EXTENDED REPORT

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), dermatomyosistis (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 60 (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 myosis, 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), dermatomyosistis (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 autoimmunity, 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 22] 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 subclasses 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 Peter[28] 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.

Table 1 Demographic data and clinical characteristics of cases and controls

	Cases	Controls	
Gender, n (%)	149	290	
Male	52 (35)	98 (34)	
Female	97 (65)	192 (66)	
Age, year (at blood sampling)			
Median (range)	56 (18–72)	41 (18–70)	
Disease duration,* (months)		–	
Median (range)	6.5 (0–368)		
Disease duration,* n (%)		–	
≤3 m	66 (44)		
>3 m	83 (56)		
Subdiagnosis, n (%)		–	
PM	76 (51)		
DM	52 (35)		
IBM	15 (10)		
JDM	6 (4)		
Autoantibodies			
Anti-Jo-1, n (%)	23 (16)	145†	–
Other AsAb‡, n (%)	3 (2)	130†	–
Anti-SSA§, n (%)	44 (34)	130†	–
Anti-SSB, n (%)	7 (6)	128†	–
Anti-Mi-2, n (%)	3 (7)	43†	–
Anti-SRP, n (%)	5 (6)	84†	–

*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-ZO.
§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 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.

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 (LIAISON 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 V.9.2 (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 (14–133) nmol/l for DM, 45.0 (11–84) nmol/l for IBM and 53.0 (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.
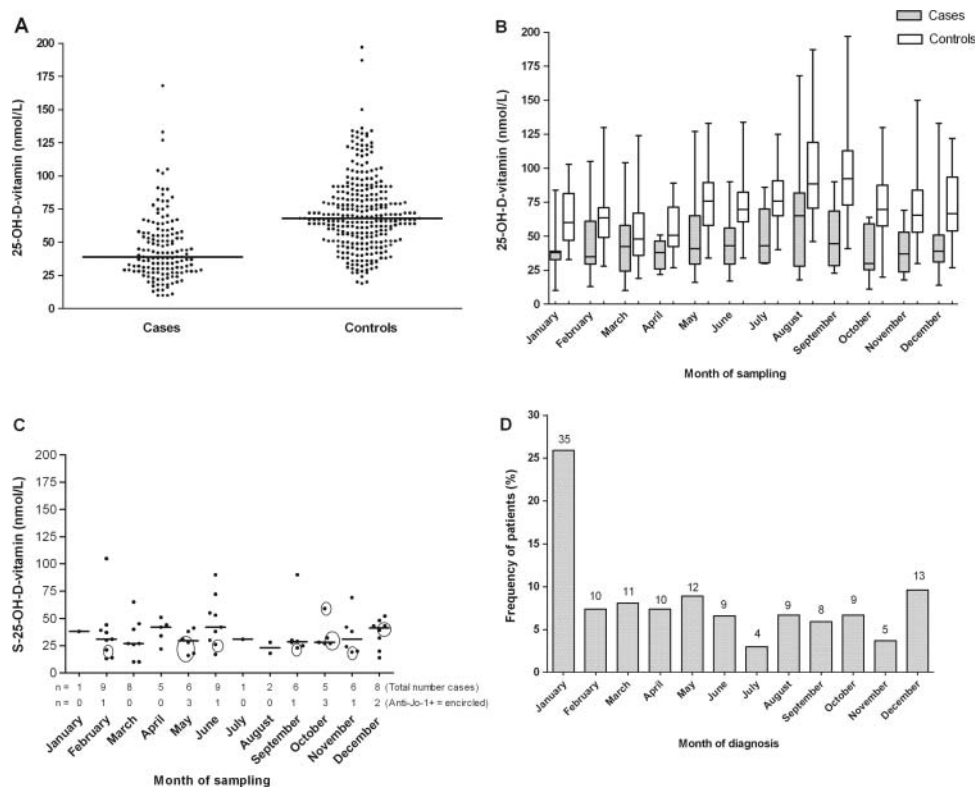
Figure 1 (A) Patients with idiopathic inflammatory myopathies (IIM) had significantly lower serum levels of vitamin D than healthy individuals (p=0.0001). (B) Median levels of monthly variations of vitamin D levels in patients with IIM and healthy controls. (C) Median levels of monthly variations of 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.

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 1B,D).

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

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

*Anti-Jo-1 autoantibody analysis was available for 145 cases.
DM, dermatomyositis; IBM, inclusion body myositis; JDM, juvenile dermatomyositis; PM, polymyositis.

Table 3 Median serum levels of vitamin D (nmol/l) in patients with disease duration ≤3 months and >3 months

| | Disease duration* | | p Value |
	≤3 months	>3 months	
Cases (n)	66	83	<0.0001
Median (range)	31 (10–105)	50 (10–168)	<0.0001
Mean±SD	36±19	53±27	<0.0001
Coefficient of variation	52.07%	51.44%	

*Disease duration calculated from the diagnosis to date of blood sampling.

DISCUSSION

We found that adult patients with IIM of all subclasses (PM, DM and IBM) have significantly lower serum levels of vitamin D compared with gender-matched population based control samples collected during the same month of the year. Furthermore, the vitamin D levels were lower in samples taken close to diagnosis than in samples taken during established disease, which suggests that low levels of vitamin D may be a risk factor for developing adult IIM. To the previously reported autoimmune diseases such as MS, RA and SLE[17][18][30] where vitamin D seems to be a risk factor, we can now add adult PM, DM and IBM.

The OR for vitamin D deficiency was high for IIM patients, even higher than that seen in MS cases in Sweden.[31] There was also a significant difference between each of the PM, DM and IBM subgroups compared with controls when we focused on the vitamin D deficiency subclass. Vitamin D levels can be considered as a proxy for sun exposure. In northern latitudes, solar radiation is not sufficient for the synthesis of enough vitamin D for almost half of the year (autumn-winter season) leading to a risk of vitamin D deficiency. As a result there is a strong seasonal variation in circulating levels of 25(OH) vitamin D in these countries.[32] Such seasonal variations in the vitamin D level were also seen in our study, but with unexpected differences between cases and controls. Cases had peak vitamin D levels in August followed by a depression in October (figure 1B) whereas controls had a depression in March and then increasing levels to the end of the spring and during the summer (figure 1B). One possible explanation could be that the vitamin D metabolism in the responsible body organs (from the gastrointestinal tract to the skin and liver) may vary between patients with IIM who have inflammation in several organs and healthy individuals. Notably, the vitamin D levels in the adult IIM cases were significantly lower than the serum levels of controls even when the same month of blood sampling was compared. This was also seen in adult cases with DM where UV light exposure is a previously reported risk factor.[11][33] This has particularly been related to the DM subgroup with anti Mi-2 autoantibodies. Notably, these autoantibodies were rarely found among our patients with DM, suggesting that there may be different risk factors for various subsets of DM and that vitamin D deficiency may constitute a risk factor for some patients with DM as well as for PM and IBM. Similar seasonal changes in vitamin D levels have been observed for patients with RA and SLE where the vitamin D levels also correlated inversely with disease activity.[34][35] In concordance with RA onset, the IIM cases were diagnosed more frequently during winter or spring (figure 1D).[36]

Strikingly, most of the anti-Jo-1 positive patients were in the group with deficient vitamin D levels (table 2). Recently, low vitamin D levels have been associated with ANA positivity in healthy controls and with anti-dsDNA titre in SLE, suggesting a role for vitamin D in autoantibody formation.[37][38] 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.[39][40] 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.[41] 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.[5][42] 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,[43][44] collagen-induced arthritis,[45] type 1 diabetes mellitus[46] or autoimmune thyroiditis.[47] In patients with MS, a vitamin D supplement has been suggested as part of the management[48] 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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