Vitamin D Status in Egyptian Patients with Rheumatoid Arthritis

R. M. Gamal1, W. Gaber2, S. Sayed2, A. M. Mohey3, S. H. Goma1, M. S. E. Mohamed4

Abstract

Background: Vitamin D is a potent regulator of calcium homeostasis and may have immunomodulatory effects. The influence of vitamin D on human autoimmune disease has not been well defined. The aim of this cross sectional study was to estimate the prevalence and determinants of vitamin D deficiency in patients with rheumatoid arthritis as compared to healthy controls and to analyze the association between 25-hydroxyvitamin D with disease activity.

Methods: The study includes 55 consecutive rheumatoid arthritis patients and 25 healthy controls, not on vitamin D supplements. Together with parameters of disease activity, all patients had serum 25-hydroxyvitamin D [25(OH)D] measured by ELISA kit in a centralized laboratory. Disease activity in rheumatoid arthritis was assessed by Disease Activity Score 28 (DAS28) and Health Assessment Questionnaire (HAQ). According to activity indexes, patients were divided into subgroups with high activity of the disease (DAS28>5.1), moderate activity of the disease (3.2<DAS28≤5.1), low activity of disease (2.6≤DAS28≤3.2) and remission (DAS28<2.6). Associations between serum levels of 25(OH)D and age, disease duration and activity were assessed.

Results: 25-hydroxyvitamin D deficiency, insufficient and sufficiency were found in 16,00, 4,00 and 80,00 % of controls, respectively, while, 25(OH)D insufficiency and sufficiency were found in 21,80 and 78,20 % of controls. According to activity indexes, patients were divided into subgroups with high activity of the disease (DAS28>5.1), moderate activity of the disease (3.2<DAS28≤5.1), low activity of disease (2.6≤DAS28≤3.2) and remission (DAS28<2.6). Associations between serum levels of 25(OH)D and disease duration and activity were assessed. According to DAS28 scoring, 9,10 % of patients showed remission (DAS28<2.6), 7,30 % showed low disease activity (2.6≤DAS28≤3.2), 63,60 % showed moderate activity (3.2<DAS28≤5.1) and 20,00 % showed high disease activity (DAS28>5.1). Disease duration was significantly longer in patients with moderate disease activity vs. those with low and high disease activity (P<0.026 and P<0.001, respectively). The difference between groups (P<0.918). Entersing to DAS28 scoring, 9,10 % of patients showed remission (DAS28<2.6), 7,30 % showed low disease activity (2.6≤DAS28≤3.2), 63,60 % showed moderate activity (3.2<DAS28≤5.1) and 20,00 % showed high disease activity (DAS28>5.1). Disease duration was significantly longer in patients with moderate disease activity vs. those with low and high disease activity (P<0.026 and P<0.001, respectively). The difference between groups (P<0.918).

Bibliography

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Zusammenfassung


Ergebnisse: Eine 25-Hydroxyvitamin D Defizienz, ein Mangel und ein suffizienter Spiegel wurden bei 16,00, 4,00 und 80,00 % der Kontrollen gefunden, während ein 25(OH)D Mangel und ein suffizienter Spiegel bei 21,80 und 78,20 % der rheumatoiden Arthritis Patienten gefunden wurden, was nicht signifikant unterschiedlich in der Gruppenanalyse war (P<0,918). Entsprechend dem DAS28 Score, waren 9,10 % der Patienten in Remission (DAS28<2,6), 7,30 % hatten eine niedrige Krankheitsaktivität (2,6≤DAS28≤3,2), und 63,60 % zeigten eine...
Introduction

Rheumatoid arthritis (RA) is the most common inflammatory arthritis, the prevalence of which is constant across the globe, regardless of geographic location and race, but there are some exceptions. For instance, in China, the occurrence of RA is somewhat lower (about 0.3%), whereas it is substantially higher in other groups, such as the Pima Indians in North America (about 5%) [1]. Likewise, RA seems more common in the North when compared to South Europe [2]. Although the etiology of RA remains a mystery, a variety of studies suggest that a blend of environmental and genetic factors are responsible; a contribution of either one is necessary but not sufficient to exactly specify the disease. It is clear that both genetic and environmental factors affect the prevalence of autoimmune diseases.

Vitamin D is widely recognized as a hormone that is important for calcium homeostasis and maintenance of skeletal health. Vitamin D also plays a role in the function of the immune system [3, 4]. The immunomodulatory effects of vitamin D have been subjected to extensive examination, leading to recent speculation that it may play a role in selected inflammatory diseases including rheumatoid arthritis (RA) [5]. Vitamin D maintains equilibrium between T helper 1 (Th1) and T helper 2 (Th2) cells. Calcitriol (1,25 dihydroxyvitamin D) can inhibit the synthesis of mRNA of macrophages-derived cytokines such as interleukin (IL)-1, IL-6, IL-12 and tumor necrosis factor alpha (TNF-α), suppress IL-2 secretion of Th1 cells and decrease the antigen-presenting activity of macrophages to lymphocytes [6–8]. Further, in vivo studies suggest that 1,25(OH)2D3 supplementation prevents the initiation and progression of inflammatory arthritis (collagen-induced arthritis) in rodents and prevents experimental autoimmune encephalomyelitis (a murine model used to determine the efficacy of drugs for the treatment of multiple sclerosis) [9, 10]. Likewise, vitamin D receptors (VDR) are constitutively expressed on activated lymphocytes, synoviocytes, macrophages, and chondrocytes in the RA lesion [11] while vitamin D metabolites namely; 1,25(OH)2D, 24,25(OH)2D and 25-OH-D are increased in RA synovial fluid [12]. In at least one epidemiological study, vitamin D intake was inversely associated with the risk of developing new-onset RA [13], though conflicting results have been reported [14, 15]. Although investigations of its association with disease activity in RA are limited, preliminary data suggest that levels of circulating vitamin D are inversely associated with disease activity in patients of European ancestry with early inflammatory arthritis [16].

The aim of this study was to assess the vitamin D status in patients with RA and look for possible correlation with disease activity.

Patients and Methods

Patients

Rheumatoid arthritis patients (n=55) regularly followed up at the outpatient clinic of Cairo and Assiut University Hospitals were invited to participate in this prospective study during the period between January 2009 and June 2010. The patient group consists of 8 males and 47 females with age range from 27–68 years (mean ± SD, 43.88 ± 12.78 years). The inclusion criteria were the following: RA diagnosis according to American College of Rheumatology (ACR) criteria [17], disease onset after 16 years of age, and use of stable medication in the last 3 months. The control group consisted of 25 age and sex-matched healthy volunteers recruited among university and hospital workers. The control group consists of 4 males and 21 females with age range from 21–62 years (mean ± SD, 42.22 ± 10.61 years). The control group had no rheumatic diseases or other skeletal symptoms based on history and clinical examination. The exclusion criteria for the patient and control groups were determined as associating inflammatory intestinal disease (Crohn’s disease, ulcerative colitis), malnutrition, hyperparathyroidism, hyperthyroidism, renal and hepatic diseases, limited physical activity and medications that might affect bone metabolism and endocrine system (e.g. thyroxin, anticonvulsants, hormone or vitamin D replacement therapy). All participants signed the informed consent form approved by the Institutional Ethics Committee.

Disease activity assessment

In the patient group, age, gender, duration of disease, peripheral joint involvement, extra-articular involvement, family history, the presence or absence of comorbidity and medication at enrollment were recorded. Disease activity was assessed according to the Disease Activity Score including 28 joint counts (DAS28). The evaluations included tender and swollen joint counts (0–28), pain (0–10), subcutaneous rheumatoid nodules (present vs. absent). Components of DAS28 are ESR, patient-assessed
global score (0–100), and swollen and tender joint counts (both 0–28). High activity of the disease was defined as a DAS28 > 5.1, moderate activity of disease was defined as 3.2 < DAS28 ≤ 5.1, and low activity of disease was defined as 2.6 ≤ DAS28 ≤ 3.2 and remission as a DAS28 < 2.60 [18, 19]. Patients were asked to complete the Stanford Health Assessment Questionnaire (HAQ, range 0–3) to measure their functional capacity [20, 21].

**Laboratory tests**

Blood samples were obtained in the morning after at least 8-h overnight fasting. 3 ml venous blood samples were withdrawn from all patients and normal controls on plain tubes, and then the sera were separated and kept at −20 °C until assay. C-reactive protein (CRP, mg/dl) was assessed with nephelometric method, and erythrocyte sedimentation rate (ESR, mm/h) was assessed with Westergren method. Rheumatoid factor (RF, IU/ml) was determined by the nephelometric method, and RF > 20 IU/ml was defined as positive. 25(OH)D plasma levels were measured by a sandwich enzyme immunoassay commercial kit, according to the manufacturer's instructions (Immundiagnostik AG, Bensheim, Germany). Vitamin D insufficiency was defined as a 25(OH)D concentration ≤ 37.5 nmol/L (15 ng/ml), a threshold used in previous investigations of older Caucasian populations [22–25]. Vitamin D deficiency, defined as a 25(OH)D level ≤ 25 nmol/L (10 ng/ml) [26].

**Statistical analysis**

All data were analyzed by SPSS 13.0 software. Data are presented as mean and standard deviations or number and percentage as appropriate. With the aim of evaluating the differences between groups, Student's “t” test for the variables with a normal distribution and nonparametric Mann-Whitney-U test for the variables with abnormal distribution were used. While studying the relations between the variables in the patient group, Pearson correlation test was used for parametric variables and Spearman correlation test was used for nonparametric variables. A P-value of <0.05 was accepted as statistically significant.

**Results**

Table 1 showed the demographic and clinical characteristics of the RA patients and controls. No significant difference was found regarding age, gender, sample season, religion, skin color, skin exposure, history of rickets, dietary vitamin D and breast fed and serum levels of 25(OH)D between RA patients and controls. 25(OH)D insufficiency and sufficiency were found in 16.00 %, 21.80 % and 78.20 % of RA patients with no significant difference between controls and patients (P < 0.918).

Table 2 showed the clinical, laboratory and treatment characteristics of rheumatoid arthritis patients. According to DAS28 scoring, 9.10 % of patients showed remission (DAS28 < 2.6), 7.30 % showed low disease activity (2.6 ≤ DAS28 ≤ 3.2), 63.60 % showed moderate activity (3.2 < DAS28 ≤ 5.1) and 20.00 % showed high disease activity (DAS28 > 5.1).
Disease duration was significantly longer in patients with moderate disease activity vs. those with low and high disease activity (P < 0.026 and P < 0.001, respectively). Morning stiffness, swollen joints count were higher in patients with moderate disease activity vs. those with remission (P < 0.023 and P < 0.009, respectively). 25(OH)D levels were lower in patients with moderate disease activity vs. those with remission (P < 0.014). Swollen joints count, tender joints count, DAS28 and HAQ were significantly higher in patients with high disease activity than remission, low disease activity and moderate disease activity.

Tender joints count, DAS28 and HAQ were significantly higher in patients with moderate disease activity than remission and those with low disease activity (Table 3).

A significant positive correlation was observed between both DAS28 and total questionnaire HAQ (Fig. 1) (r = 0.698, P < 0.0001), and morning stiffness (MS) with erythrocyte sedimentation rate (ESR) (r = 0.331, P < 0.014) (Fig. 2).

Fig. 3 shows significant positive correlation between number of swollen joints (SJno) and ESR (r = 0.432, P < 0.001) and number of tender joints (TJno) (r = 0.336, P < 0.012).

On the other hand, a significant negative correlation was found in RA patients between 25(OH)D and ESR (Table 4 and Fig. 4) while, there is no correlation between 25(OH)D and other measured parameters (Table 4).

### Table 2 Clinical, laboratory and treatment characteristics of rheumatoid arthritis patients.

<table>
<thead>
<tr>
<th>Items</th>
<th>Patients (n = 55)</th>
</tr>
</thead>
<tbody>
<tr>
<td>disease duration (years)</td>
<td>5.18 ± 4.59 (0.17–18.00)</td>
</tr>
<tr>
<td>morning stiffness (minutes)</td>
<td>50.54 ± 59.20 (0.00–240.00)</td>
</tr>
<tr>
<td>swollen joints count</td>
<td>5.56 ± 5.04 (0.00–20.00)</td>
</tr>
<tr>
<td>tender joints count</td>
<td>6.80 ± 5.93 (0.00–24.00)</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate (mm/h)</td>
<td>45.89 ± 29.34 (7.00–105.00)</td>
</tr>
<tr>
<td>DAS28</td>
<td>4.03 ± 1.25 (1.80–7.20)</td>
</tr>
<tr>
<td>remission (DAS28 &lt; 2.6)</td>
<td>5 (9.10%)</td>
</tr>
<tr>
<td>low disease activity (2.6 ≤ DAS28 ≤ 3.2)</td>
<td>4 (7.30%)</td>
</tr>
<tr>
<td>moderate activity (3.2 &lt; DAS28 ≤ 5.1)</td>
<td>35 (63.60%)</td>
</tr>
<tr>
<td>high disease activity (DAS28 &gt; 5.1)</td>
<td>11 (20.00%)</td>
</tr>
<tr>
<td>HAQ (range 0–3)</td>
<td>1.09 ± 0.56 (0.00–2.50)</td>
</tr>
</tbody>
</table>

Drugs

NSAIDs 45 (81.80%)
methotrexate 40 (72.70%)
prednisolone 21 (38.2%).
leflunamide 18 (32.70%).
chloroquine 31 (56.40%).
cyclosporine 2 (3.6%).
leflunamide 18 (32.70%).
hemoglobin (gram/dL) 11.75 ± 1.23 (9.70–14.40)
platelets 303.11 ± 82.73 (183.00–501.00)

Data are expressed as mean ± SD and range or number (%) as appropriate

DAS28: Disease Activity Score 28-joint assessment; HAQ: Health Assessment Questionnaire; NSAIDs: non steroid anti-inflammatory drugs

### Table 3 Demographic, clinical and laboratory characteristics of rheumatoid arthritis patients based on disease activity status.

<table>
<thead>
<tr>
<th>Items</th>
<th>Remission (DAS28 &lt; 2.6) (n = 5)</th>
<th>Low disease activity (2.6 ≤ DAS28 ≤ 3.2) (n = 4)</th>
<th>Moderate disease activity (3.2 &lt; DAS28 ≤ 5.1) (n = 35)</th>
<th>High disease activity (DAS28 &gt; 5.1) (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>48.00 ± 14.65 (9.10%)</td>
<td>34.25 ± 7.09 (7.30%)</td>
<td>43.46 ± 10.11 (5.10%)</td>
<td>38.55 ± 9.84 (20.00%)</td>
</tr>
<tr>
<td>disease duration (years)</td>
<td>3.67 ± 3.42 (7.30%)</td>
<td>1.86 ± 1.4 (63.60%)</td>
<td>6.84 ± 4.74 (63.60%)</td>
<td>1.79 ± 2.15 (20.00%)</td>
</tr>
<tr>
<td>morning stiffness (minutes)</td>
<td>5.00 ± 6.12 (NS)</td>
<td>8.75 ± 6.29 (NS)</td>
<td>67.29 ± 64.06 (NS)</td>
<td>32.73 ± 43.38 (NS)</td>
</tr>
<tr>
<td>swollen joints count</td>
<td>0.00 ± 0.00 (NS)</td>
<td>1.25 ± 1.26 (NS)</td>
<td>5.00 ± 3.40 (NS)</td>
<td>11.45 ± 5.91 (NS)</td>
</tr>
<tr>
<td>tender joints count</td>
<td>0.00 ± 0.00 (81.80%)</td>
<td>0.75 ± 1.50 (NS)</td>
<td>6.57 ± 4.90 (NS)</td>
<td>12.82 ± 5.44 (NS)</td>
</tr>
<tr>
<td>DAS28</td>
<td>2.13 ± 0.31 (20.00%)</td>
<td>2.88 ± 0.21 (NS)</td>
<td>4.19 ± 0.55 (NS)</td>
<td>6.15 ± 0.64 (NS)</td>
</tr>
<tr>
<td>HAQ (range 0–3)</td>
<td>0.43 ± 0.44 (NS)</td>
<td>0.55 ± 0.44 (NS)</td>
<td>1.10 ± 0.46 (NS)</td>
<td>1.59 ± 0.50 (NS)</td>
</tr>
<tr>
<td>vitamin D (nmol/ml)</td>
<td>61.90 ± 14.25 (NS)</td>
<td>50.50 ± 5.74 (NS)</td>
<td>46.04 ± 12.85 (NS)</td>
<td>50.32 ± 14.48 (NS)</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD. DAS28: Disease Activity Score 28-joint assessment; HAQ: Health Assessment Questionnaire. * significance vs. remission; ** significance vs. low disease activity; *** significance vs. moderate disease activity
Discussion

In this study, vitamin D status was assessed by measuring 25(OH)D, an accurate indicator of vitamin D levels [8]. 25(OH)D was studied rather than the more active form 1,25-dihydroxyvitamin D (1,25(OH)2D3), because reported associations with disease activity have been shown to be stronger for 25(OH)D [16]. 25(OH)D acts as a substrate for 1,25(OH)2D3, levels of which are also dependent on calcium and phosphorus status in addition to parathyroid hormone concentrations, measures not available for the participants in this study.

No significant difference was found between serum levels of 25(OH)D in RA patients and controls in this study. 25(OH)D deficiency, insufficiency and sufficiency were found in 16.00, 4.00 and 80.00% of controls, respectively and 25(OH)D insufficiency and sufficiency were found in 21.80% and 78.20% of RA patients, respectively. In consistence with our results, Turhanoflu et al. [27] reported that, 25(OH)D levels were not differing between patients with RA and healthy controls. However, they reported that vitamin D levels were lower in the patients with RA with high activity than those with low activity. A previous report in Saudi Arabia found that serum vitamin D levels in RA patients were similar to the healthy control group. However, significantly lower 25(OH)D values were found in patients who were poorly responding to treatment, and not in a state of disease remission [28]. Serum vitamin D inadequacy constitutes a largely unrecognized epidemic in many populations worldwide [29]. However, preliminary studies suggest that low levels of vitamin D may be common in rheumatoid arthritis [30–33]. More recently, vitamin D deficiency was found in 42 out of 145 postmenopausal women with RA in the USA, with the highest prevalence among African Americans [26, 34]. Preventive treatment

Table 4 Correlation between 25(OH) vitamin D and various measured parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Correlation(r)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>age (years)</td>
<td>0.223</td>
<td>0.101</td>
</tr>
<tr>
<td>disease duration (years)</td>
<td>−0.080</td>
<td>0.561</td>
</tr>
<tr>
<td>morning stiffness (minutes)</td>
<td>−0.190</td>
<td>0.165</td>
</tr>
<tr>
<td>number of swollen joints</td>
<td>−0.190</td>
<td>0.164</td>
</tr>
<tr>
<td>number of tender joints</td>
<td>−0.037</td>
<td>0.786</td>
</tr>
<tr>
<td>erythrocyte sedimentation rate (mm/h)</td>
<td>−0.441( * * )</td>
<td>0.001</td>
</tr>
<tr>
<td>HAQ total</td>
<td>−0.062</td>
<td>0.655</td>
</tr>
<tr>
<td>DAS28</td>
<td>−0.234</td>
<td>0.085</td>
</tr>
<tr>
<td>DAS status</td>
<td>−0.059</td>
<td>0.670</td>
</tr>
<tr>
<td>VAS</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>

DAS28: Disease Activity Score 28-joint assessment; HAQ: Health Assessment Questionnaire

Fig. 2 Significant positive correlations between morning stiffness (MS) and erythrocyte sedimentation rate (ESR) \(r = 0.331, P < 0.014\).

Fig. 3 Significant positive correlations between number of swollen joints (Sjno) and ESR \(r = 0.432, P < 0.001\) and number of tender joints (Tjno) \(r = 0.336, P < 0.012\).
with vitamin D of individuals considered at high risk of developing autoimmune diseases has been proposed [35]. On the other hand, a large epidemiological study did not find any association between vitamin D intake and the risk developing RA [36].

In this study, a significant negative correlation was found between 25(OH)D and ESR in RA patients. A few studies reported correlation between disease activity in RA (expressed as CRP or ESR) and 1,25(OH)2D3 metabolites levels, but not with 25(OH)D [30, 32, 37]. Moreover, some authors reported an inverse relationship between serum levels of vitamin D metabolites and disease activity or disability in patients with RA or early inflammatory polyarthritis, although conflicting results have been found [16, 26, 32, 38]. Two previous studies found no relationship between 25(OH)D and CRP or ESR [30, 32]. One small open-label intervention study demonstrated reduction in RA disease activity with 1,25(OH)2D3 supplementation [39]. Turhanoulu et al. [27] reported the relationship between disease activity and vitamin D level, and also the negative correlation between vitamin D level and DAS28, HAQ, and CRP. Yet, little is known about how vitamin D intake modifies the development of autoimmune diseases [40]. Mahon et al. [41] described that 1000 IU/day vitamin D and 800 mg Ca intake increased the anti-inflammatory transforming growth factor serum levels [41]. Moreover, synthetic vitamin D reduces the severity of the symptoms in RA [38]. RA is an immune-mediated disease, mainly driven by Th1 cells. In previous reports, higher baseline frequencies of circulating Th17 cells and serum levels of interleukin (IL)-17 were observed in active RA patients than in healthy controls [42, 43]. Th17 cells have been implicated in the pathogenesis of RA [44]. Ranganathan et al. [45] detected that vitamin D deficiency in RA may affect Th17 responses and microvascular function.

In various animal models, with 1,25(OH)2D3 vitamin treatment in early phase, collagen-induced arthritis was preventable to a certain extent [9]. With the administration of vitamin D, the progression of arthritis decreased compared with the untreated control animals. These data obtained from animal models showed that the VDR ligand along with other factors may control the development of RA [11].

Conclusions

In RA patients, vitamin D deficiency is quite common, but similar to that found in control subjects; disease activity (expressed as ESR) is inversely related to 25(OH)D levels. Our study provides new insights into the vitamin D status in RA patients as well as healthy people in Egypt.

Limitation

There were a number of limitations in our study. The RA subgroups were small. We could not properly assess seasonal differences due to the sunny winter during the study period. The average age of the population is relatively young. Finally, we were unable to estimate either the dietary calcium intake of our patients, or the prevalence of lactase deficiency, which may influence ingestion of dairy products and dietary calcium supply.

Conflict of interest:

No.

References

8 Zitterman A. Vitamin D in preventive medicine: are we ignoring the evidence? BJN 2003; 89: 552–572
12 Inaba M, Yuukioka K, Furumitsu Y et al. Positive correlation between levels of IL-1 or IL-2 and 1,25(OH)2D/25-OH-D ratio in synovial fluid of patients with rheumatoid arthritis. Life Sci 1997; 61: 977–985

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19 Van Gestel AM, Haagsma CJ, Van Riel PLCM. Validation of rheumatoid arthritis improvement criteria that include simplified joint counts. Arthritis Rheum 1998; 41: 1845–1850
35 Harel M, Shoenfeld Y. Predicting and preventing autoimmunity, myth or reality? Ann NY Acad Sci 2006; 1069: 322–345