Association between vitamin D, oestradiol and interferon-gamma in female patients with inactive systemic lupus erythematosus: A cross-sectional study

Visnja Kokic¹, Dusanka Martinovic Kaliterna², Mislav Radic², Leida Tandara³ and Dijana Perkovic²

Abstract
Objectives: To investigate possible associations between 25-hydroxyvitamin D₃ (25(OH)D₃), oestradiol (E₂) and IFN-gamma (IFNγ) in female patients with inactive systemic lupus erythematosus (SLE).

Methods: Female patients with inactive SLE and age-matched healthy controls were recruited into this cross-sectional study. Serum concentrations of 25(OH)D₃, E₂ and IFNγ were measured by radioimmunoassay with gamma-counters and enzyme-linked immunosorbent assay.

Results: 36 patients and 37 controls were enrolled. In patients with SLE, the concentration of 25(OH)D₃ was lower and E₂ was higher compared with controls. In vitamin D deficient (i.e., 25(OH)D₃ < 20 ng/ml) patients, IFNγ was 150% higher compared with patients with 25(OH)D₃ > 20 ng/ml and controls. The concentration of E₂ was higher in all patients compared with controls independently of the vitamin D level. A difference was found between patients and controls in the correlation of 25(OH)D₃ with E₂ and a positive correlation was found between E₂ and IFNγ in all participants.

Conclusions: Our results suggest that E₂ may have a strong modulating effect on vitamin D function which is significant only at low concentration of E₂.

¹Division of Endocrinology, Diabetes and Metabolic Disease, University Hospital of Split, School of Medicine, Split, Croatia
²Division of Rheumatology and Clinical Immunology, University Hospital of Split, Split, Croatia
³Department of Medical Laboratory Diagnostic, University Hospital of Split, Split, Croatia

Corresponding author: Visnja Kokic, Spinčićeva 1, Split 21000, Croatia. Email: kokicvisnja@gmail.com
Keywords
Systemic lupus erythematosus, Vitamin D, interferon-gamma, oestradiol

Introduction
Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease and involves the loss of tolerance to nuclear self-antigens, immune complex formation and chronic inflammation.1 The pathogenesis of SLE consists of a complex of cross-talk mediated by cytokines that orchestrate immune cell interactions.2 Studies have shown that interferon gamma (INFγ) plays an important role in the development and severity of SLE.3 In humoral immunity, IFNγ stimulates B-cell activation to induce immunoglobulin secretion, while an in cell-mediated immune response from IFNγ directs differentiation of T cells to a Th1 phenotype.4–6

The vitamin D status of an individual depends on access to vitamin D through dietary intake and epidermal synthesis from ultra-violet (UV) light exposure. Importantly, vitamin D reduces the risk for several diseases including severe infections, cancer and autoimmune rheumatic diseases because it regulates both innate and adaptive immunity.7 Studies have demonstrated vitamin D receptor (VDR) expression in both T- and B-lymphocytes.8 In addition, 1,25-hydroxy vitamin D3 (1,25[OH]2D3) has been shown in vitro to inhibit the action of cytokines produced by Th1 immune cells.9 Moreover, a direct effect of 1,25(OH)2D3 on B-cell homoeostasis has recently been confirmed.10 Therefore, vitamin D may have a role in B-cell-related autoimmune disorders such as SLE.

Reports suggest that patients with SLE have alterations in steroid hormone metabolism.11 It has been demonstrated that oestrogen effects are probably mediated through oestrogen receptors α and β, which are expressed in a wide range of immune cells and are involved in innate and adaptive immune responses.12 Oestrogens have specific effects on T and B cell maturation, dendritic cells and peripheral blood mononuclear cells.13 They also cause rapid maturation of B cells in bone marrow where they cause auto-reactive B cell deletion to become less efficient.14

In a previous study in females of child-bearing age with inactive SLE, an association between vitamin D, oestradiol (E2), and IFNγ was not completely established.15 Therefore, the aim of this study was to clarify the relationship further.

Methods
This was a cross-sectional study which conformed to recommendations by the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).16 Female patients with inactive SLE and age-matched healthy controls were recruited at the Daily Hospital, Division of Rheumatology and Immunology of the Clinical Hospital Centre in Split, Croatia, from June to September 2014. The study complied with the Declaration of Helsinki and was approved by the Ethical Committee of Split University Medical School for clinical studies on human subjects. Written informed consent was obtained from all participants.

Inclusion criteria were: diagnosis of SLE according to American College of Rheumatology (ACR) criteria;17 generative age (i.e., from puberty to menopause), 24h proteinuria <150 mg/day; maximum dose
of glucocorticoids ≤5 mg/day for at least 12 months; Systemic Lupus Erythematosus Disease Activity Index (SLEDAI)\(^{18}\) scores ≤2, for at least one year. Exclusion criteria included: hormonal replacement therapy; immunosuppressive drugs (e.g., azathioprine, methotrexate); anticoagulants; vitamin D supplements; smoking; primary and secondary hyperparathyroidism.

Blood samples were taken from all participants in the morning between 7:00 and 8:00. Antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-Smith (anti-Sm), parathyroid hormone (PTH) and complement components C3, and C4, were measured in patients with SLE. Reference values were: ANA- negative U/ml; anti-dsDNA > 40 IU/ml; anti-Sm > 40 AU/ml; complement C3 (0.9–1.8 g/L) and C4 (0.1–0.4 g/L). Complement components were determined using a laser nephelometry (ProSpec nephelometer, Dade Behring, Siemens Healthcare Diagnostics, Liederbach, Germany).

Circulating levels of 25(OH)D\(_3\) provide a direct reflection of vitamin D status.\(^{19}\) Serum concentrations of 25(OH)D\(_3\) were measured in samples from all participants using radioimmunoassay (RIA) and a gamma-counter (DIA source Immunoassays, Louvain-la-Neuve, Belgium catalogue number KIP 1961; P. I. Number 1700543/en; Revision nr.:130729/1). Intra-assay variation was 8.7% and inter-assay variation was 7.3%. Vitamin D deficiency was defined as <20 ng/ml, insufficiency was 21–29 ng/ml and normal range was 30–80 ng/ml.\(^{19}\) Serum concentrations of E\(_2\) were also measured using RIA and gamma-counter and intra-assay variation was 6.3%, and inter-assay variation was 10.3%. Oestrogen status was assessed from samples taken from the 3rd–5th day of the menstrual cycle. Reference values for E\(_2\) were between 0.11–0.65 nmol/L. Levels of IFN\(_\gamma\) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (eBioscience, San Diego, CA, USA) which had a detection limit of 0.99 pg/ml.

**Statistical analyses**

Sample size was estimated using PASS software (Release 11, NCSS, LLC. Kaysville, Utah, USA; 2011). Using a standard deviation (SD) of 2.08 ng/ml for the concentration of 25(OH)D\(_3\) with \(z=0.05\) and \(1-\beta\), it was estimated that 36 patients with SLE were required for the study.\(^{15}\) Statistical analyses were performed using PASS software (Release 11, NCSS, LLC. Kaysville, Utah, USA; 2011) and \(P<0.05\) was taken to indicate statistical significance.

Differences in serum 25(OH)D\(_3\), IFN\(_\gamma\), and E\(_2\) between patients and controls were analysed using the Mann–Whitney U test and area under the curve (AUC) was used as the measure of standardized effect together with absolute and relative median differences. Differences between four groups (i.e., low and high levels of vitamin D in patients and controls) were analysed using the Kruskal–Wallis test. Statistical significance from post-hoc tests were corrected by the Holm-Bonferroni method. Correlations between numeric variables were analysed using Spearman’s rank correlation. Cut-off values for serum 25(OH)D\(_3\), IFN\(_\gamma\), and E\(_2\) were determined using Receiver Operating Characteristic (ROC) curve analysis and Youden’s index J. The independent association of serum 25(OH)D\(_3\), IFN\(_\gamma\) and E\(_2\) levels with SLE was assessed using multivariate binary logistic regression.

A measurable increase in SLE disease activity in one or more organ systems involving new or worse clinical signs and symptoms and/or laboratory measurements was deemed an SLE flair.\(^{20}\) The moderating effect of serum E\(_2\) on the association of serum 25(OH)D\(_3\) and SLE flair was analysed using “Process” release 2.12 (Andrew
F. Hayes, Ohio State University, 2014). Serum E₂ values defining the region of a significant association between serum 25(OH)D₃ and SLE flair were assessed by the Johnson–Neyman technique as implemented in “Process”.

Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using a multivariate, binary logistic regression model to determine the disease characteristics associated with vitamin D deficiency, IFN-γ and E₂ levels.

Results

In total, 36 female patients with inactive SLE and 37 age-matched healthy controls were included in the study. Demographic and clinical characteristics of the participants are shown in Table 1. The median value of 25(OH)D₃ was statistically significantly lower in patients with SLE compared with controls (P = 0.001), while the median values for IFN-γ and E₂ were statistically significantly higher in patients with SLE compared with controls (P < 0.001).

Participants were further divided into two groups based on 25(OH)D₃ levels (i.e., ≤20 ng/ml and >20 ng/ml). In the control group, there were no differences in IFN-γ, E₂, and PTH between participants with 25(OH)D₃ ≤20 ng/ml or >20 ng/ml and so data were combined and compared with the two SLE subgroups (Table 2). Patients in 25(OH)D₃ ≤20 ng/ml sub group had statistically significantly higher levels of IFN-γ compared with patients in the 25(OH)D₃ >20 ng/ml sub-group or control group (P < 0.001). Both the ≤20 ng/ml and >20 ng/ml 25(OH)D₃ sub-groups of patients had statistically significantly higher levels of E₂ than the control group (P = 0.009 and P = 0.035, respectively).

For all participants, the correlation between E₂ and IFN-γ was positive and

Table 1. Demographic and clinical characteristics in patients with systemic lupus erythematosus (SLE) and controls.

|                            | SLE patients (n = 36) | Control group (n = 37) | Median difference | Statistical significance* | AUC  |
|---------------------------|----------------------|------------------------|-------------------|--------------------------|------|
| Age, years                | 40 (33–43)           | 39 (32–42)             |                   |                          |      |
| Disease duration, years   | 10 (8–13)            | –                      |                   |                          |      |
| Glucocorticoids, mg       | 2.3 (0.00–3.63)      | –                      |                   |                          |      |
| 25 (OH)D₃, ng/ml          | 16.5 (12.7–20.9)     | 22.9 (18.1–26.4)       | -6.4              | 28%                      | P = 0.001 |
| IFN-γ, pg/ml              | 2.4 (1.0–5.7)        | 1.0 (1.0–1.0)          | 2.4               | P < 0.001                | 0.77 |
| E₂, nmol/l                | 0.53 (0.36–0.61)     | 0.33 (0.29–0.40)       | 0.20              | 61%                      | P < 0.001 |
| PTH, pg/ml                | 13.9 (12.4–16.5)     | 15.1 (13.1–16.9)       | -1.2              | 8%                       | n.s. |
| ANA, U/ml                 | 26 (72.2)            | –                      |                   |                          |      |
| Anti-dsDNA, IU/ml         | 44.5 (7.3–245.3)     | –                      |                   |                          |      |
| Anti-dsDNA, >40 IU/ml     | 19 (52.8)            | –                      |                   |                          |      |
| Anti-Sm, AU/ml            | 44.5 (7.3–245.3)     | –                      |                   |                          |      |
| Anti-Sm, >40AU/ml         | 2 (5.6)              | –                      |                   |                          |      |
| C₃, g/l                   | 0.84 (0.73–0.95)     | –                      |                   |                          |      |
| C₄, g/l                   | 0.13 (0.09–0.20)     | –                      |                   |                          |      |

Data are presented as median (interquartile range) or n (%). 25(OH)D₃, 25-hydroxy vitamin D₃; IFN-γ, interferon gamma; E₂, oestradiol; PTH, parathyroid hormone; ANA, antinuclear antibody; anti-dsDNA, anti-double-stranded DNA; anti-Sm, anti-Smith; C₃ and C₄, complement components; AUC = Area under the curve; IU, international units, AU, arbitrary unit; n.s., not statistically significant

* Mann–Whitney U test
statistically significant (Spearman’s \( \rho = 0.25; \ P < 0.05; \) data not shown). In addition, there was a statistically significant (\( P = 0.03 \)) difference between patients and controls in the correlation of 25(OH)D3 with E2 (data not shown). These findings suggested an interaction between 25(OH)D3 and E2 with regard to SLE flair. A moderator analysis was performed and a statistically significant (\( P = 0.03 \)) interaction between 25(OH)D3 and E2 in regard to SLE flair was observed.

At low levels of E2 (i.e., 0.27 mmol/l; 10th percentile), the association between 25(OH)D3 and SLE flair was statistically significant (\( P = 0.002 \)) (Figure 1). The higher the 25(OH)D3, the lower the probability for SLE flair in patients with low E2 levels. However, at high values of E2 the association between 25(OH)D3 and SLE flair was not statistically significant. The Johnson–Neyman technique showed that the association between 25(OH)D3 and SLE flair was statistically significant

**Table 2.** Distribution of participants according to serum level of 25(OH)D3

|                  | SLE patients | Controls | Statistical significance* |
|------------------|--------------|----------|---------------------------|
|                  | 25(OH)D3 >20 ng/ml | 25(OH)D3 ≤20 ng/ml | 25(OH)D3 >20 ng/ml | 25(OH)D3 ≤20 ng/ml |                      |
| Patients, n      | 9            | 27       | 26                        | 11                       |
| IFN-\( \gamma \) (pg/ml) | 1.0 (1.0–4.7) | 2.5 (1.0–6.8) | 1.0 (1.0–1.0) | 1.0 (1.0–1.0) | \( P < 0.001 \)        |
| E2 (nmol/l)      | 0.58 (0.42–0.63) | 0.48 (0.35–0.59) | 0.33 (0.29–0.38) | 0.34 (0.29–0.55) | \( P = 0.001 \)        |
| PTH (pg/ml)      | 13.6 (12.9–15.4) | 14.3 (12.2–17.2) | 15.1 (12.8–16.4) | 15.0 (13.2–20.2) | n.s.                   |

Data are presented as median (interquartile range).

SLE, systemic lupus erythematosus; 25(OH)D3, 25-hydroxy vitamin D3; IFN-\( \gamma \), interferon gamma; E2, oestradiol; PTH, parathyroid hormone; n.s., not statistically significant

*Kruskal–Wallis test

**Figure 1.** Association of 25(OH)D3 with systemic lupus erythematosus (SLE) flair at different values of oestradiol (E2) in female patients (n=36) with inactive disease.
($P < 0.05$) when $E_2$ values were $\leq 0.473$ nmol/l. In our sample, 63% of patients with SLE had $E_2 \leq 0.473$ nmol/L.

The OR for an SLE flare was almost 10 times higher in patients with $\text{IFN}_\gamma > 1.3$ pg/ml compared with those with $\text{IFN}_\gamma \leq 1.3$ pg/ml (95% CI: 2.15, 45.69; $P = 0.003$). The OR for an SLE flare was 7.6 times higher in patients with $25(\text{OH})D_3 \leq 20$ ng/ml compared with those with $25(\text{OH})D_3 > 20$ ng/ml (95% CI: 2.05, 27.86; $P = 0.002$). In addition, the OR for an SLE flare was 5.4 times higher in patients with $E_2 > 0.345$ nmol/L compared with those with $E_2 \leq 0.345$ nmol/L (95% CI: 1.39, 20.87; $P = 0.015$). Therefore, all three targeted parameters were independent of each other and were statistically significantly associated with SLE flair.

**Discussion**

This study was designed to investigate the association between vitamin D, $E_2$ and $\text{IFN}_\gamma$ in female patients with inactive SLE. Vitamin D is an important hormone with immunomodulatory properties and has a vital role in many biological and biochemical pathways.\(^{22-24}\) It has been suggested that UV exposure may catalyse symptom exacerbation or flare events in patients with SLE.\(^{25,26}\) As a consequence, patients are often advised to adopt sun protective measures and use both physical and chemical barriers routinely and these measures may result in vitamin D deficiency.\(^{26}\) Indeed, many patients with SLE have been found to have a deficiency in $25(\text{OH})D_3$.\(^{27}\)

Interest in the association between $25(\text{OH})\text{D}$ and systemic SLE began in the early 2000’s following observations that vitamin D levels were associated with low bone mass in patients with SLE.\(^{28}\) Lack of sun exposure and use of hydroxychloroquine (HCQ) was thought to be responsible for the low vitamin D levels in these patients.\(^{29}\) Following these initial findings, several more studies confirmed the association between SLE and $25(\text{OH})\text{D}$.\(^{27,30,31}\) Several studies have now shown a positive association between disease activity and vitamin D deficiency in patients with SLE.\(^{32-34}\) In addition, one study found a negative correlation between the serum concentration of vitamin D and disease activity which suggests a possible protective role for vitamin D in SLE.\(^{33}\) However, data from a randomized, placebo-controlled, clinical trial involving 90 patients with Vitamin D-deficient SLE showed that vitamin D supplementation did not affect disease activity.\(^{35}\) Data from this present study, in female patients with inactive SLE showed that their blood levels of vitamin D were lower compared with healthy controls. In accordance with our previous findings, we also found that patients with low levels of vitamin D (i.e., $25(\text{OH})D_3 \leq 20$ ng/ml) had a 7.6 times higher risk for the development of SLE flare compared with patients with higher levels of vitamin D (i.e., $25(\text{OH})D_3 > 20$ ng/ml).\(^{15}\) These results suggest a beneficial effect of vitamin D on disease activity.

The importance of $\text{IFN}_\gamma$ in the pathogenesis of lupus was highlighted by a study in lupus mice that found accelerated disease in mice receiving $\text{IFN}_\gamma$ or its inducers, while those receiving anti-$\text{IFN}_\gamma$ antibodies had a significantly delayed onset of disease.\(^{36}\) The ability of $\text{IFN}_\gamma$ to promote B cell autoantibodies and activate IgG Fc receptors and complement may also contribute to disease severity.\(^{37}\) This present study showed that levels of $\text{IFN}_\gamma$ were higher in patients with inactive SLE compared with controls. In addition, in patients with low levels of vitamin D (i.e., $25(\text{OH})D_3 \leq 20$ ng/ml), $\text{IFN}_\gamma$ was 150% higher than patients with higher levels of Vitamin D (i.e., $25(\text{OH})D_3 > 20$ ng/ml). Furthermore, the risk of an SLE flare was almost 10 times higher in patients with $\text{IFN}_\gamma > 1.3$ pg/ml compared with patients.
with \( \text{IFN}_\gamma \leq 1.3 \text{ pg/ml} \). These results emphasize the importance of cytokine balance in SLE.\(^{38}\) However, studies examining the role of \( \text{IFN}_\gamma \) in SLE have been contradictory. Some have found a correlation between serum \( \text{IFN-}\gamma \) level and disease activity and a correlation between \( \text{IFN}_\gamma \) expression and severity of lupus nephritis while others show decreased \( \text{IFN}_\gamma \) levels in lupus nephritis.\(^{39,40}\) Nevertheless, monoclonal antibodies targeting \( \text{IFN}_\gamma \) are being investigated for the treatment of SLE.\(^{41}\)

Oestrogens are considered immunomodulating hormones.\(^{42}\) One study suggested that \( \text{E}_2 \) may promote innate immunity by enhancing production of \( \text{IFN}_\gamma \) from \( \text{CD11c}(+) \) cells.\(^{43}\) Others studies have proposed that \( \text{E}_2 \) has a direct effect on the production of immunoglobulin (Ig) G anti-dsDNA antibodies as well as total IgG in peripheral blood mononuclear cells from patients with SLE.\(^{44,45}\) However, opinions differ about the influence of disease activity on ovarian function. Some studies have found a relationship between SLE activity and menstrual cycle disturbances, while others have failed to confirm an association.\(^{46-49}\) In the present study, the risk of a flair was 5.4 times higher in patients with \( \text{E}_2 > 0.345 \text{ nmol/L} \) compared with those with \( \text{E}_2 \leq 0.345 \text{ nmol/L} \). Our results also showed a positive correlation between \( \text{E}_2 \) and \( \text{IFN}_\gamma \) and a difference in the correlation between \( 25(\text{OH})\text{D}_3 \) and \( \text{E}_2 \) in patients and controls. These findings suggest an interaction between \( 25(\text{OH})\text{D}_3 \) and \( \text{E}_2 \) with regard to the probability of SLE reactivation. Interestingly, at low concentrations of \( \text{E}_2 \), the association between \( 25(\text{OH})\text{D}_3 \) and SLE flair was statistically significant but the association was not significant at high concentrations of \( \text{E}_2 \). We hypothesise that the conditional association between \( 25(\text{OH})\text{D}_3 \) with SLE flair at different values of \( \text{E}_2 \) suggests a possible pro-inflammatory effect of oestradiol. Furthermore, these findings suggest that vitamin D may lose a protective effect at higher oestrogen levels. Since Vitamin D and oestrogen act via receptors on immune cells,\(^{50,51}\) including B lymphocytes which are important in the pathogenesis of SLE, we suggest that competition for receptor sites or an altered post-receptor response could explain this proposed effect. In accordance with this suggestion is the observed beneficial effects of anti-oestrogens, such as tamoxifen, in patients with SLE.\(^{52}\) It is interesting that vitamin D and oestriadiol appear to have an interwoven action in SLE. Several studies have shown that the active form of vitamin D, calcitriol, regulates the expression of aromatase.\(^{53-55}\) In addition, in young women both progesterone oestrogen levels decreased after 4 weeks of vitamin D supplementation.\(^{56}\)

Our study had several limitations. For example, the sample size was small and the study had a cross-sectional design. More longitudinal research is needed to determine the validity of the cross-sectional associations between \( 25(\text{OH})\text{D}_3 \) and \( \text{E}_2 \) observed in this study. In addition, to maintain homeostasis in patients with SLE, an evaluation of hormone and cytokine status should be considered. A better understanding of the hormonal and cytokine status in patients with SLE may lead to future strategies for disease prevention. Furthermore, more studies are required in patients with inactive SLE using high doses of vitamin D for long periods.\(^{57}\)

In conclusion, a positive correlation was found between \( \text{E}_2 \) and IFN\(_\gamma\) in all participants and a difference was observed between patients and controls in the correlation of \( 25(\text{OH})\text{D}_3 \) with \( \text{E}_2 \). Furthermore, we observed a significant interaction between vitamin D and \( \text{E}_2 \) with regard to the probability of SLE flair. Our results suggest that oestrogens have a strong modulatory effect on vitamin D function, and this dual effect has a protective effect on
SLE flair only at low oestrogen levels. Based on our results, we suggest that vitamin D supplementation may be useful in SLE patients.

**Declaration of conflicting interests**

The authors declare that there are no conflicts of interest.

**Funding**

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

**References**

1. Hagberg N, Rönnblom L. Systemic lupus erythematosus--a disease with a dysregulated type i interferon system. *Scand J Immunol* 2015; 82: 199–207.

2. Hertzog P, Forster S and Samarajiwa S. Systems biology of interferon responses. *J Interferon Cytokine Res* 2011; 31: 5–11.

3. Pollard KM, Cauvi DM, Toomey CB, et al. Interferon-gamma and systemic autoimmunity. *Discov Med* 2013; 16: 123–131.

4. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003; 8: 223–246.

5. Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. *J Biomed Biotechnol* 2011; 2011: 432595.

6. Snapper CM, Rosas F, Moorman MA, et al. IFN-gamma and systemic autoimmunity. *Discov Med* 2013; 16: 123–131.

7. Kidd P. Th1/Th2 balance: the hypothesis, its limitations, and implications for health and disease. *Altern Med Rev* 2003; 8: 223–246.

8. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357: 266–281.

9. Provvedini DM, Tsoukas CD, Deftos LJ, et al. 1,25-dihydroxyvitamin D3 receptors in human leukocytes. *Science* 1983; 221: 1181–1183.

10. Lemire JM, Archer DC, Beck L, et al. Immunosuppressive actions of 1,25-dihydroxyvitamin D3: preferential inhibition of Th1 functions. *J Nutr* 1995; 125(6 Suppl): 1704S–1708S.

11. Chen S, Sims GP, Chen XX, et al. Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. *J Immunol* 2007; 179: 1634–1647.

12. Shabanova SS, Ananieva LP, Alekberova ZS, et al. Ovarian function and disease activity in patients with systemic lupus erythematosus. *Clin Exp Rheumatol* 2008; 26: 436–441.

13. Cutolo M, Brizzolara R, Atzeni F, et al. The immunomodulatory effects of estrogens: clinical relevance in immune-mediated rheumatic diseases. *Ann N Y Acad Sci* 2010; 1193: 36–42.

14. Tanriverdi F, Silveira LF, MacColl GS, et al. The hypothalamic-pituitary-gonadal axis: immune function and autoimmunity. *J Endocrinol* 2003; 176: 293–304.

15. Kokic V, Martinovic Kaliterna D, Radic M, et al. Relationship between vitamin D, IFN-γ, and E2 levels in systemic lupus erythematosus. *Lupus* 2016; 25: 282–288.

16. von Elm E, Altman DG, Egger M, et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* 2007; 370: 1453–1457.

17. Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1997; 40: 1725.

18. Bombardier C, Gladman DD, Urowitz MB, et al. Derivation of the SLEDAI: a disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. *Arthritis Rheum* 1992; 35: 630–640.

19. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. *J Clin Endocrinol Metab* 2011; 96: 1911–1930.

20. Ruperto N, Hanrahan LM, Alarcón GS, et al. International consensus for a definition of disease flare in lupus. *Lupus* 2011; 20: 453–462.
21. Hayes AF, Matthes J. Computational procedures for probing interactions in OLS and logistic regression: SPSS and SAS implementations. *Behav Res Methods* 2009; 41: 924–936.

22. Borges MC, Martini LA and Rogero MM. Current perspectives on vitamin D, immune system, and chronic diseases. *Nutrition* 2011; 27: 399–404.

23. Baeke F, Takiishi T, Korf H, et al. Vitamin D: modulator of the immune system. *Curr Opin Pharmacol* 2010; 10: 482–496.

24. Guillot X, Semerano L, Sainedenberg-Kermanac’h N, et al. Vitamin D and inflammation. *Joint, Bone, Spine* 2010; 77: 552–557.

25. Cooper GS, Wither J, Bernatsky S, et al. Occupational and environmental exposures and risk of systemic lupus erythematosus: silica, sunlight, solvents. *Rheumatology* 2010; 49: 2172–2180.

26. Bogaczewicz J, Sysa-Jedrzejowska A, Arkuszewska C, et al. Vitamin D status in systemic lupus erythematosus patients and its association with selected clinical and laboratory parameters. *Lupus* 2012; 21: 477–484.

27. Toloza SM, Cole DE, Gladman DD, et al. Vitamin D insufficiency in a large female SLE cohort. *Lupus* 2010; 19: 13–9.

28. Becker A, Fischer R and Schneider M. Bone density and 25-OH vitamin D serum level in patients with systemic lupus erythematosus. *Z Rheumatol* 2001; 60: 352–358 [in German, English Abstract].

29. Huisman AM, White KP, Algra A, et al. Vitamin D levels in women with systemic lupus erythematosus and fibromyalgia. *Ann Rheum Dis* 2010; 69: 1155–1157.

30. Mok CC, Birmingham DJ, Ho LY, et al. Vitamin D deficiency as marker for disease activity and damage in systemic lupus erythematosus: a comparison with anti-dsDNA and anti-C1q. *Lupus* 2012; 21: 36–42.

31. Karimzadeh H, Shirzadi M and Karimifar M. The effect of Vitamin D supplementation in disease activity of systemic lupus erythematosus patients with Vitamin D deficiency: A randomized clinical trial. *J Res Med Sci* 2017; 22: 4.

32. Jacob CO, van der Meide PH and McDevitt HO. In vivo treatment of (NZB X NZW)F1 lupus-like nephritis with monoclonal antibody to gamma interferon. *J Exp Med* 1987; 166: 798–803.

33. Malek TR. The biology of interleukin-2. *Curr Drug Targets* 2008; 9: 1033–1040.

34. Straub RH. The complex role of estrogens in inflammation. *Endocr Rev* 2007; 28: 521–574.

35. Siracusa MC, Overstreet MG, Housseau F, et al. 17beta-estradiol alters the activity of
conventional and IFN-producing killer dendritic cells. *J Immunol* 2008; 180: 1423–1431.

44. Murakami M, Kumagai S, Sugita M, et al. In vitro induction of IgG anti-DNA antibody from high density B cells of systemic lupus erythematosus patients by an HLA DR-restricted T cell clone. *Clin Exp Immunol* 1992; 90: 245–250.

45. Kanda N, Tsuchida T and Tamaki K. Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. *Arthritis Rheum* 1999; 42: 328–337.

46. Pasoto SG, Mendonca BB and Bonfa E. Menstrual disturbances in patients with systemic lupus erythematosus without alkylating therapy: clinical, hormonal and therapeutic associations. *Lupus* 2002; 11: 175–180.

47. Silva CA, Leal MM, Leone C, et al. Gonadal function in adolescents and young women with juvenile systemic lupus erythematosus. *Lupus* 2002; 11: 419–425.

48. Cabral de Sousa D, das Chagas Medeiros MM, Trindade Viana VS, et al. Anti-corpus luteum antibody and menstrual irregularity in patients with systemic lupus erythematosus and Hashimoto’s thyroiditis. *Lupus* 2005; 14: 618–624.

49. Brunner HI, Bishnoi A, Barron AC, et al. Disease outcomes and ovarian function of childhood-onset systemic lupus erythematosus. *Lupus* 2006; 15: 198–206.

50. Kassi E and Moutsatsou P. Estrogen receptor signaling and its relationship to cytokines in systemic lupus erythematosus. *J Biomed Biotechnol* 2010; 2010: 317452.

51. Aranow C. Vitamin D and the immune system. *J Investig Med* 2011; 59: 881–886.

52. Sthoeger ZM, Zinger H and Mozes E. Beneficial effects of the anti-oestrogen tamoxifen on systemic lupus erythematosus of (NZBxNZW)F1 female mice are associated with specific reduction of IgG3 autoantibodies. *Ann Rheum Dis* 2003; 62: 341–346.

53. Barrera D, Avila E, Hernández G, et al. Estradiol and progesterone synthesis in human placenta is stimulated by calcitriol. *J Steroid Biochem Mol Biol* 2007; 103: 529–532.

54. Krishnan AV, Swami S, Peng L, et al. Tissue-selective regulation of aromatase expression by calcitriol: implications for breast cancer therapy. *Endocrinology* 2010; 151: 32–42.

55. Yague JG, Garcia-Segura LM and Azcoitia I. Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone, and mifepristone in human glioma cells. *Endocrine* 2009; 35: 252–261.

56. Knight JA, Wong J, Blackmore KM, et al. Vitamin D association with estradiol and progesterone in young women. *Cancer Causes Control* 2010; 21: 479–483.

57. Aranow C, Kamen DL, Dall’Era M, et al. Randomized, Double-Blind, Placebo-Controlled Trial of the Effect of Vitamin D3 on the Interferon Signature in Patients With Systemic Lupus Erythematosus. *Arthritis Rheumatol* 2015; 67: 1848–1857.