

ASSOCIATION OF VITAMIN D WITH LYMPHOCYTE COUNT AND CALCIUM LEVELS IN PATIENTS WITH SJÖGREN SYNDROME PATIENTS

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ABSTRACT

Background: Previous studies have shown that there is a lower vitamin D levels in patients with Sjögren syndrome (SS) compared to healthy people. Lymphopenia is one of the frequent occurrences of SS, which is associated with the disease activity. However, there are limited that examine association between vitamin D levels, lymphocyte count, and serum calcium levels in patients with SS.

Aim: This study aimed to determine association between vitamin D levels with lymphocyte count and calcium levels in patients with SS.

Methods: A cross-sectional design that includes 24 outpatients with Sjögren syndrome (SS) was adopted at Dr. Moewardi General Hospital from January 2021 to December 2022. The ELFA method (ng/ml) was used to obtain the measurement of vitamin D levels while the flow cytometer method (%) was used to determine lymphocyte count. Calcium levels was determined using the ISE direct method (mmol/L) and the normality test uses Shapiro-Wilk, followed by the Spearman rank test.

Results: Association between vitamin D and lymphocyte count in Sjögren syndrome (SS) patients was $r = 0.066$, with a $p\text{-value} = 0.760$ ($p\text{-value} > 0.05$). Furthermore, association between vitamin D and calcium levels in patients was $r = -0.023$ with a $p\text{-value} = 0.914$ ($p\text{-value} > 0.05$).

Conclusion: In conclusion, vitamin D was not related to lymphocyte count and blood calcium levels in Sjögren syndrome (SS) patients.

Keywords: calcium serum; lymphocyte count; Sjögren syndrome; vitamin D

INTRODUCTION

Sjögren syndrome (SS) is a systemic inflammatory autoimmune disease that primarily attacks the tear and salivary glands. The etiology of SS remains unclear and its characteristic is the presence of sicca symptoms, which can be accompanied by other organ manifestations. It occurs most often in women with a ratio of 9:1 to 19:1, with a global prevalence of around 0.1-0.6% [1,2]. The specific cause of SS disease remained unknown, though several factors contribute, including environment, genetics, hormonal, changes in B lymphocyte, or innate immunity. Furthermore, its pathogenesis is quite complex, including genetic factors, immune cell activation, and autoantibody production [3,4].

Vitamin D has both calcemic and non-calcemic actions in the body. In its calcemic action, vitamin D increases calcium concentrations by inducing the proteins in active intestinal absorption and phosphate absorption. It also maintains normal blood calcium concentrations by

mobilizing calcium from bones (requires parathyroid hormone) and induces calcium reabsorption in the distal renal tubules (also requires hormone parathyroid). The compound $1,25(\text{OH})_2\text{D}_3$ also plays a significant role in modulating the innate and adaptive immune and influencing the endocrine system [2].

Association was observed between vitamin D deficiency and supplementation and immune-mediated diseases. Several studies have shown that patients with autoimmune diseases have lower $25(\text{OH})\text{D}_3$ levels than healthy populations. However, the cause-and-effect association remains unclear, whether low levels of $25(\text{OH})\text{D}_3$ have a risk of autoimmune disease or are a consequence of the disease [2].

Sha et al. [5] showed that SLE patients had lower total serum calcium levels than normal controls. The study also showed that serum calcium levels were negatively correlated with SLE disease activity. Calcium homeostasis is regulated by $1,25(\text{OH})_2\text{D}_3$, which is the active form of vitamin D. Autoimmune patients, including SS, often experience vitamin D defi-

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ciency, necessitating supplementation in autoimmune disease activity [5]. Available evidence suggests that vitamin D levels in SS patients are lower than in normal controls. However, there is a need for more studies to determine the mechanisms underlying the importance of vitamin D in the development and degree of SS activity. Serum calcium levels and their role in SS disease need further investigation due to the importance of calcium signaling in autoimmune diseases and vitamin D levels. Until now, no study has measured serum calcium levels in SS patients.

METHODS

Sample and study design

This study uses an observational analytical method with a cross-sectional design. The subjects were 24 SS patients treated at Dr. Moewardi General Hospital from January 2021 to December 2022. These subjects include outpatients at the Internal Medicine Polyclinic at RSUD Dr. Moewardi Surakarta, who is ≥ 18 years old. Medical records of patients, including vitamin levels, lymphocyte count, and serum calcium levels. Outpatients diagnosed with SS aged ≥ 18 years at Dr. Moewardi General Hospital from January 2021 to December 2022 were included in the study. Patients underwent laboratory examinations of vitamin D levels, lymphocyte count, and serum calcium levels. On the other hand, the exclusion criteria are patients who are experiencing infections, suffering from chronic kidney disease, or hematological malignancies.

SS patients

SS patients met the diagnostic criteria according to the American European Consensus Group criteria (AECG) or ACR/EULAR. All of these patients were diagnosed with SS from January 2021 to December 2022.

Vitamin D levels

The measurement of serum vitamin D levels was carried out with the mini vidas device using the ELFA (Enzyme Linked Fluorescent Assay) method. ELFA uses a fluorogenic compound as a substrate, where the

presence of antigen and antibody complexes causes a color glow (fluorescence). Furthermore, it is measured by a fluorometer with appropriate excitation and emission filters at specific wavelengths.

Lymphocyte count

Lymphocyte count was the total levels of lymphocyte from the number of leukocytes. Normal levels range from 22.00 to 44.00%, and the measuring instrument used is a flow cytometer.

Serum calcium levels

Calcium levels are the amount of calcium in venous blood (serum) expressed in units (mmol/L), and measured using the ISE (Ion Selective Electrode) method. The normal value of calcium level is 1.17-1.29 mmol/L.

Ethical Approval

Ethical approval with number: 1.596/VIII/HREC/2023 was obtained from the Health Research Ethics Committee of Dr. Moewardi General Hospital.

Statistic analysis

Data were analyzed using the Statistical Product and Service Solution (SPSS) version 26.00 for Windows. First, vitamin D levels, lymphocyte count, and serum calcium levels were evaluated using descriptive analysis. Second, the Shapiro-Wilk test was used to evaluate data normality and homogeneity. Data is said to meet the normality assumption when the Shapiro-Wilk test has a p-value > 0.05 . This normality test was used as the basis for selecting a correlation analysis to determine association between vitamin D and lymphocyte count and calcium levels. Pearson's product-moment was used for normally distributed data and Spearman's rank was adopted when the data is not normal.

RESULTS

Description of basic data characteristics of study subjects

A total of 24 SS patients participated in this study and the basic description of this data includes vitamin D, lymphocyte count, and serum calcium levels.

Table 1. Base Characteristics of Research Subjects

Variable	Mean \pm sd	Minimum	Maximum
Vitamin D levels	29.11 \pm 21.00	3.90	81.60
Lymphocyte count	25.75 \pm 15.30	4.80	79.60
Calcium levels	1.21 \pm 0.07	0.98	1.34

Based on Table 1, SS patients who checked their vitamin D levels received an average of 29.11 \pm 21.00 ng/ml with a range between 3.90 ng/ml to 81.60 ng/ml. In lymphocyte count examination, the average was 25.75

\pm 15.30%, ranging from 4.80% to 79.60%, and calcium level was 1.21 \pm 0.07 mmol/L with a range between 0.98 mmol/L to 1.34 mmol/L.

Normality test

The result in Table 2 shows that the variables, namely vitamin D, lymphocyte count, and calcium levels from the Saphiro-wilk test obtained a p-value <0.05,

or the data is not normally distributed. Therefore, the Spearman rank test was used to determine the correlation between variables.

Table 2. Normality Test

Variable	Shapiro-Wilk		Note
	Statistic	p-value	
Vitamin D levels	0.913	0.040	Abnormal
Lymphocyte count	0.848	0.002	Abnormal
Calcium levels	0.888	0.012	Abnormal

Association between vitamin D and lymphocyte count in SS patients

The scatter plot showed the overview of the data on association between vitamin D and lymphocyte count with the following results:

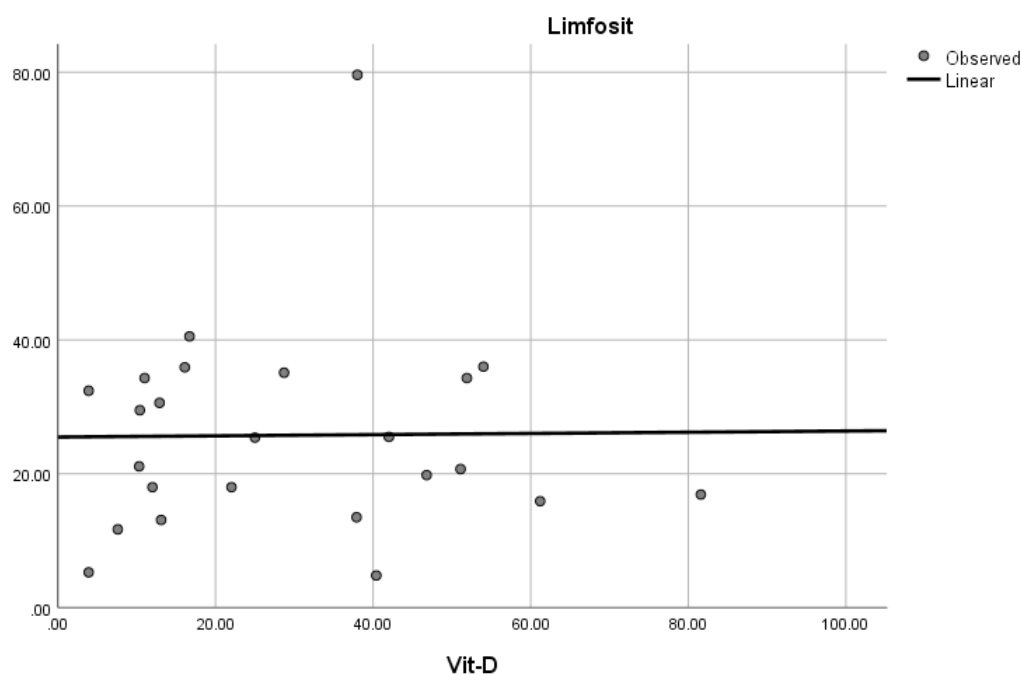


Figure 1. Scatterplot of the Association between Vitamin D and Lymphocyte Count

The scatter plot distribution of association between vitamin D and lymphocyte count forms shows a linear line from bottom left to top right. This indicate an increase in vitamin D levels and lymphocyte count. This shows that there is a positive association between vitamin D and lymphocyte count. However, the slope of the linear line is almost horizontal, showing a very weak correlation between the two variables.

The Spearman rank test was used to determine the correlation between vitamin D and lymphocyte count because these two variables did not meet the assumption of normality. The results of statistical analysis of association between vitamin D and lymphocyte count are as follows:

Table 3. Association between Vitamin D and Lymphocyte Count in SS Patients

Variable	Lymphocyte Count	
	r	p-value
Vitamin D level	0,066	0.760

Note: Spearman rank correlation test

Based on Table 3, the correlation between vitamin D and lymphocyte count in SS patients has a value of $r=0.066$, showing a positive and very weak association ($r=0.000-0.199$). The $p\text{-value} = 0.760 > 0.05$ shows association is not statistically significant. Therefore, the hypothesis that states “there is association between vitamin D levels and lymphocyte count in SS patients” is not proven.

Association between vitamin D and calcium levels in SS patients

An overview of the data on association between vitamin D and calcium levels in SS patients is shown in a scatter plot with the following results:

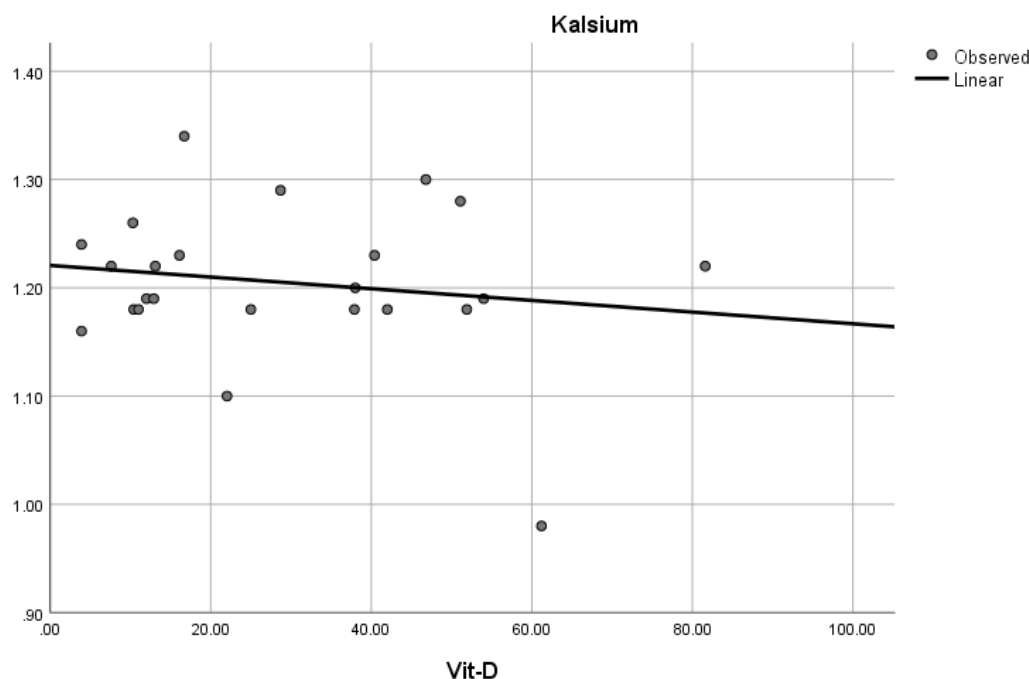


Figure 2. Scatterplot of the Association between Vitamin D and Calcium Levels

The scatter plot data distribution of association between vitamin D and serum calcium levels in Figure 2 forms a linear line from top left to bottom right. This shows that an increase in vitamin D levels is accompanied by a decrease in calcium. However, the slope of the linear line is almost horizontal, showing a very weak association between the two variables.

The Spearman rank test was used to determine the correlation between vitamin D and calcium levels because both variables were not normally distributed. The statistical analysis results of association between vitamin D and calcium are as follows:

Table 4. Association between Vitamin D and Calcium Levels in SS Patients

Variable	Calcium	
	r	p-value
Vitamin D level	-0,023	0.914*

Note: Spearman rank correlation test: *) significant at $p < 0.05$

Based on Table 4, association between vitamin D and calcium levels in SS patients has a value of $r=-0.023$, showing a negative and very weak association ($r=0.023-0.914$). The corresponding $p\text{-value}$ of $0.914 > 0.05$ shows that association is not statistically significant. Therefore, the hypothesis that “there is association between vitamin D levels and calcium in SS patients” is not proven.

DISCUSSION

In this study, the average result of examining vitamin D levels was 29.11 ng/ml, showing that average SS patients had insufficiency. This result is consistent with the report of Radic et al. [2] and Erten et al. [6] that SS patients have lower vitamin D levels than healthy controls. Sandhya et al. [7] measured vitamin D levels in 235 patients with pSS at a tertiary hospital

in South India and the results showed that 60%, 25.5%, and 14.5% experienced vitamin D deficiency, insufficiency, and deficiency, respectively. A cohort found association between low vitamin D levels and peripheral neuropathy and the presence of lymphoma [8]. Low vitamin D levels (≤ 30 ng/ml) were also associated with a higher risk of positive oral biopsy grade and Rheumatoid Factor (RF). However, vitamin D/25(OH)D levels were associated with lower ESSDAI scores and exhibited reduced impact on the lung [7].

The presence of peripheral lymphopenia in SS patients is associated with higher disease activity and mortality. Patients with lymphopenia also have a higher risk of developing non-Hodgkin's lymphoma. Lymphopenia is a disease that occurs in SS patients due to insufficient CD4+ T cells. In healthy individuals, the production of T cells by the thymus and the proliferation of peripheral T cells compensate for the naturally occurring decrease [9].

The result of this study showed a positive but weak association between vitamin D levels in SS patients and lymphocyte count. This implies that lower vitamin D levels will reduce lymphocyte count. Although not significantly significant, these results may have clinical significance. A decrease in vitamin D is associated with an increased risk of lymphoma and peripheral neuropathy in SS patients. According to Lee et al. [10], ESSDAI was negatively correlated with serum vitamin D levels in SS patients. This implies that lower vitamin D levels result in an increased degree of SS activity. The presence of a lower lymphocyte count/lymphopenia was also associated with increased SS activity and mortality. The compensatory mechanism for the decrease in lymphocyte in SS patients by the thymus is still unknown. Therefore, further studies are needed to determine the mechanisms underlying the role of vitamin D in the development and degree of SS activity. Calcium signaling plays a significant role in many pathways related to immunotolerance and inflammation. It contributes to the development and survival of B cells, which is an important aspect of immunotolerance. Calcium signaling can regulate the activation of cGMP-AMP synthase from the interferon gene to modulate innate immunity through type 1 interferon. An abnormal calcium signaling is associated with several autoimmune diseases due to its effect on innate and adaptive immunity. This emphasizes the significance of investigating the correlation between serum calcium levels and disease activity in SLE patients. The result of this study showed that low serum calcium levels were negatively correlated with SLE disease activity. Calcium response in lymphocyte of SLE patients increases along with the role of antigen receptors. In SLE patients T lymphocyte, binding to the T cell receptor increased the production of inositol 1,4,5-triphosphate (IP₃) and calcium release from the endoplasmic reticulum. The responses of B cell receptor-mediated calcium also increase [5]. However, the result did not show association between vitamin D and blood calcium levels in SS patients. It can be concluded that calcium levels cannot be directly linked

to the degree of SS disease activity, even though it is related to levels of vitamin D.

The limitations of this study include the small sample size due to the low prevalence of SS and the absence of a positive control group. Future studies with a larger sample size including other centers with a control group and measuring the degree of SS disease activity are recommended.

CONCLUSION

In conclusion, vitamin D levels were not related to lymphocyte count and blood calcium levels in SS patients. Consequently, more significant studies are required along with the inclusion of variables that play a greater role in the inflammatory process, to establish them as markers of SS disease activity.

Data Availability Statement

All data relevant to the study are included or uploaded as online supplementary information. The data that support the results of this study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare no competing interests.

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REFERENCES

1. Stefanski A, Tomiak C, Pleyer U et al. The diagnosis and treatment of Sjögren's syndrome. *Dtsch Arztebl Int* 2017;114: 354–61.
2. Radić M, Kolak E, Dogas H et al. Vitamin D, and Sjögren's disease: revealing the connections-A systematic review and meta-analysis. *Nutrients* 2023; 15:497.
3. Espinoza-espinoza LA, Moreno-Quispe LA, Moreno RM. Vitamins A, D, and E in the primary Sjögren syndrome compared with the control group. *Saudi J Biomed Res* 2019;4(1):1–4.
4. Athanassiou P, Mavragani C, Athanassiou L et al. Vitamin D deficiency in primary Sjögren syndrome: association with clinical manifestations and im-

- mune activation markers. *Mediterr J Rheumatol* 2022;33(1):106-8.
5. Sha Y, Rui Z, Dong Y et al. Total serum calcium level is negatively correlated with systemic lupus erythematosus activity. *Dose Response* 2020;18(2):1559325820926764.
 6. Erten S, Sahin A, Altunoğlu A et al. Comparison of plasma vitamin D levels in patients with Sjögren's syndrome and healthy subjects. *Int J Rheum Dis* 2014;18(1):70-5.
 7. Sandhya P, Mahasampath G, Mashru P et al. Vitamin D levels and associations in Indian patients with primary Sjögren's syndrome. *J Clin Diagn Res* 2017;11(9):OC33-OC36.
 8. Agmon-Levin N, Kivity S, Tzioufas AG et al. (2012). Low levels of vitamin D are associated with neuropathy and lymphoma among patients with Sjögren's syndrome. *J Autoimmun* 2012;39(3),234-9.
 9. Fessler J, Fasching P, Raicht A et al. Lymphopenia in primary Sjögren's syndrome is associated with premature aging of naïve CD4+ T cells. *Rheumatol* 2020; 0:1-10.
 10. Lee SJ, Oh HJ, Choi BY et al. Serum 25-hydroxyvitamin D₃ and BAFF levels are associated with disease activity in primary Sjögren's syndrome. *J Immunol Res* 2016;5781070

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