.4049/jimmunol.175.12.8392
- Li W, Deng C, Yang H, Wang G. The Regulatory T Cell in Active Systemic Lupus Erythematosus Patients: A Systemic Review

- and Meta-Analysis. *Front Immunol.* 2019;10. Accessed September 18, 2022. <https://www.frontiersin.org/articles/10.3389/fimmu.2019.00159>
6. Correa-Rodriguez M, Pocovi-Gerardino G, Callejas-Rubio JL, et al. Vitamin D Levels are Associated with Disease Activity and Damage Accrual in Systemic Lupus Erythematosus Patients. *Biol Res Nurs.* 2021;23(3):455-463. doi:10.1177/1099800420983596
 7. Mak A. The Impact of Vitamin D on the Immunopathophysiology, Disease Activity, and Extra-Musculoskeletal Manifestations of Systemic Lupus Erythematosus. *Int J Mol Sci.* 2018;19(8):2355. doi:10.3390/ijms19082355
 8. Ji J, Zhai H, Zhou H, Song S, Mor G, Liao A. The role and mechanism of vitamin D-mediated regulation of Treg/Th17 balance in recurrent pregnancy loss. *Am J Reprod Immunol.* 2019;81(6):e13112. doi:10.1111/aji.13112
 9. Smolders J, Thewissen M, Peelen E, et al. Vitamin D Status Is Positively Correlated with Regulatory T Cell Function in Patients with Multiple Sclerosis. *PLOS ONE.* 2009;4(8):e6635. doi:10.1371/journal.pone.0006635
 10. Gabbay MAL, Sato MN, Finazzo C, Duarte AJS, Dib SA. Effect of Cholecalciferol as Adjunctive Therapy With Insulin on Protective Immunologic Profile and Decline of Residual β -Cell Function in New-Onset Type 1 Diabetes Mellitus. *Arch Pediatr Adolesc Med.* 2012;166(7):601-607. doi:10.1001/archpediatrics.2012.164
 11. Prietl B, Treiber G, Mader JK, et al. High-dose cholecalciferol supplementation significantly increases peripheral CD4⁺ Tregs in healthy adults without negatively affecting the frequency of other immune cells. *Eur J Nutr.* 2014;53(3):751-759. doi:10.1007/s00394-013-0579-6
 12. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. *Am J Clin Nutr.* 2008;87(4):1080S-6S. doi:10.1093/ajcn/87.4.1080S
 13. Kriegel MA, Manson JE, Costenbader KH. Does Vitamin D Affect Risk of Developing Autoimmune Disease?: A Systematic Review. *Semin Arthritis Rheum.* 2011;40(6):512-531.e8. doi:10.1016/j.semarthrit.2010.07.009
 14. Shoenfeld N, Amital H, Shoenfeld Y. The effect of melanism and vitamin D synthesis on the incidence of autoimmune disease. *Nat Clin Pract Rheumatol.* 2009;5(2):99-105. doi:10.1038/ncprheum0989
 15. ARSHAD A, MAHMOOD SBZ, AYAZ A, Al Karim MANJI A, AHUJA AK. Association of vitamin D deficiency and disease activity in systemic lupus erythematosus patients: Two-year follow-up study. *Arch Rheumatol.* 2020;36(1):101-106. doi:10.46497/ArchRheumatol.2021.8178
 16. Roffe-Vazquez DN, Huerta-Delgado AS, Castillo EC, et al. Correlation of Vitamin D with Inflammatory Cytokines, Atherosclerotic Parameters, and Lifestyle Factors in the Setting of Heart Failure: A 12-Month Follow-Up Study. *Int J Mol Sci.* 2019;20(22):5811. doi:10.3390/ijms20225811
 17. Sanlier N, Guney-Coskun M. Vitamin D, the immune system, and its relationship with diseases. *Egypt Pediatr Assoc Gaz.* 2022;70(1):39. doi:10.1186/s43054-022-00135-w
 18. Gallo D, Baci D, Kustrimovic N, et al. How Does Vitamin D Affect Immune Cells Crosstalk in Autoimmune Diseases? *Int J Mol Sci.* 2023;24(5):4689. doi:10.3390/ijms24054689
 19. Khairallah MK, Makarem YS, Dahpy MA. Vitamin D in active systemic lupus erythematosus and lupus nephritis: a forgotten player. *Egypt J Intern Med.*

- 2020;32(1):16. doi:10.1186/s43162-020-00016-x
20. Petri M, Bello KJ, Fang H, Magder LS. Vitamin D in SLE: Modest Association with Disease Activity and Urine Protein/Creatinine Ratio. *Arthritis Rheum.* 2013;65(7):1865-1871. doi:10.1002/art.37953
 21. Schoindre Y, Jallouli M, Tanguy ML, et al. Lower vitamin D levels are associated with higher systemic lupus erythematosus activity, but not predictive of disease flare-up. *Lupus Sci Med.* 2014;1(1):e000027. doi:10.1136/lupus-2014-000027
 22. Giltiay NV, Chappell CP, Clark EA. B-cell selection and the development of autoantibodies. *Arthritis Res Ther.* 2012;14(Suppl 4):S1. doi:10.1186/ar3918
 23. Moulton VR, Suarez-Fueyo A, Meidan E, Li H, Mizui M, Tsokos GC. Pathogenesis of Human Systemic Lupus Erythematosus: A Cellular Perspective. *Trends Mol Med.* 2017;23(7):615-635. doi:10.1016/j.molmed.2017.05.006
 24. Fisher SA, Rahimzadeh M, Brierley C, et al. The role of vitamin D in increasing circulating T regulatory cell numbers and modulating T regulatory cell phenotypes in patients with inflammatory disease or in healthy volunteers: A systematic review. *PLoS ONE.* 2019;14(9):e0222313. doi:10.1371/journal.pone.0222313
 25. Di Liberto D, Scazzone C, La Rocca G, et al. Vitamin D increases the production of IL-10 by regulatory T cells in patients with systemic sclerosis. *Clin Exp Rheumatol.* 2019;37 Suppl 119(4):76-81.
 26. Mizui M, Tsokos GC. Targeting Regulatory T Cells to Treat Patients With Systemic Lupus Erythematosus. *Front Immunol.* 2018;9. Accessed April 13, 2023. <https://www.frontiersin.org/articles/10.3389/fimmu.2018.00786>
 27. Piantoni S, Andreoli L, Scarsi M, et al. Phenotype modifications of T-cells and their shift toward a Th2 response in patients with systemic lupus erythematosus supplemented with different monthly regimens of vitamin D. *Lupus.* 2015;24(4-5):490-498. doi:10.1177/0961203314559090
 28. Terrier B, Derian N, Schoindre Y, et al. Restoration of regulatory and effector T cell balance and B cell homeostasis in systemic lupus erythematosus patients through vitamin D supplementation. *Arthritis Res Ther.* 2012;14(5):R221. doi:10.1186/ar4060
 29. Marinho A, Carvalho C, Boleixa D, et al. Vitamin D supplementation effects on FoxP3 expression in T cells and FoxP3+/IL-17A ratio and clinical course in systemic lupus erythematosus patients: a study in a Portuguese cohort. *Immunol Res.* 2017;65(1):197-206. doi:10.1007/s12026-016-8829-3
 30. Rothen JP, Rutishauser J, Walter PN, Hersberger KE, Arnet I. Oral intermittent vitamin D substitution: influence of pharmaceutical form and dosage frequency on medication adherence: a randomized clinical trial. *BMC Pharmacol Toxicol.* 2020;21(1):51. doi:10.1186/s40360-020-00430-5