Abnormal vitamin D₃ metabolism in patients with primary Sjögren’s syndrome

K Müller, P Oxholm, O H Sørensen, M Thymann, M Høier-Madsen, K Bendtzen

Abstract
Recent studies have suggested that vitamin D₃ may have an immunoregulatory role in vitro. The vitamin D₃ metabolism in 35 patients with primary Sjögren’s syndrome was investigated by measuring blood concentrations of 1α,25-dihydroxyvitamin D₃ (1α,25-(OH)₂D₃) and 25-hydroxyvitamin D₃ (25-OHD₃), in normal serum levels. There was no significant difference between the distribution of Gc phenotypes in the patients with primary Sjögren’s syndrome and normal controls. Likewise, blood concentrations of Gc globulin corresponded to normal values. Among patients with increased concentrations of IgM rheumatoid factor there was a significant negative correlation between the serum values of IgM rheumatoid factor and 25-OHD₃ concentrations.

Methods
Thirty-five consecutive female patients with primary Sjögren’s syndrome were studied. Their ages ranged from 27 to 69 years, mean 51 years. All patients fulfilled the Copenhagen diagnostic criteria for primary Sjögren’s syndrome. —that is, keratoconjunctivitis sicca evaluated by the Schirmer-I test, break-up time, and van Bijsterveld score, and xerostomia determined by unstimulated sialometry, salivary gland scintigraphy, and lower lip biopsy. The patients were not treated with immunosuppressive drugs other than non-steroidal anti-inflammatory drugs (n=4). The most common extraglandular manifestations were as follows: arthralgia/synovitis (n=18, 51%), myalgia/myositis (n=7, 20%), Raynaud’s phenomenon (n=8, 23%) and pulmonary signs (n=7, 20%). Only a limited number of the patients showed other extraglandular manifestations such as purpura, interstitial nephritis, cutaneous vasculitis, intermittent non-infectious fever, and lymphoproliferative disorders. Serum samples were stored at -20°C until assay.

Serum 25-OHD₃ was examined by a radioassay using rachitic rat kidney cytosol as a binding protein. The normal mean (SD) was 28.2 (12.4) ng/ml (taken from healthy volunteers of both sexes, aged 18 to 60 years, from whom blood samples were obtained throughout the year; the mean therefore represents a sex, age, and seasonal average). Serum 1α,25-(OH)₂D₃ was measured by a modification of a method described previously. Calf thymus receptors were used as a source of binding protein. Normal mean (SD) was 33.1 (15.3) pg/ml (healthy volunteers of both sexes aged 18–86 years). In these assays vitamin D₃ is measured in addition to vitamin D₁.

Ionised calcium was measured, as described previously. The normal range (pH=7.4) is 1.15–1.35 mmol/l.

Gc typing was performed by isoelectric focusing, followed by specific immunofixation on cellulose acetate. Gc levels were determined by rocket immunoelectrophoresis using purified human Gc globulin (Sigma Chemical Company, St Louis, Missouri, USA) as standard and polyclonal rabbit anti-Gc-antibody (Dakopatts, Denmark) (normal range 0.4–0.7 mg/ml). The concentrations of IgA and IgM rheumatoid factors were determined by an enzyme linked immunosorbent assay (ELISA), as described previously. Concentrations of IgM rheumatoid factor were determined by relating the content in the samples to an international laboratory standard with a known concentration.
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of IgM rheumatoid factor expressed in international units (IU) (normal value <8 IU/ml).

IgA rheumatoid factor concentrations were related to an internal laboratory standard serum and the content expressed in arbitrary units (AU) (normal value <25 AU/ml).

Titres of circulating antinuclear antibodies of the IgG class (determined by an indirect fluorescence technique) and concentrations of plasma immunoglobulins were recorded at the same time.

The Pearson correlation coefficient and the χ² of test was used. A level of 5% was regarded as significant.

Results

The blood concentrations of 1α,25-(OH)2D3 in patients with Sjögren's syndrome (median 26.0, Q1 19.0, Q3 42.0 pg/ml) were normal (mean (SD) 33±1 (15.3) pg/ml). In contrast, the concentrations of 25-OHD3 were reduced (median 9.0, Q1 5.0, Q3 16.0 ng/ml), (normal mean: 28±2 (12-4) ng/ml). Undetectable concentrations of 25-OHD3 were found in nine patients, and six of these had serum 1α,25-(OH)2D3 concentrations lower than 15 pg/ml (figure).

There was no significant difference between the distribution of Gc phenotypes in the patients with primary Sjögren's syndrome and in 1674 controls (χ²=1.65) (table). The serum levels of Gc globulin (median 0.6 mg/ml, Q1 0.55, Q3 0.65) corresponded to normal values (0.4-0.7 mg/ml serum).

Increased concentrations of IgM rheumatoid factor were found in 25 (72%) patients, with a median concentration of 118 IU/ml (Q1 41, Q3 189). Increased concentrations of IgA rheumatoid factor were found in 21 (60%) of the patients, with a median concentration of 92 AU/ml (Q1 55, Q3 105). Among patients with increased concentrations of IgM rheumatoid factor there was a significant negative correlation (r=-0.42, p<0.05) between the titres of IgM rheumatoid factor and the concentration of 25-OHD3, but not those of 1α,25-(OH)2D3. This was, however, not found within the group of IgA rheumatoid factor positive patients.

Twenty four (69%) of the patients exhibited antinuclear antibodies. The concentration of 1α,25-(OH)2D3 and 25-OHD3 within this group did not differ significantly from the group of patients as a whole.

Sixteen (46%) had hypergammaglobulinemia (IgG, IgA, IgM). The median IgG level was 14.9 g/l (Q1 12.7, Q3 25.2 g/l). Neither immunoglobulin levels nor the presence of extraglandular manifestations correlated with the vitamin D3 concentrations.

Ionised calcium concentration in 12 patients from whom additional serum samples were available (median 1.19 mmol/l, Q1 1.14, Q3 1.23) was normal (1.15-1.35 mmol/l).

Discussion

Primary Sjögren's syndrome was chosen for two reasons to examine the hypothesis that vitamin D metabolism is changed in patients with systemic autoimmune diseases. Firstly, polyclonal B cell activation is a pronounced and convenient marker of disturbed immune regulation in this disease. Secondly, patients with primary Sjögren's syndrome are normally not treated with immunosuppressive drugs that may affect metabolic rate. To our knowledge, this study is the first investigation of vitamin D metabolism in patients with primary Sjögren's syndrome. We found that the serum concentrations of 25-OHD3 were significantly below those of normal subjects in most patients with primary Sjögren's syndrome. In contrast, the median concentration of 1α,25-(OH)2D3 was normal. Serum immunoglobulins and immune complexes do not interfere with the vitamin D3 assay. It is questionable whether the changed vitamin D metabolism affects immune reactivities and contributes to the polyclonal B cell activation that is characteristic of these patients. Because 1α,25-(OH)2D3 is, immunologically, the most active vitamin D metabolite in vitro, it is conceivable that an abnormal concentration of this compound might result in changed immune function. Low concentrations (<18 pg/ml), however, were only found in eight of the 35 patients and did not correlate with the titres of immunoglobulin or autoantibodies. 25-OHD3 has been found to be about a thousand times less active than 1α,25-(OH)2D3 in vitro, and it has a higher affinity for Gc globulin. 

Gc phenotype distribution in 35 patients with primary Sjögren's syndrome

<table>
<thead>
<tr>
<th>Number</th>
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</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>1S</td>
</tr>
<tr>
<td>Observed</td>
<td>0 13 3 8 8</td>
</tr>
</tbody>
</table>

*Calculated from Gc prevalence determined in 1674 unrelated Danish adults.

Distribution of 1α,25-dihydroxyvitamin D3 (1α,25-(OH)2D3) and 25-hydroxyvitamin D3 (25-OHD3) in 35 patients with primary Sjögren's syndrome.

(1α,25-(OH)2D3: normal mean (SD) is 33±1 (15.3) pg/ml; 25-OHD3: normal mean (SD) is 28±2 (12.4) ng/ml.

= patients with undetectable concentrations of 25-OHD3.
the normal blood concentrations of 25-OHD3 are about a thousand times higher than those of 1α,25-(OH)2D3, the observed low concentrations of 25-OHD3 might have affected immune reactivity in these patients. It is interesting, therefore, that a highly significant correlation between the concentration of circulating 25-OHD3 and IgG titres has been reported. This was not found in our study, but the negative correlation between 25-OHD3 concentrations and IgM rheumatoid factor suggests that vitamin D3 metabolism may be related to immunopathological processes in primary Sjögren’s syndrome.

What might be the cause of the observed low 25-OHD3 concentrations? One possibility might be an impaired ability of Gc globulin to bind D3 vitamins. Thus certain phenotypes of Gc globulin have been associated with other immunoinflammatory diseases, such as rheumatoid arthritis, multiple sclerosis, and liver disease. Eales and colleagues also recently reported an association between different Gc alleles and the susceptibility to, and the clinical manifestation of, human immunodeficiency virus (HIV) infection. Subsequent studies, however, were unable to confirm this finding.

In patients with primary Sjögren’s syndrome the distribution of Gc phenotypes did not differ significantly from that in the normal population. Moreover, the serum concentrations of Gc globulin were normal. Therefore, changed Gc globulin binding of vitamin D3 is unlikely to account for the low 25-OHD3 concentrations found in patients with primary Sjögren’s syndrome.

Low concentrations of vitamin D3 may also result from severely impaired liver function but our patients had no signs of liver disease which might have explained the concentrations of 25-OHD3 we found.

As the primary aim of this investigation was to study the vitamin D3 metabolism, and because the concentrations of ionised calcium were normal, the parathyroid hormone and calcitonin metabolism were not likely to have been disturbed and were therefore not studied.

Finally, polyclonal B cell activation may itself have contributed to the low concentrations of 25-OHD3, because 25-OHD3 is metabolised in activated monocytes and consumed by activated lymphocytes through binding to vitamin D receptors of these cells.

In conclusion, we found severely diminished blood concentrations of 25-OHD3 in patients with primary Sjögren’s syndrome. It is unclear whether the changed vitamin D3 metabolism is related to the immunopathology of this disease or is merely an epiphenomenon.
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