Low serum levels of vitamin D in idiopathic inflammatory myopathies

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ABSTRACT

Objectives To evaluate serum levels of 25(OH) vitamin D in patients with idiopathic inflammatory myopathies (IIM) (polymyositis (PM), dermatomyositis (DM), inclusion body myositis (IBM) and juvenile DM (JDM)) and to compare these with healthy controls.

Methods Serum samples from 149 patients with IIM and 290 healthy controls matched for gender and the month of blood sampling were analysed for 25(OH) vitamin D. ORs for vitamin D classes with 95% CI were calculated using a matched (conditional) logistic regression model. Groups were compared by the Kruskal–Wallis test and p values <0.05 were considered significant.

Results Patients with IIM had significantly lower serum levels of 25(OH) vitamin D than healthy controls (median 39 (10–168) nmol/l vs 68 (19–197) nmol/l; p=0.0001). There was no significant difference in vitamin D levels between the myositis subgroups. When vitamin D levels were subclassified into deficient (<50 nmol/l), insufficient (50–74 nmol/l) and normal (≥75 nmol/l), most of the patients with PM (68%), DM (65%) and IBM (53%) had deficient levels compared with only 80 (21%) healthy individuals. In patients with IIM the OR for deficient versus normal was 17.7 (95% CI 8.1 to 38.6) and the OR for insufficient versus normal was 2.4 (95% CI 1.2 to 4.7).

Conclusions Low serum levels of vitamin D were found in most patients with IIM and may confer a risk factor for developing adult myositis, similar to some other autoimmune diseases.

INTRODUCTION

Idiopathic inflammatory myopathies (IIM) are chronic inflammatory disorders characterised clinically by symmetrical progressive muscle weakness and histologically by inflammatory cell infiltrates in muscle tissue. Based on different clinical and histopathological features, IIM can be classified into three major subgroups: polymyositis (PM), dermatomyositis (DM) and inclusion body myositis (IBM).1 2 The inflammatory infiltrates in muscle tissue are predominantly composed of T lymphocytes, macrophages, dendritic cells and B lymphocytes.3 Other organ manifestations are often present such as skin rash in DM or interstitial lung disease in both PM and DM. Autoantibodies are frequently detected in PM and DM (up to 80%),4 but less often in patients with IBM (20%).5 Some autoantibodies are myositis-specific, of which the anti-histidyl tRNA synthetase (anti-Jo-1) is the most common and can be found in all subsets of IIM.6 Based on the observations of immune cells in muscle tissue and the frequent presence of autoantibodies, PM and DM are considered autoimmune disorders whereas, for IBM, the role of the immune system in the pathogenesis is more debatable.7 8 Both genetic and environmental factors are likely to contribute to the aetiology of IIM, although their relative contribution to disease susceptibility has not been clarified.9–12 In DM, ultraviolet (UV) light is one suggested risk factor.13 14 There are also seasonal variations for the onset of PM and DM, with the onset of anti-Jo-1 positive myositis seeming to occur preferentially in the spring whereas the anti-Mi-2 positive DM has a peak of onset during the summer months.13 15

In the context of autoimmune, vitamin D is an interesting factor as low levels of vitamin D have been associated with several autoimmune diseases including type 1 diabetes mellitus, multiple sclerosis (MS), inflammatory bowel disease, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA).16–19 1,25-Dihydroxy vitamin D, the final active metabolite of vitamin D, is converted by 7-dehydrocholesterol upon UV-B radiation. As a member of the class II steroid hormones, it exerts immune regulating, mainly suppressive properties, acting through vitamin D receptor.20 1,25-Dihydroxy vitamin D inhibits T lymphocyte proliferation,21 particularly Th1,23 inhibits cytokine secretion such as interleukin 2 (IL-2) and interferon γ (IFNγ) by CD4 T lymphocytes and suppresses antibody secretion and autoantibody production from B lymphocytes.24 Antigen presenting cells such as dendritic cells and macrophages are also affected by 1,25-dihydroxy vitamin D. It is one of the most powerful blockers of dendritic cell differentiation and IL-12 secretion in vitro.25 26 In addition, vitamin D may induce monocyte differentiation into macrophages and modulate the macrophage response such as release of inflammatory cytokines and chemokines.27

In this study we aimed to investigate whether low levels of vitamin D could be a risk factor for patients with IIM living in a northern country with seasonal variations of UV light exposure and vitamin D levels. We compared serum levels of vitamin D between patients with IIM and healthy controls. As serum levels of vitamin D vary with the season, we also matched for month of serum sampling. Furthermore, we wanted to investigate whether there was a difference between the IIM subtypes DM, PM, IBM and juvenile onset DM (JDM) as well as between patients with or...
without autoantibodies. Moreover, we wanted to investigate if the vitamin D levels differed between patients in early disease and those with established disease.

METHODS

Subjects

This was a cross-sectional retrospective observational case–control study. All cases were identified from the myositis register at the Rheumatology Unit, Karolinska University Hospital, Solna, Stockholm. A total of 169 patients fulfilled the criteria for definitive, probable or possible PM, DM or JDM according to Bohan and Peter28 or criteria for IBM.2 From 149 of these cases, non-thawed frozen serum samples were available and were included in this study. Seventy-six patients (51%) were classified as PM, 52 (35%) as DM, 6 (4%) as JDM and 15 (10%) as IBM. Patient characteristics are presented in table 1. For the cases we aimed to analyse the blood sample which was taken at the time of diagnosis. For patients with a long disease duration, blood samples from the time of diagnosis were not always available (n=95). In these cases the serum sample that was available closest to the diagnosis date was selected for the analysis. For one case there was no information on date of diagnosis. Sixty-six cases (44%) had a disease duration ≤3 months from diagnosis at blood sampling and were considered as early cases and 83 (56%) had a disease duration >3 months and were considered as having established disease. The serum samples had been stored frozen at –80°C.

Vitamin D levels

Serum/plasma samples from the cases and controls were analysed for 25(OH) vitamin D at the same laboratory, Clinical Chemistry Laboratory, Karolinska University Hospital Solna, Stockholm, Sweden (LIASON 25OH Vitamin D TOTAL analysis, DiaSorin Inc, Stillwater, Minnesota, USA), which uses the chemiluminescence immunoassay technique for quantification of 25(OH) vitamin D. The concentration is expressed as ng/ml and the result can be transformed to SI units using the formula: ng/ml×2.5=nmol/l. The reference range for vitamin D levels was 75–250 nmol/l.

Clinical and laboratory data

Clinical data were retrieved from medical records and from a myositis register at the Rheumatology Unit, Karolinska University Hospital. For 135 patients, autoantibody profiles for myositis-specific and myositis-associated autoantibodies were analysed by line blot assay (Euroimmune AG, Lübeck, Germany) at the Kennedy Institute, London. For the remaining 14 patients the Multiplex Antinuclear Antibody Assay (BioPlex 2200 System, Bio-Rad Inc Laboratories, Hercules, CA, USA) was used, tested as clinical routine at the Department of Clinical Immunology, Karolinska University Hospital.

Statistical analyses

GraphPad Prism 4.0 statistical software (GraphPad, San Diego, California, USA) was used for the following tests. Vitamin D levels were compared by the Kruskal–Wallis test between multiple groups. The Mann–Whitney U test was used to compare vitamin D levels between the two groups. The Spearman rank correlation coefficient was used to test for correlations. p Values ≤0.05 were considered significant. ORs for vitamin D classes with 95% CI were calculated by means of a matched (conditional) logistic regression model. The SAS software package V9.2 (SAS Institute, Cary, North Carolina, USA) was used to calculate ORs and 95% CI.

RESULTS

Patients with IIM had significantly lower serum levels of 25(OH) vitamin D than healthy controls (median 38.5 (range 10–168) nmol/l vs 68.0 (range 19–197) nmol/l, p=0.0001; figure 1A).

In the IIM subgroups the median (range) vitamin D levels were 35.5 (10–168) nmol/l for PM, 43.9 (14–135) nmol/l for DM, 45.0 (11–84) nmol/l for IBM and 55.0 (18–78) nmol/l for JDM. There was no significant difference in vitamin D levels between the myositis subgroups. There was no difference in the vitamin D levels between men and women in the IIM group but, in the control group, men had lower levels than women.

The controls were selected from a population-based randomly drawn control group that had been identified as controls for the Epidemiological Investigation of Multiple Sclerosis, a Swedish case-control study for MS.16 29 The controls had been identified through a national population registry and encompassed 1194 Swedish controls with Scandinavian ancestry. They had received a kit through the mail that contained sample tubes and a referral letter to a laboratory where the blood was drawn. For each IIM case, two gender-matched controls were selected where possible. The controls were also matched for the month of blood sampling (only men born in November got one control each). Two hundred and ninety controls were included in the study (table 1).

The study was approved by the regional ethics committee of Karolinska University Hospital, Stockholm, Sweden and patients and controls gave their consent for the study.

Table 1 Demographic data and clinical characteristics of cases and controls

<table>
<thead>
<tr>
<th></th>
<th>Cases</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td>149</td>
<td>290</td>
</tr>
<tr>
<td>Male</td>
<td>52 (35)</td>
<td>98 (34)</td>
</tr>
<tr>
<td>Female</td>
<td>97 (65)</td>
<td>192 (66)</td>
</tr>
<tr>
<td>Age, year (at blood sampling)</td>
<td>56 (18–72)</td>
<td>41 (18–70)</td>
</tr>
<tr>
<td>Disease duration,* (months)</td>
<td>6.5 (0–368)</td>
<td>–</td>
</tr>
<tr>
<td>Subdiagnosis, n (%)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>PM</td>
<td>76 (51)</td>
<td>–</td>
</tr>
<tr>
<td>DM</td>
<td>52 (35)</td>
<td>–</td>
</tr>
<tr>
<td>IBM</td>
<td>15 (10)</td>
<td>–</td>
</tr>
<tr>
<td>JDM</td>
<td>6 (4)</td>
<td>–</td>
</tr>
<tr>
<td>Autoantibodies</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Jo-1, n (%)</td>
<td>23 (16)</td>
<td>1451</td>
</tr>
<tr>
<td>Other AsAb†, n (%)</td>
<td>3 (2)</td>
<td>1301</td>
</tr>
<tr>
<td>Anti-SSA‡, n (%)</td>
<td>44 (34)</td>
<td>1301</td>
</tr>
<tr>
<td>Anti-SSB, n (%)</td>
<td>7 (6)</td>
<td>1281</td>
</tr>
<tr>
<td>Anti-Mi-2, n (%)</td>
<td>3 (7)</td>
<td>43†</td>
</tr>
<tr>
<td>Anti-SRP, n (%)</td>
<td>5 (6)</td>
<td>84†</td>
</tr>
</tbody>
</table>

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* Disease duration calculated from diagnosis to date of blood sampling.
† Total number of cases with available results from antibody analysis.
‡ Anti-PL-7, anti-PL-12, anti-EJ, anti-OJ, anti-KS, anti-ZD.
§ SSA/Ro52 and/or SSA/Ro60: six patients had an overlap syndrome (rheumatoid arthritis in 1, systemic sclerosis in 2, Sjögren’s syndrome in 2, mixed connective tissue disease in 1).
¶ AsAb, anti-synthetase antibody; DM, dermatomyositis; IBM, inclusion body myositis; JDM, juvenile dermatomyositis; PM, polymyositis; SSA, Sjögren’s syndrome antigen A; SSB, Sjögren’s syndrome antigen B; SRP, signal recognition particle.
The vitamin D levels were subclassified into three categories: deficient (<50 nmol/l), insufficient (50–74 nmol/l) and normal (≥75 nmol/l). Most of the patients with PM (69%), DM (65%) and IBM (53%) had deficient levels while most of the population-based controls had normal or insufficient vitamin D levels (table 2). There were significant differences in vitamin D levels when the PM, DM and IBM subgroups were compared with the controls (p<0.001). The difference in vitamin D levels between JDM cases and controls was not significant. The ORs for the different subclasses of vitamin D levels in the whole IIM cohort were 17.7 (95% CI 8.1 to 38.6) for deficient versus normal and 2.4 (95% CI 1.2 to 4.7) for insufficient versus normal. After adjustment for age, the ORs changed to 13.6 (95% CI 5.8 to 31.7) for deficient versus normal and 2.1 (95% CI 1.0 to 4.4) for insufficient versus normal. No correlation was observed between age at sampling and vitamin D levels for cases (p=0.37) or for controls (p=0.56).

Anti-Jo-1 autoantibody analysis was available for 145 cases, 23 of whom (16%) were positive for anti-Jo-1 autoantibodies. Most of the anti-Jo-1 positive patients (61%) were in the group with deficient levels of vitamin D, the second largest group of patients was in the category with insufficient vitamin D levels and the lowest number of patients was in the normal category of vitamin D levels (table 2). Patients with anti-Jo-1 autoantibodies had significantly lower median vitamin D levels than controls (38.0 (range 10–168) nmol/l vs 68.0 (range 19–197) nmol/l, p<0.0001). When the median levels of vitamin D in all autoantibody positive patients (40.0 (range 10–168) nmol/l) were compared with those in all autoantibody negative patients (38.0 (range 11–104) nmol/l), no significant difference was seen.

There was also a difference in vitamin D levels with disease duration, with lower levels in patients with disease duration ≤3 months than in those with disease duration >3 months (table 3). There was a positive correlation between disease duration in months and vitamin D levels (r=0.300, p=0.0001).

Finally, we analysed levels of vitamin D during the different months of serum sampling. As expected, the vitamin D levels varied over the year, being lowest in the winter and spring months. The pattern of seasonal variation was different between cases and controls in this respect (figure 1B,C). When assessing the month of diagnosis of the patients, the highest frequency of myositis diagnosis was made during January, which is during the period of seasonal low vitamin D levels (figure 1D).

Table 2  Number (%) of cases and controls with deficient, insufficient or normal serum levels of vitamin D

<table>
<thead>
<tr>
<th></th>
<th>Deficient (&lt;50 nmol/l)</th>
<th>Insufficient (50–74 nmol/l)</th>
<th>Normal (≥75 nmol/l)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>60 (21)</td>
<td>113 (39)</td>
<td>117 (40)</td>
<td>290 (100)</td>
</tr>
<tr>
<td>Cases</td>
<td>96 (64)</td>
<td>35 (24)</td>
<td>18 (12)</td>
<td>149 (100)</td>
</tr>
<tr>
<td>PM</td>
<td>52 (69)</td>
<td>14 (18)</td>
<td>10 (13)</td>
<td>76 (51)</td>
</tr>
<tr>
<td>DM</td>
<td>34 (65)</td>
<td>12 (23)</td>
<td>6 (12)</td>
<td>52 (35)</td>
</tr>
<tr>
<td>IBM</td>
<td>8 (53)</td>
<td>6 (40)</td>
<td>1 (7)</td>
<td>15 (10)</td>
</tr>
<tr>
<td>JDM</td>
<td>2 (33)</td>
<td>3 (50)</td>
<td>1 (17)</td>
<td>6 (4)</td>
</tr>
<tr>
<td>Anti-Jo-1 +*</td>
<td>14 (61)</td>
<td>6 (26)</td>
<td>3 (13)</td>
<td>23 (16)</td>
</tr>
</tbody>
</table>

*Anti-Jo-1 autoantibody analysis was available for 145 cases.

DM, dermatomyositis; IBM, inclusion body myositis; JDM, juvenile dermatomyositis; PM, polymyositis.

Figure 1  (A) Patients with idiopathic inflammatory myopathies (IIM) had significantly lower serum levels of vitamin D than healthy individuals (p=0.0001). (B) Median levels of monthly variations of vitamin D levels in patients with IIM and healthy controls. (C) Median levels of monthly variations of vitamin D levels in patients with IIM positive for anti-Jo-1 autoantibodies and with disease duration ≤3 months. (D) Month of diagnosis of patients with IIM.
a role for vitamin D in autoantibody formation. Patients with SLE with vitamin D deficiency had higher IFNα and B cell activity than patients without vitamin D deficiency. Notably, patients with anti-Jo-1 positivity were associated with type I IFN activity and high serum levels of B cell activating factor, which might indicate a role for vitamin D deficiency in autoantibody production in these patients. In patients with JDM, higher IFNα levels were seen with shorter disease duration but information on vitamin D levels was not included in this report. Measurement of interferon activity was beyond the scope of our study and the number of JDM cases was too low to allow any conclusions. The role of autoimmunity in patients with IBM is controversial, although a subgroup of patients with IBM had features of other autoimmune diseases and autoantibodies. In our rheumatology setting we cannot exclude a bias towards this subset.

We observed that vitamin D levels correlated positively with disease duration (≤3 months). One explanation for the low levels of vitamin D in the early phase of disease might be disability and difficulties in being outdoors because of musculoskeletal symptoms. This may be particularly relevant for patients with IBM who often have a very slowly progressive muscle weakness and a long delay before being diagnosed, but this could not be answered by our study. The higher serum levels of vitamin D in patients with established disease than in those with early disease could possibly be explained by the administration of a calcium vitamin D supplement together with glucocorticoid treatment as prophylaxis against osteoporosis. However, the vitamin D dosage given as a supplement with calcium is lower than the recommended dose for treatment of vitamin D deficiency, so the supplement is not likely to explain the higher levels during established disease. Details of the use of a vitamin D supplement were missing in many patients which prevented us from calculating achieved doses.

Interestingly, vitamin D supplements could be therapeutically effective in autoimmune diseases, as demonstrated in some studies with animal models—for example, in mice with experimental autoimmune encephalomyelitis, collagen-induced arthritis, type 1 diabetes mellitus or autoimmune thyroiditis. In patients with MS, a vitamin D supplement has been suggested as part of the management but the documentation on therapeutic intervention with vitamin D in already established MS is limited. Whether the therapeutic effectiveness of vitamin D is true in other autoimmune diseases is not known.

Our study has limitations. First, the controls were generally younger than the cases but this is not likely to explain the differences between patients and controls as no correlation between age and vitamin D levels was found among the controls and the significant difference between patients and controls persisted after adjustment for age. Another general limitation is that the low vitamin D levels may be a consequence of the disease rather than a cause, and at least some of the cases may have taken vitamin D supplements for different lengths of time before blood sampling, particularly after IIM diagnosis, with glucocorticoid treatment. To overcome this problem, we divided the cases into two groups with disease duration shorter or longer than 3 months. Indeed, the patients with shorter disease duration had lower levels of vitamin D than those with established treated disease. This supports our hypothesis that low levels of vitamin D could be one of several risk factors for the development of IIM.

In summary, low serum levels of vitamin D were found in most adult patients with IIM. The deficient vitamin D levels in patients with disease of shorter duration (<3 months) and the
seasonality of the disease support the role of vitamin D deficiency as a risk factor in autoimmune/inflammatory rheumatic diseases such as RA and now also including IIM. Whether low levels of vitamin D have a role in the pathogenesis or affect the prognosis is not known and will need further investigation. The results provide guidance for future studies looking at a potential role for vitamin D in the prevention and/or treatment of IIM.

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Contributors PA, IEL: conceived and designed the study, collected and monitored the data, reviewed and analysed the data and wrote the manuscript. SBH: data collection and analysis, drafting the article. PFA: conceived and designed the study and drafting the article. PC: data collection, drafting the article. All authors gave final approval of the version to be published.

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Competing interests None.

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