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RESEARCH PROTOCOL

Vitamin D Deficiency in Primary Sjögren's Syndrome: Association with Clinical Manifestations and Immune Activation Markers

Panagiotis Athanassiou[1], Clio Mavragani[2], Lambros Athanassiou[2,3], Ifigenia Kostoglou-Athanassiou[4], Michael Koutsilieris[5]

[1]Department of Rheumatology, St. Paul's Hospital, Thessaloniki, Greece, [2]Molecular Physiology-Clinical Application Unit, Department of Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece, [3]Department of Rheumatology, Asclepeion Hospital, Voula, Athens, Greece, [4]Department of Endocrinology, Asclepeion Hospital, Voula, Athens, Greece, [5]Department of Physiology, Medical School, National and Kapodistrian University of Athens, Athens, Greece

ABSTRACT

Vitamin D is an agent involved in bone and mineral homeostasis. It has been recognized as a potent immunomodulator. It has immune-enhancing properties, and it induces immune tolerance. Vitamin D deficiency has been shown to be related to the development of autoimmune disorders. Vitamin D deficiency has been observed in patients with rheumatoid arthritis (RA) and it has been shown to be related with disease activity. Vitamin D deficiency has also been found in patients with systemic lupus erythematosus (SLE) and it was shown to be related to disease activity and renal involvement. Vitamin D deficiency has also been observed in multiple sclerosis. Vitamin D has been found to act as a supplemental therapeutic agent in multiple sclerosis. Sjögren's syndrome is a systemic autoimmune disease affecting the exocrine glands, known as an autoimmune epithelitis. The disease has a complex pathogenesis, requiring a genetic background, immune cell activation, and autoantibody production. The disease affects the exocrine glands, lacrimal, and salivary glands leading to ocular and oral dryness. Vitamin D levels have been measured in patients with Sjögren's syndrome and an association was observed between low vitamin D levels, peripheral neuropathy and the presence of lymphoma. In other cohorts, such as a Turkish cohort, vitamin D deficiency was observed in patients with Sjögren's syndrome. The aim is to measure serum vitamin D levels in consecutive patients with primary Sjögren's syndrome and investigate the relationship between vitamin D levels and the presence of immunologic markers, clinical, serological, and histopathological characteristics.

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Corresponding Author:
Panagiotis Athanassiou
Department of Rheumatology
St. Paul's Hospital, Thessaloniki, Greece
Tel.: +30 6944 757 675
E-mail: pathanassiou@yahoo.gr

INTRODUCTION

Sjögren's syndrome is a systemic autoimmune disease affecting the exocrine glands,[1,2] known as autoimmune epithelitis,[3,4] given the presence of lymphocytic infiltrates around affected epithelia. The disease is characterized by a complex pathogenesis as a genetic background is required along with immune cell activation and autoantibody production. The disease affects the exocrine glands mainly lacrimal and salivary glands

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leading to generation of oral and ocular dryness known as "sicca syndrome"[5,6], while systemic manifestations are not uncommon. Hashimoto's thyroiditis is commonly observed in patients with Sjögren's syndrome.[5] Loviselli et al. studied thyroid function parameters and thyroid antibodies in a cohort of patients with primary and secondary Sjögren's syndrome. They found higher prevalence of thyroid antibodies in patients with Sjögren's syndrome, which was more pronounced in primary Sjögren syndrome.[7] It has been suggested that Sjögren's syndrome and autoimmune thyroid disease may be the two sides of the same coin.[8]

Vitamin D is an agent involved in the regulation of bone/mineral metabolism[9] as well as in immune pathways.[10-12] It has immune-enhancing properties,[13,14] and is also known to induce immune tolerance.[15-17] Vitamin D deficiency has been related to the development and disease activity of autoimmune disorders,[18,19] including rheumatoid arthritis,[20,21] systemic lupus erythematosus,[22] and multiple sclerosis.[23] In the latter group, vitamin D supplementation may act as a supplemental therapeutic agent.[24] Agmon-Levin et al.[25] measured vitamin D levels in a cohort of patients with Sjögren's syndrome, and they found an association between low vitamin D levels, peripheral neuropathy, and the presence of lymphoma, while in a cohort of Turkish patients with Sjögren's, lower vitamin D levels were observed[26] and in a cohort of Indian patients low vitamin D levels were related to a high risk for high lip grading and rheumatoid factor positivity.[27]

AIM

The aim was to measure serum vitamin D levels in consecutive patients with Sjögren's syndrome and investigate the relationship between vitamin D levels and the presence of immunologic markers, clinical, serological, and histopathological characteristics.

PATIENTS AND METHODS

For our study, $25(OH)D_3$ levels will be retrospectively measured in stored sera from consecutive patients with primary Sjögren's syndrome (SS) in the "Molecular Physiology-Clinical Application Unit", Department of Physiology, National and Kapodistrian University of Athens as well as in prospectively collected sera from the Department of Rheumatology St. Paul's Hospital, Thessaloniki, Greece and Department of Rheumatology, Asclepeion Hospital, Voula, Athens, Greece following written consent to participate in the study. All primary SS subjects participating in the study fulfil the revised international criteria for the classification of primary SS.[28] Healthy individuals of similar age and sex distribution to the patients with primary SS will be also included. Exclusion criteria for all participants include pregnancy, age <18 years, and renal dysfunction (serum creatinine levels >3 mg/dl, creatinine clearance <30 ml/minute).

Demographic data, clinical features, and therapeutic regimens will be recorded in all participating patients and controls. Demographic data including age, sex, and BMI will be recorded. Clinical manifestations including the presence of subjective and objective oral and ocular dryness (documented by unstimulated salivary flow rates and Schirmer's test/Rose Bengal staining, respectively); dry cough; dyspareunia; fever; arthralgias; arthritis; carpal tunnel syndrome (documented by physical examination and nerve conduction studies); Raynaud's phenomenon; lymphadenopathy; splenomegaly; purpura; pulmonary involvement (small airway disease or interstitial lung disease documented by pulmonary function tests and high resolution computed tomography scans); pleuritis; pericarditis; renal involvement including interstitial nephritis (documented by urine-specific gravity <1.010 or pH >5.5 on at least two consecutive measurements after fluid restriction) and glomerulonephritis documented by renal biopsy; liver involvement (documented by liver biopsy showing changes compatible with primary biliary cirrhosis in the setting of increased liver enzymes or anti-mitochondrial antibodies); peri-epithelial disease (defined as peribronchial, interstitial nephritis, autoimmune cholangitis); myositis (documented by muscle biopsy in the setting of increased aldolase or creatinophosphokinase); peripheral neuropathy (documented by nerve conduction studies in patients with clinical symptoms or signs suggestive of neuropathy); central nervous involvement; lymphoma (documented by biopsy) will be recorded. Sjögren's syndrome disease activity index (ESSDAI) will be determined.[29]

$25(OH)D_3$ will be measured by an electrochemiluminescence binding assay (Elecsys Vitamin D total, for cobas e 411 analyser, Roche Diagnostics GmbH, Mannheim, Germany). The assay is based on a competition principle. In summary, after a first incubation, so that the bound 25(OH)D could be released from the vitamin D binding protein, a second incubation is performed. During the second incubation, the pre-treated sample is incubated with a ruthenium labelled vitamin D binding protein. Thus, a complex is formed between 25(OH)D and the ruthenium labelled vitamin D binding protein, the ruthenylated vitamin D binding protein. Thereafter, a third incubation is performed. During the third incubation, streptavidin microparticles and vitamin D labelled with biotin are added, and unbound ruthenium labelled vitamin D binding proteins become occupied. A complex consisting of the ruthenylated vitamin D binding protein and the biotinylated vitamin D is formed and it is bound to the solid phase of the assay via interaction of biotin and streptavidin. The reaction mixture is then aspirated into the cell where the microparticles are magnetically captured onto the surface of an electrode. Unbound substances are then removed. Application of a voltage to the electrode then induces a chemiluminescent emission, which is detected

by a photomultiplier. The sensitivity of the assay is 10.03 nmol/L. The within run CV of the assay ranges from 3.1% at 70.0 nmol/L to 7.8% at 16.9 ng/ml.

Statistical evaluation

Statistical analysis will be performed by SPSS v.21 package. Two-group comparisons of continuous data will be assessed using t-tests, or the Mann-Whitney test, when the data do not have a normal distribution. Comparisons between groups will be performed by Fisher's exact two tailed test and Mann Whitney test. Difference is considered statistically significant if $p<0.05$.

SIGNIFICANCE

Should vitamin D levels found to be associated with the presence of specific immunologic markers in patients with Sjögren's syndrome, vitamin D substitution may be considered, and underlying mechanisms will be further explored.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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