Original article

On lupus, vitamin D and leukopenia

Juliana A. Simioni, Flavia Heimovski, Thelma L. Skare*

Rheumatology Unit, Hospital Universitário Evangélico de Curitiba, Curitiba, PR, Brazil

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ABSTRACT

Background: Immune regulation is among the noncalcemic effects of vitamin D. So, this vitamin may play a role in autoimmune diseases such as systemic lupus erythematosus (SLE).

Objectives: To study the prevalence of vitamin D deficiency in SLE and its association with clinical, serological and treatment profile as well as with disease activity.

Methods: Serum OH vitamin D3 levels were measured in 153 SLE patients and 85 controls. Data on clinical, serological and treatment profile of lupus patients were obtained through chart review. Blood cell count and SLEDAI (SLE disease activity index) were measured simultaneously with vitamin D determination.

Results: SLE patients have lower levels of vitamin D than controls (p = 0.03). In univariate analysis serum vitamin D was associated with leukopenia (p = 0.02), use of cyclophosphamide (p = 0.007) and methotrexate (p = 0.03). A negative correlation was verified with prednisone dose (p = 0.003). No association was found with disease activity measured by SLEDAI (p = 0.88). In a multiple regression study only leukopenia remained as an independent association (β = 4.04; p = 0.02). A negative correlation of serum vitamin level with granulocyte (p = 0.01) was also found, but not with lymphocyte count (p = 0.33).

Conclusion: SLE patients have more deficiency of vitamin D than controls. This deficiency is not associated with disease activity but with leukopenia (granulocytopenia).

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ACERCA DE LÚPUS, VITAMINA D E LEUCOPENIA

RESUMO

Introdução: A regulação imune está entre os efeitos não calcêmicos da vitamina D. Assim, essa vitamina pode influenciar em doenças autoimunes, como o lúpus eritematoso sistêmico (LES).

Objetivos: Estudar a prevalência da deficiência de vitamina D no LES e sua associação com o perfil clínico, sorológico e de tratamento, bem como com a atividade da doença.

Métodos: Mensuraram-se os níveis séricos de OH-vitamina D3 em 153 pacientes com LES e 85 controles. Os dados sobre o perfil clínico, sorológico e de tratamento de pacientes com...
Introduction

Vitamin D has immune modulatory properties. The main source of vitamin for humans is the conversion of 7-dehydrocholesterol in pre vitamin D3 in the skin, which occurs by exposure to ultraviolet radiation. A small portion comes from the diet, mainly seafood. Vitamin D3 must first undergo a hydroxylation in the liver resulting in 25 OH vitamin D3 or calcidiol which is the circulating form of the vitamin and that it was the form used for serum measurement at present. The most active form of vitamin D is 1,25 (OH)2 vitamin D synthesized in the kidney. It is known that this vitamin plays an inhibitory role of dendritic cells, CD4, CD8, B lymphocytes and the production of cytokines, such as IFN-γ, IL-2, IL-6, TNF-α, and increases the number of T regulatory cells and synthesis of other cytokines such as IL-4, IL-10 and TGF-B. Thus it is conceivable that serum vitamin D levels exert influence on autoimmune diseases such as SLE. However existing studies in this area are contradictory. Birmingham et al. reported that patients with acute disease flare have lower levels of vitamin D but a cause-effect relationship could not be established. Others noted that deficiency of vitamin D has been associated with increased disease activity, while others denied it. Some clinical manifestations have been associated with vitamin D levels such as leukopenia renal involvement, photosensitivity and the presence of anti-ds DNA.

The vitamin D receptor gene polymorphism has been linked with SLE susceptibility in Asian, Polish and Egyptian patients. In Brazilians this association could not be demonstrated although it seemed to influence the appearance of cutaneous and articular manifestations and in the presence of anti-ds DNA.

Some factors contribute to a higher prevalence of vitamin D deficiency in SLE patients than in the general population. The photoprotection, held as part of the disease treatment, can block the synthesis of cholecalciferol induced by UVB radiation on the skin. Antimalarials, widely used in this disease, are described by some authors to be associated with its deficiency due to its photoprotective effect. Others believe that this medication hinders the production of dihydroxvitamin D as they inhibit the alpha-hydroxylase enzyme responsible for transformation of mono dihydroxvitamin D. This action could increase circulating levels of the level of monohydroxy vitamin D3 in detriment of dihydroxy active form.

In the present study we aimed to determine whether the levels of vitamin D in a Southern Brazilian population with SLE are associated with disease activity, serological, clinical profile and with medications used for treatment.

Methods

This study was approved by the local Ethics Committee in Research and all participants signed an informed consent. One hundred and fifty three patients with at least 4 of 1997 modified American College of Rheumatology (ACR) classification criteria for SLE were invited to participate during the period of six months, according to the order of consultation in the clinic and willingness to participate in the study. We excluded patients using anticonvulsants with creatinine greater than 1.3 mg/dL and pregnant women. None of the included patients had made replacement of vitamin D in the last year and none made use of more than 600IU of vitamin D3/day, which is routinely done in the service for all glucocorticoids users. Demographic, clinical and serological data were collected through chart review. Clinical data were considered a cumulative way and defined according to the 1997 ACR classification criteria for SLE. Disease activity was calculated by SLEDAI (or SLE Disease activity index). Also, data on creatinine levels and blood cell count were collected simultaneously with vitamin D as well as the drugs considered for study were those used at the moment of vitamin D determination.

As controls, we included 85 self-reported healthy individuals from the same geographical area, matched for age and gender.

The serum vitamin D (25 OH vitamin D3) was analyzed by chemiluminescence by the Liaison 25OH Vitamin D Assay (DiaSorin Inc., Stillwater, MN, USA). Value ≥30 mg/dL were
considered normal; between 20 and 29 mg/dL were considered as vitamin insufficiency and values below 20 mg/dL as deficiency.

Data were collected on the frequency and contingency tables. Study of data distribution was performed by the Kolmogorov–Smirnov test and central tendency was expressed as median and interquartile range (IQR) for non-parametric and mean and standard deviation for parametric data. Association studies of vitamin D levels with clinical, serological and demographic variables were made by Fisher’s and chi-square when data was nominal and for unpaired t test when nominal. Correlation studies of SLEDAI values, age, prednisone dose, granulocyte and lymphocyte blood count with values of serum vitamin D were done using Pearson and Spearman test. All variables with p < 0.1 in univariate analysis were further studied through multiple linear regression to access its independency. Calculations were made with help of software Medcalc version 12.1.3.0 and the significance was set at 5%.

Results

Description of the studied sample

In the sample of 153 patients with SLE, 11/153 (7.1%) were men and 142/153 (92.8%) were women aged 19–65 years (median 42 years, IQR = 31–49) and disease duration from 6 to 244 months (median 36 months; IQR = 12–72). The SLEDAI values ranged from 0 to 20 (median 0, IQR = 0–2). About 43/126 (34.1%) were smokers. Patients’ clinical, serological and treatment profile is seen in Table 1.

In this sample the levels of serum vitamin D ranged from 4.0 to 57.2 (mean 22.5 ± 9.2). In 29/153 (18.9%) patients the values were normal; in 59/153 (38.5%) were insufficient and 65/153 (42.4%) were deficient. In 125/153 (81.6%) had values below the normal range.

The 85 included controls had mean age 41.8 ± 16.4 years; 985 were men and 76/85 women and they were matched for age (p = 0.49) and gender (p = 0.36) with patients. The comparison between the levels of vitamin D between SLE patients and controls can be seen in Fig. 1.

Vitamin D in SLE population according to demographic, clinical, serological and treatment variables

The values of serum vitamin D levels according to studied variables are seen in Table 2.

The correlation of vitamin D levels with prednisone dose showed a negative value with rho = −0.23 (95% CI = −0.38 to −0.07; p = 0.003) and a trend was observed with patients age with rho = 0.07 (95% CI = −0.08 to 0.23; p = 0.360). No correlation between vitamin D levels and SLEDAI was found (rho = −0.01, 95% CI = 0.15 to −0.17; p = 0.88).

When variables with p < 0.1 in univariate analysis were studied by multiple linear regression (prednisone, cyclophosphamide, methotrexate, leupokemia, anti Sm and Lupus anticoagulant) only leupokemia remained as an independent variable (B = 4.04; p = 0.02).

Study of leupokemia with serum levels of vitamin D

The study of vitamin D levels and leupocyte cell count showed no correlation of lymphocyte count with serum vitamin D

Table 1 – Clinical, serological and treatment profile of 153 patients with systemic lupus erythematosus.

<table>
<thead>
<tr>
<th>n</th>
<th>Arthritis</th>
<th>Serositis</th>
<th>SLEDAI</th>
<th>Methotrexate use</th>
<th>Prednisone use</th>
</tr>
</thead>
<tbody>
<tr>
<td>124/153</td>
<td>76.0%</td>
<td>32.3%</td>
<td>20.2%</td>
<td>22.9%</td>
<td>37.9%</td>
</tr>
</tbody>
</table>

Fig. 1 – Vitamin D levels in systemic lupus erythematosus patients (mean value 22.4 ± 9.16 mg/dL) and controls (mean value 25.6 ± 10.2 mg/dL) with p = 0.03.
levels (rho = 0.07; 95% CI = −0.07 to 0.22; p = 0.33). The association study with granulocyte count is on Fig. 2.

Discussion

The result of the present study confirms the findings of other authors, showing that low levels of vitamin D are more common in lupus patients than in the normal population from the same geographical area. This fact would be expected since these patients are advised to avoid sunlight, the main source of its synthesis. Autoantibodies against vitamin D have been described in SLE, although they do not seem to affect the vitamin circulating levels. Also it was not possible to identify any difference respecting antimalarial use ratifying the findings of Fragoso et al. So the use of this medication cannot be implied in the vitamin D deficiency.

At present, no correlation of vitamin D levels with disease activity measured by SLEDAI was found but an interesting association of vitamin D and leukopenia was confirmed. Bogaczewicz et al. have found that lower vitamin D level was linked to leukopenia in 49 SLE patients. The same finding was observed in 76 females with primary Sjögren syndrome by Baldini et al. The importance of such finding is highlighted when one notes that infections are a leading cause of death in SLE patients. Bacterial infections are most frequent, followed by viral and fungal infections.

Vitamin D has a powerful effect in the differentiation of cells of the myeloid lineage favoring monocyte differentiation. Cultures of bone marrow cells in the presence of this vitamin result in a dramatic increase of monocytes and macrophages from 12% in controls to 68% in treated cultures. Furthermore, vitamin D suppresses neutrophil differentiation. In a study of vitamin D-induced differentiation in cultures of normal fetal liver blast cells, a reciprocal promotion of maturation of cells from monocyte lineage and a suppression of maturation of cells restricted

<table>
<thead>
<tr>
<th>Table 2 – Comparison of vitamin D serum levels according to clinical demographic, serological and treatment variables.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (female)</td>
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<tr>
<td>Tobacco use</td>
</tr>
<tr>
<td>Arthritis</td>
</tr>
<tr>
<td>Serositis</td>
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<tr>
<td>Hemolytic anemia</td>
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<tr>
<td>Leukopenia</td>
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<tr>
<td>Thrombocytopenia</td>
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<tr>
<td>Glomerulonephritis</td>
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<tr>
<td>Disoid lesions</td>
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<tr>
<td>Malar rash</td>
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<tr>
<td>Photosensitivity</td>
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<tr>
<td>Oral ulcers</td>
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<tr>
<td>Seizures</td>
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<tr>
<td>Psychosis</td>
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<tr>
<td>Anti Ro</td>
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<tr>
<td>Anti La</td>
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<td>Anti Sm</td>
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<tr>
<td>Anti dsDNA</td>
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<tr>
<td>Anti RNP</td>
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<tr>
<td>Anticardiolipin IgG</td>
</tr>
<tr>
<td>Anticardiolipin IgM</td>
</tr>
<tr>
<td>Lupus anticoagulant</td>
</tr>
<tr>
<td>Antimalarial use</td>
</tr>
<tr>
<td>Azathioprine use</td>
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<tr>
<td>Methotrexate use</td>
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<tr>
<td>Mophethyl mycophenolate use</td>
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<tr>
<td>Cyclophosphamide use</td>
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</tbody>
</table>

Fig. 2 – Correlation studies of serum vitamin D levels and granulocyte count (rho = −0.19; 95% CI = −0.19 to −0.33; p = 0.01).
to neutrophil development was observed. When treated with (OH)₂ vitamin D, these cultures produced 10 times the number of monocytes than corresponding control cultures. This was reflected by the control cultures holding five times as many neutrophils as the treated cultures.²⁵ Ratifying these studies we found, at present, a negative association of vitamin D levels with granulocyte peripheral count. Nevertheless no association of this vitamin serum level could be found with lymphocyte count. It is important to remember that lymphocyte counts in SLE patients may be altered by other interfering factors such as the presence of lymphocytotoxic autoantibodies. These autoantibodies appear in 36–90% of lupus patients and are associated with lower count of leucocytes and with higher disease activity.²⁶,²⁷ Reduced surface expression of complement regulatory proteins such as CD55 and CD59 has also been implicated in the pathogenesis of lupus lymphopenia as this deficiency will turn the cells susceptible to complement-mediated lysis.²⁸

Summarizing, the present study shows that vitamin D serum levels are lower in lupus patients than in controls. No relationship of this vitamin serum level with disease activity could be found but a negative relation with leucocytes number was established. Further studies are needed in order to clarify the importance of this vitamin deficiency in the prevalence of infections in these patients.

Conflicts of interest

The authors declare no conflicts of interest.

References

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