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# Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: Northern versus Southern Europe

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## ABSTRACT

**Background.** *Greater intake of vitamin D has been associated with a lower risk of rheumatoid arthritis (RA) and low serum vitamin D together with higher prevalence of RA seem common among North European people when compared to Southern Europe.*

**Objectives.** *To evaluate serum 25-hydroxyvitamin D [25(OH)D] levels in female RA patients from North (Estonia) and South (Italy) Europe and to correlate them with the disease activity score (DAS28) during winter and summer.*

**Methods.** *Fifty-four RA Italian patients (IP) and 64 RA Estonian patients (EP) were evaluated for serum 25(OH)D levels in winter and summer time, as well as for DAS28 score. Normal female controls (C) were 35 (IC) and 30 (EC) age-matched subjects, respectively. 25(OH)D concentrations were measured by a competitive radioimmunoassay. Statistical analysis was performed by "r" Pearson correlation, "t" Student with Bonferroni correction and by repeated ANOVA measures (summer and winter) with two factors (country and clinical status).*

**Results.** *25(OH)D levels were found significantly higher in IP versus EP ($p = 0.0116$) both in winter and in summer time. Differences were observed also in controls. The variations (increase) of 25(OH)D levels between winter and summer were found significant ($p = 0.0005$) in both IP and EP. Differences were observed also in controls.*

*No significant differences were found concerning 25(OH)D levels between RA patients and their controls in either country.*

*Interestingly, a significant negative correlation between 25(OH)D and DAS28, was found in summer only in IP ($r = -0.57$, $p < 0.0001$) and in winter in EP ($r = -0.40$, $p < 0.05$).*

**Conclusion.** *Significantly lower 25-(OH)D serum levels were observed in RA patients from North versus South Europe with a circannual rhythm in winter and summer time. In addition, 25(OH)D values showed a significant correlation (negative) with RA clinical status (DAS28) in both North and South European RA patients, suggesting possible effects of vitamin D among other factors on disease activity.*

## Introduction

It is clear that both genetic and environmental factors affect prevalence of autoimmune diseases. The data link vitamin D and insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), inflammatory bowel diseases (IBD), and rheumatoid arthritis (RA) (1,2).

The fact that vitamin D has been implicated as a factor in several different autoimmune diseases suggests that vitamin D might be one of the environmental factors that among others normally participates in the control of self tolerance (2).

Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations (3). However, its highest concentration is found in the immature immune cells of the thymus and the mature CD-8 T lymphocytes.

The significant role of vitamin D compounds as selective immunosuppressants is also illustrated by their ability to either prevent or markedly suppress animal models of autoimmune diseases (4).

RA is an autoimmune disorder of multifactorial etiology in which both genetic and nongenetic factors (i.e. infectious, hormonal, environmental) contribute to disease susceptibility.

Vit D may exert immunomodulatory effects and hypovitaminosis D together with higher prevalence of RA seems common amongst North when compared to South Europe (5). Recently, greater intake of vit D was associated with a lower risk of rheumatoid arthritis (RA), as well as lower vitamin D was found associated with higher disease activity (6, 7).

In the present study, we decided to evaluate serum 25-hydroxyvitamin D [25(OH)D] levels in female RA patients from North (Estonia) and South (Italy) Europe and to correlate them with the disease activity score (DAS28) during summer and winter time.

## Patients and methods

A total number of 54 Italian RA patients (IP, 58.5 ± 1.1 years,) and 64 Estonian RA patients (EP, 56.3 ± 2.3 y), were evaluated in winter and summer

for 25-hydroxyvitamin D 25(OH)D, as well as for disease activity score (DAS 28). Clinical evaluations and blood samples were collected at the end of each season. In particular, the sample collection was carried out mainly from December to February in the winter, and mainly from July to August in the summer.

Normal female controls included 35 (IC, 59.9 ± 0.9 y) and 30 (EC, 57.1 ± 3.8 y) age-matched subjects, respectively. No vitamin D or calcium, nor drugs affecting calcium metabolism were administered from 2 months before the study starting. No liver or kidney insufficiency was present. Treatment with low dose glucocorticoids and disease-modifying antirheumatic drugs (DMARDs) was constant from at least 3 months.

25(OH)D concentrations were measured by a competitive radioimmunoassay (DiaSorin, Stillwater, Minnesota, USA). The current standard of disease activity evaluation was the EULAR Disease Activity Score (DAS), as well as the modified DAS 28 based on a 28-joint assessment (8).

Informed consent from all the patients, as well as the Ethical Committee approval were obtained. Statistical analysis was performed by *r* Pearson correlation, *t*-Student with Bonferroni correction and by repeated ANOVA measures (summer and winter) with two factors (country and clinical status). Log transformed data were used for the analysis. Significant level was considered to be $p = 0.05$.

## Results

As reported in Table I, 25(OH)D levels were found significantly higher in IP versus EP ($p = 0.0116$) both in winter and in summer. Differences were observed also among the controls both in winter and in summer (Table I).

The variations (increase) of 25(OH)D levels between winter and summer time (Table I), were found significant in both IP and EP ($p = 0.0005$). Differences were observed also in the controls between winter and summer.

No significant differences were found concerning 25(OH)D levels between Italian and Estonian RA patients and their controls.

Interestingly, a significant negative correlation between 25(OH)D and DAS28, was found in summer in IP ($r = -0.57$, $p < 0.0001$) and in winter in EP ($r = -0.40$, $p < 0.05$).

However, the mean DAS28 scores were found generally higher in EP vs IP (NS) and the difference was more evident in winter (Table I).

## Discussion

Significantly lower 25(OH)D serum levels were observed in RA patients from North (Estonia) versus South (Italy) Europe with a circannual rhythm, namely in winter and summer time. In addition, low 25(OH)D values showed in summer a significant moderate negative correlation with RA clinical status (DAS28) in South European patients, whereas in Estonian RA patients the significant negative correlation was found in winter, suggesting possible effects of vitamin D, among other factors, on RA disease activity.

Vitamin D may exert immunomodulatory effects and hypovitaminosis D together with higher prevalence of RA seems common amongst Northern patients when compared to South Europe (7).

As a matter of fact, hypovitaminosis D linked to higher latitude further characterizes Greenlanders versus Danes (plasma 25(OH)D < 40 nmol/l) (9).

A possible beneficial role for ultraviolet radiations (UVR) on three Th1-mediated autoimmune diseases: multiple sclerosis, type 1 diabetes and RA in relation to recent developments in photoimmunology has been analyzed (2). Recent work suggests that UVR exposure may be one factor that can attenuate the autoimmune activity leading to these three diseases through several pathways involving UVB and UVA irradiation, UVR-derived vitamin D synthesis and other routes such as alpha-melanocyte-stimulating hormone, and melatonin (2, 10). Therefore, ecological features, particularly a gradient of increasing prevalence of RA (Finland nearly 0.8 % and 0.3 % in Italy) and other autoimmune diseases with higher latitude, provide some support for a beneficial role of UVR (11-15).

In addition, there may be a higher vitamin D requirement for patients at risk for developing autoimmunity and those that already have an autoimmune disease such as systemic lupus erythematosus (SLE) (16).

The optimal amount of vitamin D to support the immune response may be different from the amount required to prevent vitamin D deficiency or to maintain calcium homeostasis. New evidence from human, animal, and *in vitro* mechanistic experiments suggest that vitamin D may play a role in the etiology of autoimmunity (4). Therefore, although chronic excessive exposure to sunlight increases the risk of melanoma skin cancer, the limited and/or avoidance of all direct sun exposure (i.e. darkness) increases the risk of vitamin D deficiency, which in turn can play a role in autoimmunity (17).

Interestingly, polymorphisms in the vitamin D receptor (VDR) have been correlated with increased susceptibility of RA (18). Furthermore, a recent study in North European people (Finnish) reported the association between poly-

**Table I.** RA patients and control characteristiques. 25(OH)D serum levels and DAS28 scores in Italian and Estonian RA patients in winter and in summer. 25(OH)D serum levels in Italian and Estonian controls. Average ± SE.

| | Italy** RA patients | Estonia** RA patients | Italy** Controls | Estonia** Controls |
|---|---|---|---|---|
| -Patients (n°) | 53 | 64 | 35 | 30 |
| Mean age (years) | 58.5 ± 1.1 | 56.3 ± 2.3 | 59.9 ± 0.9 | 51.1 ± 3.8 |
| 25(OH)D Winter (nmol/L)* | 58.9 ± 5.4 | 35.1 ± 1.9 | 54.5 ± 5.5 | 43.3 ± 2.6 |
| 25(OH)D Summer (nmol/L)* | 65.2 ± 5.4 | 46.4 ± 2.3 | 68.9 ± 6.1 | 47.4 ± 3.1 |
| DAS28 Winter | 3.73 ± 1.69 | 4.19 ± 1.24 | | |
| DAS28 Summer | 3.48 ± 0.25 | 3.99 ± 1.46 | | |

*ANOVA: winter versus summer $p = 0.0005$; ** ANOVA: Italy versus Estonia $p = 0.0116$.

morphisms of VDR gene and individual risk of hand osteoarthritis (OA) (19). However, recently, a significant clinical improvement was strongly correlated with the immunomodulating potential in vitamin D-treated RA patients (20).

In conclusion, prolonged daily darkness, different genetic background (i.e. vitamin D receptor polymorphism) and nutritional factors, may explain lower 25(OH)D levels observed in North Europe (i.e. Estonia). Low 25(OH)D levels might be also partially linked, among other factors, to the recognized local increased prevalence of autoimmune diseases such as RA by considering the potential immunosuppressive role of Vit D (2,10).

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