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Original Article

Clinical significance of vitamin D deficiency and receptor gene polymorphism in systemic lupus erythematosus patients

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ABSTRACT

Introduction: The vitamin D receptor (VDR) gene is a candidate for susceptibility to autoimmune disorders.
Aim of the work: To study the frequency of vitamin D deficiency in Egyptian systemic lupus erythematosus (SLE) patients and investigate the association of *Bsm*I and *Fok*I VDR gene polymorphisms with disease susceptibility, activity and damage.
Patients and methods: Forty-five SLE patients and 40 controls were enrolled. SLE Disease Activity Index (SLEDAI) and Systemic Lupus International Collaborating Clinics (SLICC) damage index were assessed for the patients. Serum vitamin D levels were measured in all subjects. Genotyping for the VDR *Bsm*I and *Fok*I gene polymorphisms was performed by polymerase chain reaction and restriction fragment length polymorphism for only 34 patients and 16 controls.
Results: The mean age of SLE patients was 28.8 ± 7.9 years and disease duration 11.3 ± 9.8 years. Vitamin D level was significantly lower in patients than control ($p < 0.001$) and significantly correlated with C3 and C4 levels ($p < 0.001$) and inversely with SLEDAI ($p < 0.001$), SLICC ($p = 0.005$), anti-ds DNA ($p < 0.001$) and ESR ($p = 0.011$). There were no significant differences in genotype and allelic frequencies of *Fok*I and *Bsm*I polymorphisms between patients and controls. There was a significant relation of *Fok*I polymorphisms with serum vitamin D level ($p = 0.002$), SLEDAI ($p = 0.021$) and SLICC ($p = 0.002$). *Bsm*I polymorphisms showed significant associations with neuropsychiatric damage, low complement, fever and mucosal ulcers.
Conclusions: VDR *Fok*I polymorphism in SLE patients is significantly related to low vitamin D level in SLE patients and both are associated with increasing disease activity and damage denoting important implications in this disease.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune inflammatory disorder characterized by generation of autoantibodies against nuclear antigens and deposition of immune complexes in various organs resulting in chronic inflammation and tissue damage [1]. Although the underlying cause still remains unclear, it is thought to result from interactions between environmental factors, disease- prone genetic background and a variety of pathogen eliciting innate and adaptive immune responses [2].

The possible involvement of vitamin D deficiency in the development of autoimmune diseases has provided new insights into the function of this vitamin. The immunomodulatory effects of vitamin D include down regulating the T helper cell (Th1) immune responses, and reducing Th induction of immunoglobulin production by B cells, thus altering the cytokine profile toward the Th2 phenotype and preserving innate immune response [3]. Each of these immunologic properties has great potential implications for SLE patients [4]. Lower vitamin D levels were significantly associated with increased cardiovascular risk, higher disease activity and damage in Egyptian SLE patients [5].

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Vitamin D (1, 25 dihydroxyvitamin D3) exerts many of its actions through interaction with vitamin D receptor (VDR) [6]. The wide distribution and expression of VDR in most immune cells such as peripheral lymphocytes, macrophages, monocytes, dendritic cells, natural killer cells and T and B lymphocytes [7,8] as well as its effect on cell proliferation and differentiation makes vitamin D a possible candidate for regulation of immune response [8].

The VDR gene, located on chromosome 12 and composed of 9 exons has emerged as a candidate gene for susceptibility to autoimmune disorders [9]. The VDR gene contains more than 470 single nucleotide polymorphisms (SNPs), a number of which modulate the uptake of 1, 25(OH)2D3 uptake. The most frequently studied VDR polymorphisms include *Fok*I situated in exon 2, *Bsm*I located in intron 8, *Taq*I and *Apa*I with the latter being situated in exon 9 and intron 9 [10].

The role of VDR polymorphisms in the development of SLE and its clinical manifestations have been demonstrated with conflicting results. The aim of the present study was to assess vitamin D status in Egyptian patients with SLE and to investigate whether BsmI and FokI VDR gene polymorphisms could be susceptibility markers for the disease activity and severity.

2. Patients and methods

This cross-sectional case-control study was conducted on 45 SLE (38 females and 7 males) patients fulfilling the fulfilled the Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [11] and 40 age and sex matched healthy controls. All subjects were recruited from Internal Medicine and Rheumatology outpatient clinics of Kasr Al Ainy University Hospitals. All the patients provided an informed consent and the study was approved by the local ethical committee conforming to the 1995 Helsinki declaration.

Patients with hepatic dysfunction or disease, renal disease (other than SLE), malignancy or those taking drugs known to affect vitamin D metabolism (such as anticonvulsants, cimetidine, antituberculosis agents, theophylline, orlistat and drugs for AIDS) were excluded. All subjects were subjected to full medical history including age, body mass index (BMI), history of photosensitivity and dress code, concurrent diseases (such as diabetes or hypertension), post menopausal history as well as history of pregnancy and lactation. Detailed drug history was obtained from all patients and included duration and daily doses of medications as well as calcium and vitamin D supplementations.

The disease activity was assessed in SLE patients by the SLE Disease Activity Index (SLEDAI) [12] and organ damage was assessed by Systemic Lupus International Collaborating Clinics/American College of Rheumatology Damage Index (SLICC/ACR DI) [13]. All subjects underwent routine laboratory investigations which included complete blood picture (CBC), Erythrocyte sedimentation rate (ESR), serum calcium (total and ionized), serum phosphorous, alkaline phosphatase, serum creatinine and glomerular filtration rate (GFR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and complete urine analysis with 24 h urinary protein. Specific laboratory investigations for SLE patients included antinuclear antibody (ANA), anti-double stranded deoxyribonucleic acid (anti-dsDNA) with titer, serum complement components 3 and 4 (C3 and C4).

The level of serum 25-hydroxyvitamin D [25(OH) D3] was quantitatively measured by enzyme linked immunosorbent assay (ELISA) for all subjects. A serum level $\geqslant$30 ng/ml was considered *normal,* between 10 and 30 ng/ml as *insufficiency* and < 10 ng/ml as *deficiency*.

Only 34 SLE patients and 16 age- and sex- matched controls were genotyped for the VDR BsmI and FokI gene polymorphisms based on the polymerase chain reaction and restriction fragment length polymorphism (PCR/RFLP) due to financial constraints.

Statistical analysis

The data was coded and entered using the statistical package SPSS version 15. The data was summarized using descriptive statistics: mean, standard deviation, minimal and maximum values for quantitative variables and number and percentage for qualitative values. Statistical differences between the groups were tested using the Chi-Square test for qualitative variables, independent sample *t*-test for quantitative normally distributed variables; while the non-parametrical Mann-Whitney test was used for quantitative variables which were not normally distributed. Correlations were done to test for linear relations between quantitative variables.

3. Results

The age, BMI and laboratory data of the SLE patients and their age- and sex- matched controls are shown in Table 1. There were no significant differences between SLE patients and controls as regard sex and post menopausal status (5 patients and 4 control) ($p = 0.81$ and 0.4 respectively). The mean disease duration of the patients was 11.3 ± 9.8 years. Patients suffered from peripheral vascular damage (40%), followed by cardiac, neuropsychiatric, musculoskeletal and skin damage (33.3%, 20%, 15.5%, and 15.5% respectively). Only one patient suffered pulmonary damage (2.2%) and none suffered from malignancy.

Serum levels of vitamin D were significantly lower in SLE patients (12 ± 2.28 ng/ml) compared to the controls (21.13 ± 3.2 ng/ml) ($p < 0.001$). Table 2 shows comparison between SLE patients and the controls as regard the vitamin D status. There was a significant difference between SLE patients with vitamin D deficiency and insufficiency as regards the total and ionized calcium ($p = 0.009$ and 0.039 respectively). Patients with deficiency showed higher mean anti-ds DNA titer (90.2 ± 37.8 IU/ml) compared to those with insufficiency (61.5 ± 10.2 IU/ml) ($p < 0.001$). The daily and cumulative doses of steroids were non-significantly higher in vitamin D deficient compared to insufficient patients. The cumulative doses of HCQ, AZA and cyclophosphamide tended to be higher in vitamin D insufficient patients. Vitamin D deficient patients had significantly greater SLEDAI and damage scores than insufficient patients ($p = 0.001$ and $p = 0.035$ respectively) (Fig. 1). As regard gender, post-menopausal status, photosensitivity, dress code and vitamin D supplementation, no significant differences were observed between patients with vitamin D deficiency or insufficiency. A significant difference was found between vitamin D deficient and insufficient patients as regard

Table 1
The age, body mass index and laboratory investigations in systemic lupus erythematosus patients and control.

Variable mean ± SD	SLE patients (n = 45)	Controls (n = 40)	p
Age (years)	28.8 ± 7.9	30.4 ± 9.6	0.4
BMI	22 ± 2.7	22.5 ± 1.95	0.38
Hb (g/dl)	8.2 ± 1.9	12.4 ± 1.2	<0.001
TLC (10^3/μL)	6.3 ± 2.2	6.3 ± 0.9	0.62
Creatinine (mg/dl)	1.4 ± 1.4	0.8 ± 0.21	<0.001
Calcium (mg/dl)	7.6 ± 0.9	9.1 ± 0.21	<0.001
Ionized	3.7 ± 0.6	4.96 ± 0.2	<0.001
25(OH)D3 (ng/ml)	12 ± 2.3	21.1 ± 3.2	<0.001

SLE: systemic lupus erythematosus, BMI: body mass index, Hb: haemoglobin, TLC: total leucocytic count. Significance is considered at p value < 0.05