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ORIGINAL ARTICLE

Vitamin D status of patients with early inflammatory arthritis

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Abstract The present study aimed to investigate the vitamin D status in patients with early inflammatory arthritis (EIA). We conducted a retrospective study among patients who presented with EIA at the outpatient rheumatology clinic of a tertiary referral center between March 2012 and February 2013. In total, 101 subjects with EIA (≥1 swollen joint and symptom duration of ≤6 months, not explained by another disease) and 101 healthy controls matched for age, sex, and the month of serum vitamin D measurements were enrolled. Serum 25-hydroxy vitamin D (25-OHD) concentrations were assessed by radioimmunoassay. Vitamin D "deficiency" and "severe deficiency" were defined as serum 25-OHD levels <20 and <10 ng/mL, respectively. Among EIA patients, rheumatoid arthritis (RA) was classified according to the 2010 American College of Rheumatology/European League against Rheumatism criteria. Vitamin D deficiency was highly prevalent among EIA patients, but no significant differences in the frequency of vitamin D deficiency of EIA patients and controls were observed (75.2 vs 65.3 %, p=0.106). Additionally, in spring and summer, EIA patients had significantly lower serum 25-OHD concentrations than controls, but the opposite trend was observed in autumn. Among 101 EIA patients, 38 (37.1 %) were classified as having RA. Severe vitamin D deficiency in EIA patients was significantly associated with the higher likelihood of being classified as having RA. In conclusion, the frequency of vitamin D deficiency in EIA patients was comparable to that in controls, but severe vitamin D deficiency was associated with the presence of RA among EIA patients.

Keywords Early inflammatory arthritis · Rheumatoid arthritis · Seasons · Vitamin D

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Introduction

There is increasing evidence that vitamin D fulfills an immunoregulatory role, and in recent years, several studies have investigated the association between hypovitaminosis D and the onset as well as severity of rheumatic diseases including rheumatoid arthritis (RA). Previous studies have reported that vitamin D deficiency is highly prevalent among patients with RA [1, 2] and correlates with higher disease activity [1, 3–6], lower functional status [4, 5], and even increased cardiometabolic risk [7, 8]. However, it is not yet clear whether vitamin D deficiency is an independent risk factor for the onset of RA, because studies have reported conflicting results [9, 10]. Although a recent meta-analysis showed that low vitamin D intake is associated with increased RA incidence [11], many studies failed to find significant differences in the serum vitamin D concentrations of RA patients and healthy individuals [3, 5, 12]. Therefore, the true immunomodulating role of vitamin D in the onset of RA has not been fully elucidated and it warrants further investigation.

When compared with "established" RA, little attention has been given to vitamin D deficiency in early inflammatory arthritis (EIA) or unclassified arthritis, which can lead to the development of RA. We speculated that knowledge of vitamin D status among patients with EIA could be valuable for understanding the role of vitamin D in the onset and

development of RA. Considering this hypothesis, our study was designed to compare the serum 25-hydroxy vitamin D (25-OHD) levels of EIA patients and healthy individuals matched for age, sex, and sampling month. In addition, we evaluated the association of vitamin D deficiency and the likelihood of being classified as having RA among EIA patients.

Methods

Study design and subjects

We conducted a retrospective study among consecutive patients who presented with EIA at the outpatient rheumatology clinic of the Pusan National University Hospital in Busan, South Korea, between March 2012 and February 2013. Pusan National University Hospital is a tertiary referral center in South Korea and Busan is a harbor city with a temperate climate located in the southeastern part of South Korea at a latitude of 34 ° north.

In total, 101 subjects with EIA, as well as 101 healthy controls, matched for age (±1 year), sex, and the month of serum 25-OHD measurement were enrolled; none of the participants were receiving vitamin D supplementation. "EIA" was defined as follows: ≥1 swollen joint, with a symptom duration of ≤6 months, which could not be attributed to another disease. Healthy controls were randomly selected from individuals who underwent annual health check-ups at the same hospital, and none of them had a history of rheumatic and musculoskeletal diseases. Serum 25-OHD levels at the initial visit were available for all study subjects. Further, all subjects were naïve to disease-modifying anti-rheumatic drugs (DMARDs) including hydroxychloroquine, sulfasalazine, methotrexate, and leflunomide, and none of them had a history of chronic kidney disease or were taking medications that could have affected vitamin D metabolism including vitamin D, calcium, glucocorticoids, bisphosphonate, or thyroxine. The procedures of our study were approved by the Research and Ethical Review Board of the Pusan National University Hospital, which waived informed patient consent.

Assessment

The medical records including clinical and laboratory parameters of all participants were reviewed. Serum 25-OHD levels were assessed by radioimmunoassay. Vitamin D "deficiency" and "severe deficiency" were defined as serum 25-OHD levels <20 and <10 ng/mL, respectively [2, 13]. The month of serum 25-OHD measurement was further classified as spring (March to May), summer (June to August), autumn (September to November), and winter (December to February) [14].

For patients with EIA, clinical parameters including swollen joint count (SJC), tender joint count (TJC), and symptom duration, as well as their assessment of general health and physical function, were obtained and recorded. General health was assessed using a visual analog scale (VAS) that was rated from 0–100 and physical function was assessed using the Korean version of health assessment questionnaire (K-HAQ) [15]. For each EIA subjects, disease activity score assessed by using 28-joint counts for swelling and tenderness (DAS28)-erythrocyte sedimentation rate (ESR) score was calculated by the following formula: DAS28-ESR score $=[0.56\times\sqrt{(\text{TJC }28)}]+[0.28\times\sqrt{(\text{STC }28)}]+[0.70\times\ln\text{ESR}]+[0.0014\times\text{VAS}]$ [16]. We also reviewed the following laboratory parameters of EIA subjects, including serum ESR, C-reactive protein (CRP), immunoglobulin M—rheumatoid factor (RF), and anti-cyclic citrullinated peptide (CCP) antibody. CRP was measured by particle-enhanced immunoturbidimetric assay (Tina-quant C-reactive protein, Roche Diagnostics, Switzerland) using a P-800 Modular (Roche, Switzerland). RF was assessed by particle-enhanced immunoturbidimetric assay (range 0–14 IU/ml) and anti-CCP was measured using chemiluminescent microparticle immunoassay (range 0–5 U/mL).

Patients with EIA were classified at their initial visit by one rheumatologist (Lee) as rheumatoid arthritis (RA) and non-RA. The criteria for the 2010 American College of Rheumatology/European League against Rheumatism (ACR/EULAR) criteria were used for this classification [17]. Non-RA subjects were further categorized by the same rheumatologist as having osteoarthritis (OA), undifferentiated inflammatory arthritis (UIA), or other rheumatic or musculoskeletal diseases.

Statistical analysis

Descriptive statistics included means and standard deviations (with normal distribution) and medians and interquartile ranges (with non-normal distribution) for continuous variables and number of cases with percentages for categorical variables. Kolmogorov-Smirnov tests were used to evaluate the normal distribution of data. For group comparisons, we used the two-tailed Student's t tests or the Mann-Whitney U tests for continuous variables and the χ^2 analysis or the Fisher's exact tests for categorical variables, as appropriate. Spearman's analysis was used to evaluate the correlations between serum 25-OHD levels and other clinical and laboratory parameters in EIA patients. We used multivariable logistic regression analyses with backward model selection of demographic variables such as age and gender as well as variables that had $p\leq 0.1$ in univariable analysis, to assess the relationship between serum 25-OHD deficiency and the higher likelihood of EIA subjects being classified as having

RA. Odds rations (ORs) with 95 % confidence intervals (95 % CI) were calculated to assess the association between variables and the presence of RA. p Value less than 0.05 was considered statistically significant and all statistical analyses were carried out using PASW 18.0 for windows (Chicago, IL, USA) and STATA 11.0 for windows (StataCorp LP, College Station, TX, USA).

Results

Clinical characteristics of subjects with EIA and healthy controls are summarized in Table 1. Of note, the majority of subjects, both EIA patients and healthy subjects, were vitamin D deficient (75.2 and 65.3 % of EIA and healthy subjects, respectively). We found no significant differences between the serum 25-OHD levels or the proportion of vitamin D deficient subjects and severely vitamin D deficient subjects in the two groups. In addition, the serum 25-OHD levels of EIA and healthy subjects did not differ according to sex (data not shown). In spring and in summer, the serum 25-OHD levels of EIA subjects were significantly lower than those of healthy controls (12.7 (8.9–15.6) vs 15.5 (10.7–22.3), p=0.039, for spring and 13.7 (11.1–19.2) vs 20.0 (16.5–26.2), p=0.047, for summer, respectively). In contrast, in autumn, serum 25-OHD levels of EIA subjects tended to be higher than that of healthy controls (19.0 (14.0–33.3) vs 14.8 (11.0–22.3), p=0.056), but statistical significance was not reached (Fig. 1). We found no difference in serum 25-OHD levels between the two groups in the winter months and our findings suggest that there could be seasonal variation in the differences of serum 25-OHD levels between patients with EIA and healthy individuals.

Table 1 Demographics and disease characteristics of patients with early inflammatory arthritis and healthy controls

Variables	EIA (n=101)	Controls (n=101)	p value
Age, years	56.5±12.2	56.6±12.1	0.949
Female, n (%)	86 (85.1)	86 (85.1)	1.000
Serum 25-OHD, ng/mL	14.2 (10.9–20.3)	16.3 (11.5–23.3)	0.299
Vitamin D deficiency (<20 ng/mL), n (%)	76 (75.2)	66 (65.3)	0.166
Severe vitamin D deficiency (<10 ng/mL), n (%)	18 (17.8)	15 (14.9)	0.704
Classification			
RA, n (%)[a]	38 (37.6)		
Non-RA, n (%)	63 (62.4)		
OA, n (%)	37 (36.6)		
UIA, n (%)	24 (23.8)		
Other, n (%)	2 (2.0)		

Values are mean±SD, median (interquartile range) and number (percentage) for counts

EIA early inflammatory arthritis, *25-OHD* 25-hydroxy vitamin D, *RA* rheumatoid arthritis, *OA* osteoarthritis, *UIA* undifferentiated inflammatory arthritis, *ACR/EULAR* American College of Rheumatology/European League Against Rheumatism

[a] RA was classified as 2010 ACR/EULAR criteria

As shown in Table 1, among 101 patients with EIA, 38 patients (37.6 %) were classified as having RA and all but two patients with RA tested positive for RF or anti-CCP antibody. Among EIA subjects, there were no seasonal differences in the proportion of subjects classified as having RA (54.5 % in spring, 30.4 % in both summer and autumn, and 27.3 % in winter, p=0.109). All subjects with RA were treated with one or more DMARDs after their initial visit, based on the decision of the rheumatologist (Lee). After considering the clinical and radiological finding at the initial visit, the remaining 63 subjects (62.4 %, non-RA patients) were categorized as having OA (n=37, 36.6 %), UIA (n=24, 23.8 %), peripheral spondyloarthritis (n=1, 1.0 %) and dermatomyositis (n=1, 1.0 %). Of the 24 subjects with UIA, DMARD treatment was initiated in 16 subjects within 1 year of their initial visit, and all of them had negative results for both RF and anti-CCP antibody at their initial visit. There were no significant differences in the frequency of vitamin D deficiency and serum 25-OHD levels between subjects who started the DMARD treatment within 1 year (n=54) and those who had never received this treatment until the end of study (n=47, data not shown).

Spearman's correlation analyses showed that in EIA subjects, the serum 25-OHD levels were significantly negatively correlated with TJC, RF titer, patient's VAS, and DAS-ESR scores (Table 2). Lower serum 25-OHD levels in subjects with EIA tended to be associated with higher CRP, anti-CCP titer and K-HAQ score, but statistical significance was not reached.

In our subgroup analysis of EIA subjects, those classified as having RA had significantly higher ESR, CRP, SJC, TJC, RF, and anti-CCP antibody titers than did non-RA subjects (Table 3), these clinical markers were included in the categories of the 2010 ACR/EULAR classification criteria for RA. In addition, RA patients had significantly higher DAS 28-ESR scores than did non-RA patients. Of interest, serum 25-OHD levels in subjects with RA were significantly lower than those in non-RA subjects.

Table 4 shows the relationship between serum 25-OHD levels and the presence of RA in subjects with EIA. As serum 25-OHD levels declined, the possibility of having RA tended to increase, but statistical significance was not reached in univariable and multivariable logistic regression analyses (p=0.054). In univariable analysis, severe vitamin D deficiency (<10 ng/mL) was significantly associated with a higher likelihood of being classified as having RA. This association remained significant after adjusting for confounding factors, including age, sex, K-HAQ score, and sampling season (Table 4). Interaction between severe vitamin D deficiency and sampling season did not show statistical significance in our logistic regression models and

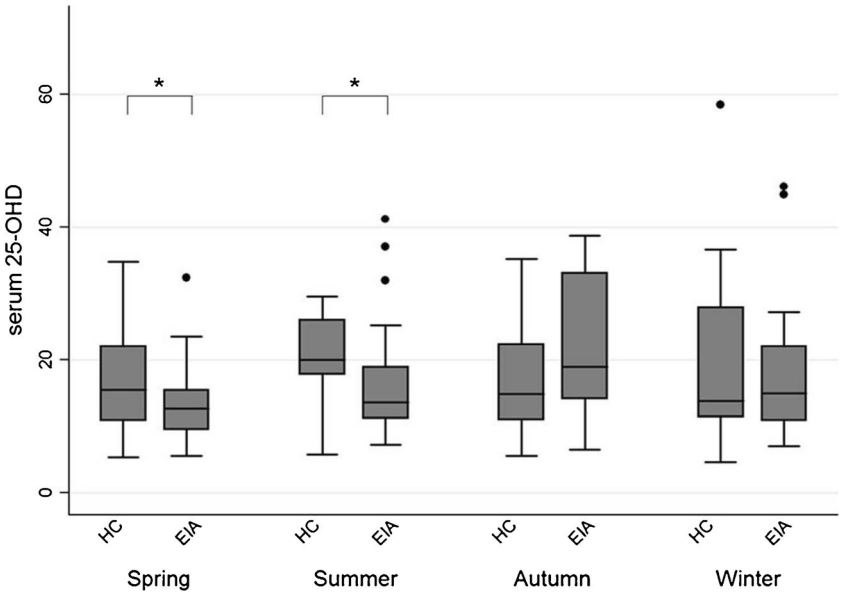

Fig. 1 Comparisons of serum 25-hydroxy vitamin D in patients with early inflammatory arthritis and healthy controls according to sampling season based on the definition of spring (March to May), summer (June to August), autumn (September to November), and winter (December to February) *$p<0.05$. *25-OHD* 25-hydroxy vitamin D, *HC* healthy controls, *EIA* early inflammatory arthritis

vitamin D deficiency (<20 ng/mL) was not correlated with the presence of RA (data not shown). In multivariable regression models, neither K-HAQ score nor sampling season remained statistically significant.

Discussion

In the present study, vitamin D deficiency was highly prevalent among subjects with EIA. Although the 25-OHD levels of EIA subjects were comparable to those of healthy controls, we found significant seasonal variation in serum 25-OHD levels between the two groups. EIA subjects classified as having RA according to criteria of the 2010 ACR/EULAR classification had significantly lower serum 25-OHD levels than those classified as non-RA. Further, in EIA subjects, severe vitamin D deficiency (<10 ng/mL) was independently associated with a higher likelihood of being classified as having RA in EIA patients, while low-serum 25-OHD levels had a weak association with the presence of RA ($p=0.054$). These findings suggest that hypovitaminosis D contributes to the presence of RA in patients who initially present with EIA.

Table 2 Correlation of serum 25-hydroxy vitamin D levels with clinical parameters in patients with early inflammatory arthritis

	Spearman's correlation coefficient	*p* value
Age, years	0.047	0.638
ESR, mm/hr	−0.160	0.101
CRP, mg/dL	−0.191	0.055
SJC	−0.149	0.101
TJC	−0.214	0.031
RF, IU/mL	−0.196	0.049
Anti-CCP antibody, U/mL	−0.194	0.052
Patient VAS scale	−0.202	0.043
K-HAQ	−0.177	0.077
DAS 28-ESR	−0.224	0.024

ESR erythrocyte sedimentation rate, *CRP* C-reactive protein, *SJC* swollen joint count, *TJC* tender joint count, *RF* rheumatoid factor, *CCP* cyclic citrullinated peptide, *VAS* visual analogue scale, *K-HAQ* Korean version of health assessment questionnaire, *DAS 28* disease activity score 28

Table 3 Comparisons of clinical characteristics of patients with early inflammatory arthritis according to the presence or absences of rheumatoid arthritis

	Non-RA (*n*=63)	RA (*n*=38)	*p* value
Age, years	56.1±12.1	57.3±12.6	0.616
Female, *n* (%)	54 (85.7)	32 (84.2)	0.837
Serum 25-OHD, ng/mL	15.5 (12.4–22.3)	12.1 (8.2–16.8)	0.046
ESR, mm/hr	13.0 (7.0–25.0)	45.5 (24.0–75.8)	<0.001
CRP, mg/dL	0.05 (0.03–0.14)	0.8 (0.2–2.64)	<0.001
SJC	2 (2–3)	4 (2–7)	<0.001
TJC	4(2–7)	6 (5–11)	0.001
RF, IU/mL	7.4 (7.0–11.2)	61.2 (28.8–143.0)	<0.001
Anti-CCP antibody, U/mL	0.5 (0.5–0.8)	79.0 (9.4–200.0)	<0.001
Disease duration, month	4.0 (2.0–6.0)	3.5 (1.0–6.0)	0.647
K-HAQ	0.25 (0–0.75)	0.5 (0–1)	0.053
DAS 28-ESR	3.4±0.9	4.7±0.9	<0.001

Values are mean±SD, median (interquartile range) and number (percentage) for counts

25-OHD 25-hydroxy vitamin D, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein, *SJC* swollen joint count, *TJC* tender joint count, *RF* rheumatoid factor, *CCP* cyclic citrullinated peptide, *K-HAQ* Korean version of health assessment questionnaire, *DAS 28* disease activity score 28

Table 4 Association between serum 25-hydroxy vitamin D levels and the presence of rheumatoid arthritis in early inflammatory arthritis

Variables	Crude OR (95 % CI)	*p* value	Adjusted OR (95 % CI)	*p* value
Severe vitamin D deficiency (<10 ng/mL)	3.26 (1.14–9.34)	0.028	3.26 (1.14–9.34)[a]	0.028
Age, years	1.00 (0.98–1.04)	0.612		
Female (ref. male)	0.89 (0.29–2.73)	0.837		
K-HAQ	1.85 (0.96–3.53)	0.064		
Season				
Spring	1.0			
Summer	0.37 (0.12–1.12)	0.078		
Autumn	0.37 (0.12–1.12)	0.078		
Winter	0.31 (0.10–1.00)	0.050		

K-HAQ Korean version of health assessment questionnaire

[a] Estimated using multivariable logistic regression analyses with backward selection, adjusted for age, sex, K-HAQ, and sampling season

Although the vitamin D status of patients with "established" RA has been studied extensively, knowledge regarding vitamin D deficiency in patients presenting with EIA is sparse. In the present study, more than 70 % of subjects with EIA were vitamin D deficient (<20 ng/mL). Our results are similar to those of previous reports, which estimated the prevalence of vitamin D deficiency in RA patients to be between 50 and 84 % [1, 2, 6, 18]. It can therefore be presumed that vitamin D deficiency is an increasingly common problem in rheumatology clinics.

Although we did not find a significant difference in the serum 25-OHD levels of EIA subjects and healthy controls in our study, Heidari et al. found that patients with UIA had significantly lower serum 25-OHD levels than did healthy individuals (25.1 vs 33.2 ng/mL, p=0.04) [19]. We propose that the difference between our results and those of Heidari et al. could partly be due to the variations in characteristics of study subjects enrolled in the two studies. For instance, Heidari et al. defined UIA as an inflammatory arthritis or inflammatory joint disease in one or more joints, which after clinical, radiological, and laboratory examination, did not fulfill definite diagnostic criteria [19]. EIA subjects in our study had various rheumatic diseases, including RA, OA, and UIA. Symptom duration in EIA subjects in our study was ≤6 months, but there were no information about disease duration in the UIA patients included in the study by Heidari et al. Further, we recruited controls that matched for age, sex, and the month of sampling; therefore, we believe that results of our study provide accurate information on the vitamin D status of patients with "early" inflammatory joint diseases. However, further research is clearly needed to determine whether EIA patients have lower serum 25-OHD than healthy individuals.

We found significant differences in seasonal variation of serum 25-OHD levels in EIA subjects and healthy controls (Fig. 1). In spring and in summer, EIA subjects had significantly lower serum 25-OHD levels than controls, whereas the opposite trend was observed in autumn. A possible hypothesis for this finding is that there were differences in vitamin D metabolism in patients with rheumatic diseases and in healthy individuals, as suggested by Azali et al. [20]. In contrast to our study, previous studies found similar pattern of seasonal variations in serum vitamin D levels in patients with RA [3] and idiopathic inflammatory myopathies [20] when compared with healthy individuals. However, in patients with EIA, the pattern and clinical implication of seasonal variation in vitamin D have not yet been fully elucidated.

In our study, there was an inverse relationship between serum 25-OHD levels and disease activity in EIA subjects (Table 2). Hypovitaminosis D was associated with higher TJC (p=0.031), CRP (p=0.055), and patient VAS scores (p=0.043); all of these markers contributed to higher disease activity in our EIA subjects. Similar findings were also observed in a previous study that included patients with early inflammatory polyarthritis [21]. Taken together, these findings support the notion that serum 25-OHD are influenced by the acute-phase response [22]. Previous studies have shown that in RA patients, vitamin D deficiency is related to higher disease activity [1, 3–6], but contradicting data also exists [18, 23, 24]. Differences in the relationship between vitamin D levels and inflammatory status may well be explained by variations in the vitamin D status of patients with early arthritis and in those with longstanding disease. The EIA subjects enrolled in the present study were not previously exposed to DMARDs or vitamin D supplementations. Therefore, our results are not affected by the effect of drugs on vitamin D status.

We found a weak inverse association between serum 25-OHD levels and RF as well as anti-CCP antibody titer in our EIA subjects (Table 2). Kerr et al. also reported that in patients with RA, anti-CCP antibody positivity is related to a twofold increase in the risk of 25-OHD insufficiency (<30 ng/mL) [6]. Taken together, hypovitaminosis D is seems to be associated with autoantibody production in RA. Experimental data showing the inhibitory effect of 1,25-hydroxy vitamin D (1,25 OH2D) on the in vitro synthesis of immunoglobulin

[25–27] may support this notion. However, in some studies, the relationship between vitamin D deficiency and RA-specific autoantibody production could not be corroborated [12, 28]. Differences in study population, study design, and assay methods for serum 25-OHD may have contributed to the seemingly contradictory results. Nonetheless, the role of hypovitaminosis D in autoantibody production in rheumatic diseases deserves greater attention in future research.

Interestingly, in our subgroup analysis of EIA subjects, severe vitamin D deficiency (<10 ng/mL) was associated with a higher probability of meeting the criteria of the 2010 ACR/EULAR classification for RA and low-serum 25-OHD levels, per se, were weakly associated with the presence of RA among EIA patients (p=0.054). Therefore, vitamin D deficiency appears to be a risk factor for the onset of RA in subjects who present with EIA. Our results should be interpreted with caution. Low-serum 25-OHD levels were associated with a higher TJC, higher acute-phase reactant levels, and increased RF and anti-CCP titers, which are all subcategories of the 2010 ACR/EULAR classification criteria for RA. Therefore, vitamin D deficiency can act as a mediator or surrogate marker of RA, thus merely reflecting the degree of inflammation or the autoantibody titer levels, rather than being an independent cause of RA. Further, only a few of our subjects had severe vitamin D deficiency, and the OR for the presence of RA in this group varied considerably. Therefore, extrapolating the results to a larger population might not be valid. Nonetheless, the association between hypovitaminosis D and the higher likelihood of EIA subjects being classified as having RA was noticeable.

Although our results cannot fully demonstrate the hypothesis that hypovitaminosis D is a risk factor for initiating RA, vitamin D deficiency could mechanistically play a role in the ongoing development and worsening of RA. 25-OHD, which is recognized as an indicator for "vitamin D status," is converted to active 1,25-OH2D by 1-α-hydroxylase. After binding vitamin D receptor (VDR), 1,25-OH2D exerts its physical role. The immunomodulatory effect of vitamin D is mediated by VDR expressed on immune cells including monocytes and dendritic cells. By decreasing interleukin 6 and increasing interleukin 10 production in monocytes, 1,25 OH2D could induce dendritic cells with tolerogenic properties as well as modulate T cell response [29]. Recently, Feng et al. showed that 1,25 OH2D could upregulate the osteoprotegerin/receptor activator of nuclear factor κB ligand ratio and mediate the anti-inflammatory effect in an inflammatory milieu of synoviocyte, which suggests the inhibitory effect of vitamin D on inflammation induced osteoclastogenesis in RA [30]. Thus, further research is needed to explore the association between vitamin D and progression of RA.

Nearly all vitamin D metabolites circulate bound to the vitamin D binding protein (DBP). [31]. As DBP-bounded vitamin D metabolites have a limited access to target cells, DBP is a key factor in regulating the availability of serum 25-OHD [32]. In addition, because DBP is a highly polymorphic protein, DBP phenotype is important to determine the serum levels of vitamin D metabolites [33]. Therefore, at a given level of serum 25-OHD, DBP concentration and phenotype could influence a biological response of vitamin D in immune system. Several studies showed the association between DBP phenotype and susceptibility of diseases such as tuberculosis [34] and RA [35]. Thus, it would be interesting to investigate whether DBP concentration and phenotype vary between subjects with EIA and health controls.

Our study had several potential limitations. First, we did not record the sun exposure time of study subjects. Sun exposure time is an established risk factor for vitamin D deficiency and the association between serum 25-OHD levels and clinical markers may be confounded by the differences in the sun exposure time of study subjects. In addition, sun exposure time can be influenced by various factors such as lifestyle and climate. In particular, subjects with EIA who have low functional capacity are prone to have insufficient sun exposure time, eventually leading to vitamin D deficiency. In this light, we could not fully adjust confounding factors that can affect serum 25-OHD levels. Second, because of our retrospective design, causality between vitamin D and diagnostic outcome in EIA patients should be interpreted with caution. Although the present study does not establish that vitamin D deficiency is an independent risk factor for the presence of RA or a reflection of inflammation or autoantibody titers, it provides a "snapshot" of the status of vitamin D in subjects who present with EIA. Longitudinal studies are needed to determine the role of vitamin D in the initiation of RA. Lastly, we recruited all our study subjects at a single tertiary hospital, which could have resulted in a selection bias.

In summary, similar to RA patients and those with other rheumatic diseases, vitamin D deficiency was highly prevalent among subjects who initially presented with EIA. We therefore suggest that in clinical practice, rheumatologists and other treating doctors should pay special attention to patients who have hypovitaminosis D. Although the frequency and magnitude of hypovitaminosis D in EIA patients were similar to those in healthy controls, seasonal variation was apparent in serum 25-OHD levels between the two groups. In addition, severe vitamin D deficiency (<10 ng/mL) in EIA patients was associated with a higher probability of being classified as having RA. This finding suggests a potential role of hypovitaminosis D in the initiation of RA.

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Disclosures None.

References

1. Rossini M, Maddali Bongi S, La Montagna G, Minisola G, Malavolta N, Bernini L, Cacace E, Sinigaglia L, Di Munno O, Adami S (2010) Vitamin D deficiency in rheumatoid arthritis: prevalence, determinants and associations with disease activity and disability. Arthritis Res Ther 12:R216
2. Furuya T, Hosoi T, Tanaka E, Nakajima A, Taniguchi A, Momohara S, Yamanaka H (2013) Prevalence of and factors associated with vitamin D deficiency in 4,793 Japanese patients with rheumatoid arthritis. Clin Rheumatol 32:1081–1087
3. Cutolo M, Otsa K, Laas K, Yprus M, Lehtme R, Secchi ME, Sulli A, Paolino S, Seriolo B (2006) Circannual vitamin D serum levels and disease activity in rheumatoid arthritis: northern versus southern Europe. Clin Exp Rheumatol 24:702–704
4. Haque UJ, Bartlett SJ (2010) Relationships among vitamin D, disease activity, pain and disability in rheumatoid arthritis. Clin Exp Rheumatol 28:745–747
5. Turhanoglu AD, Guler H, Yonden Z, Aslan F, Mansuroglu A, Ozer C (2011) The relationship between vitamin D and disease activity and functional health status in rheumatoid arthritis. Rheumatol Int 31(7): 911–914
6. Kerr GS, Sabahi I, Richards JS, Caplan L, Cannon GW, Reimold A, Thiele GM, Johnson D, Mikuls TR (2011) Prevalence of vitamin D insufficiency/deficiency in rheumatoid arthritis and associations with disease severity and activity. J Rheumatol 38:53–59
7. Goshayeshi L, Saber H, Sahebari M, Rezaieyazdi Z, Rafatpanah H, Esmaily H (2012) Association between metabolic syndrome, BMI, and serum vitamin D concentrations in rheumatoid arthritis. Clin Rheumatol 31:1197–1203
8. Haque UJ, Bathon JM, Giles JT (2012) Association of vitamin D with cardiometabolic risk factors in rheumatoid arthritis. Arthritis Care Res (Hoboken) 64:1497–1504
9. Nielen MM, van Schaardenburg D, Lems WF, van de Stadt RJ, de Koning MH, Reesink HW, Habibuw MR, van der Horst-Bruinsma IE, Twisk JW, Dijkmans BA (2006) Vitamin D deficiency does not increase the risk of rheumatoid arthritis: comment on the article by Merlino et al. Arthritis Rheum 54:3719–3720
10. Cutolo M, Otsa K, Yprus M, Seriolo B (2007) Vitamin D and rheumatoid arthritis: comment on the letter by Nielen et al. Arthritis Rheum 56:1719–1720
11. Song GG, Bae SC, Lee YH (2012) Association between vitamin D intake and the risk of rheumatoid arthritis: a meta-analysis. Clin Rheumatol 31:1733–1739
12. Feser M, Derber LA, Deane KD, Lezotte DC, Weisman MH, Buckner JH, Mikuls T, O'Dell J, Gregersen PK, Holers VM, Norris JM (2009) Plasma 25, OH vitamin D concentrations are not associated with rheumatoid arthritis (RA)-related autoantibodies in individuals at elevated risk for RA. J Rheumatol 36:943–946
13. Holick MF (2007) Vitamin D deficiency. N Engl J Med 357:266–281
14. Welsh P, Peters MJ, McInnes IB, Lems WF, Lips PT, McKellar G, Knox S, Michael Wallace A, Dijkmans BA, Nurmohamed MT, Sattar N (2011) Vitamin D deficiency is common in patients with RA and linked to disease activity, but circulating levels are unaffected by TNFalpha blockade: results from a prospective cohort study. Ann Rheum Dis 70:1165–1167
15. Bae SC, Cook EF, Kim SY (1998) Psychometric evaluation of a Korean Health Assessment Questionnaire for clinical research. J Rheumatol 25:1975–1979
16. Prevoo ML, Hof MA V 't, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL (1995) Modified disease activity scores that include twenty-eight-joint counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum 381:44–48
17. Aletaha D, Neogi T, Silman AJ, Funovits J, Felson DT, Bingham CO 3rd, Birnbaum NS, Burmester GR, Bykerk VP, Cohen MD, Combe B, Costenbader KH, Dougados M, Emery P, Ferraccioli G, Hazes JM, Hobbs K, Huizinga TW, Kavanaugh A, Kay J, Kvien TK, Laing T, Mease P, Menard HA, Moreland LW, Naden RL, Pincus T, Smolen JS, Stanislawska-Biernat E, Symmons D, Tak PP, Upchurch KS, Vencovsky J, Wolfe F, Hawker G (2010) 2010 Rheumatoid arthritis classification criteria: an American College of Rheumatology/European League Against Rheumatism collaborative initiative. Ann Rheum Dis 69:1580–1588
18. Craig SM, Yu F, Curtis JR, Alarcon GS, Conn DL, Jonas B, Callahan LF, Smith EA, Moreland LW, Bridges SL Jr, Mikuls TR (2010) Vitamin D status and its associations with disease activity and severity in African Americans with recent-onset rheumatoid arthritis. J Rheumatol 37:275–281
19. Heidari B, Hajian-Tilaki K, Heidari P (2012) The status of serum vitamin D in patients with rheumatoid arthritis and undifferentiated inflammatory arthritis compared with controls. Rheumatol Int 32: 991–995
20. Azali P, Barbasso Helmers S, Kockum I, Olsson T, Alfredsson L, Charles PJ, Piehl Aulin K, Lundberg IE (2013) Low serum levels of vitamin D in idiopathic inflammatory myopathies. Ann Rheum Dis 72:512–516
21. Patel S, Farragher T, Berry J, Bunn D, Silman A, Symmons D (2007) Association between serum vitamin D metabolite levels and disease activity in patients with early inflammatory polyarthritis. Arthritis Rheum 56:2143–2149
22. Welsh P, Peters MJ, Sattar N (2011) Is vitamin D in rheumatoid arthritis a magic bullet or a mirage? The need to improve the evidence base prior to calls for supplementation. Arthritis Rheum 63:1763–1769
23. Higgins MJ, Mackie SL, Thalayasingam N, Bingham SJ, Hamilton J, Kelly CA (2013) The effect of vitamin D levels on the assessment of disease activity in rheumatoid arthritis. Clin Rheumatol 32:863–867
24. Baker JF, Baker DG, Toedter G, Shults J, Von Feldt JM, Leonard MB (2012) Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. Clin Exp Rheumatol 30: 658–664
25. Shiozawa K, Shiozawa S, Shimizu S, Fujita T (1985) 1 alpha,25-dihydroxyvitamin D3 inhibits pokeweed mitogen-stimulated human B-cell activation: an analysis using serum-free culture conditions. Immunology 56:161–167
26. Provvedini DM, Tsoukas CD, Deftos LJ, Manolagas SC (1986) 1 alpha,25-Dihydroxyvitamin D3-binding macromolecules in human B lymphocytes: effects on immunoglobulin production. J Immunol 136:2734–2740
27. Chen S, Sims GP, Chen XX, Gu YY, Lipsky PE (2007) Modulatory effects of 1,25-dihydroxyvitamin D3 on human B cell differentiation. J Immunol 179:1634–1647
28. Orbach H, Zandman-Goddard G, Amital H, Barak V, Szekanecz Z, Szucs G, Danko K, Nagy E, Csepany T, Carvalho JF, Doria A, Shoenfeld Y (2007) Novel biomarkers in autoimmune diseases: prolactin, ferritin, vitamin D, and TPA levels in autoimmune diseases. Ann N Y Acad Sci 1109:385–400
29. Peelen E, Knippenberg S, Muris AH, Thewissen M, Smolders J, Tervaert JW, Hupperts R, Damoiseaux J (2011) Effects of vitamin D on the peripheral adaptive immune system: a review. Autoimmun Rev 10:733–743

30. Feng X, Lv C, Wang F, Gan K, Zhang M, Tan W (2013) Modulatory effect of 1,25-dihydroxyvitamin D 3 on IL1 beta -induced RANKL, OPG, TNF alpha, and IL-6 expression in human rheumatoid synoviocyte MH7A. Clin Dev Immunol 2013:160123. doi:10.1155/2013/160123
31. Chun RF, Lauridsen AL, Suon L, Zella LA, Pike JW, Modlin RL, Martineau AR, Wilkinson RJ, Adams J, Hewison M (2010) Vitamin D-binding protein directs monocyte responses to 25-hydroxy- and 1,25-dihydroxyvitamin D. J Clin Endocrinol Metab 95:3368–3376
32. Speeckaert M, Huang G, Delanghe JR, Taes YE (2006) Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chem Acta 372:33–42
33. Lauridsen AL, Vestergaard P, Hermann AP, Brot C, Heickendorff L, Mosekilde L, Nexo E (2005) Plasma concentrations of 25-hydroxy-vitamin D and 1,25-dihydroxy-vitamin D are related to the phenotype of Gc (vitamin D-binding protein): a cross-sectional study on 595 early postmenopausal women. Calcif Tissue Int 77:15–22
34. Martineau AR, Leandro AC, Anderson ST, Newton SM, Wilkinson KA, Nicol MP, Pienaar SM, Skolimowska KH, Rocha MA, Rolla VC, Levin M, Davidson RN, Bremner SA, Griffiths CJ, Eley BS, Bonecini-Almeida MG, Wilkinson RJ (2010) Association between Gc genotype and susceptibility to TB is dependent on vitamin D status. Eur Respir J 35:1106–1112
35. Papiha SS, Pal B (1985) Gc (vitamin D binding protein) subtypes in rheumatoid arthritis. Hum Genet 70:278–280