The Relationship between Vitamin D and Disease Activity in Egyptian Patients with Rheumatoid Arthritis

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Abstract - Rheumatoid arthritis (RA) is an autoimmune disorder of unknown etiology. Vitamin D plays an important role in bone metabolism and may also have immunomodulatory effects. Vitamin D deficiency has been found to be associated with disease activity in patients with RA. The aim of this study was to estimate the prevalence of vitamin D deficiency in patients with rheumatoid arthritis (RA) as compared to healthy controls and to analyze the correlation between 25-hydroxyvitamin D (25(OH)D) with disease activity. The study includes 60 RA patients (85% women) and 40 controls, not on vitamin D Supplements. Together with parameters of disease activity, all patients had serum 25(OH)D measured. Results: The prevalence of 25(OH)D insufficiency (≤ 60 nmol/L or 24 ng/ml) was 76.7%. Vitamin D concentrations were inversely associated with VAS scores, swollen joint counts, tender joint counts, HAQ, and DAS-28. Therby, vitamin D insufficiency is highly prevalent and linked to disease severity in patients with RA.

I. INTRODUCTION
Rheumatoid arthritis (RA) is an autoimmune disease of unknown aetiology [1]. Both T and B lymphocytes are involved in the pathogenesis of the disease [2]. The role of T lymphocytes as well as that of B lymphocytes in the pathogenesis of RA has been further proved by the therapeutic efficacy of methods affecting both T and B lymphocytes, namely the biological agents[3,4]. Vitamin D deficiency may increase the risk for the development of RA [5]. Recently, the role of vitamin D deficiency in the pathogenesis of RA, as well as the relationship between vitamin D deficiency and the activity of RA is discussed [6,7]. Vitamin D and its analogues have been shown to suppress T-cell proliferation and inhibit the expression of pro-inflammatory cytokines involved in RA pathogenesis including interleukin (IL)-2 and interferon-γ [8].

II. MATERIALS AND METHODS
This study included 60 adult patients (49 females, 11 males) aged 31.1±6.2 (range 23–42 years), presenting at outpatient Rheumatology clinics of Ain-Shams University Hospitals. These patients met the American College of Rheumatology (ACR) RA classification criteria [12]. Forty apparently healthy individuals matched in age and sex were also included as controls.

The laboratory work was conducted at Clinical Pathology Department, Ain-Shams University Hospital after taking informed consent from all subjects. This study was approved by the Committee of Ethics and Research of the University Hospital.

Physical examination, a medical history of patients, and blood biochemistry were evaluated in all patients to exclude chronic diseases, namely diabetes mellitus,
systemic lupus erythematosus, chronic liver disease and renal disease.

Disease activity was assessed according to the Disease Activity Score including 28 joint counts (DAS28). Components of DAS28 are ESR, patient’s general health on a Visual Analog Scale (VAS) (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 a 3.2 < DAS28 ≤ 5.1, and low activity of disease was defined as a DAS28 ≤ 3.2. Patients were asked to complete the Stanford Health Assessment Questionnaire (HAQ) to measure their functional capacity.

Three ml of venous blood were withdrawn aseptically into a sterile disposable syringe from every patient and control, collected in plane tube to be clotted and centrifuged. The yielding serum was used for the detection of rheumatoid factor (RF), C-reactive protein (CRP) and vitamin D. CRP (mg/dl) was assessed with the nephelometric method using MININEPH HUMAN C-REACTIVE PROTEIN KIT supplied by BINDING SITE (UK), and erythrocyte sedimentation rate (ESR, mm/h) was assessed with the Westergren method. RF (IU/ml) was also determined by the nephelometric method using MININEPH HUMAN Rheumatoid factor KIT supplied by BINDING SITE, and RF > 27 IU/ml was defined as positive.

Serum 25(OH)D concentration was analyzed using an enzyme linked immunosorbent assay (ELISA) kit (Immundiagnostik AG, Bensheim, Germany). The assay could detect 25(OH)D concentrations as low as 6.4 nmol/L. The intra-and inter-assay coefficients of variation for the ELISA were both 7.0%. 25(OH)-D was studied rather than the more active form (1,25-dihydroxyvitamin D [1,25(OH)2D]), because reported associations with disease activity have been shown to be stronger for 25(OH)-D [13]. 25(OH)-D acts as a substrate for 1,25(OH)2D, levels of which are also dependent on calcium and phosphorus status in addition to parathyroid hormone concentrations, measures of calcium and phosphorus are not available for the participants in this study.

III. RESULTS

The mean age was 31.1±6.2 years in the patients with RA and 28.05±6.18 years in the healthy controls. There was no significant difference between the two groups as regards to gender and age (p > 0.05). Women comprised 85% of the study participants; RA patients had a mean of disease duration of 5 years. The mean of the 25-OH Vitamin D levels was 47.65±21.80 nmol/l in patients with RA (n = 60) and 93.60±61.82 nmol/l in healthy controls (n = 40). We found that the mean of the 25-OH D vitamin levels of the patients with RA was significantly lower than that of controls (p < 0.01) (Table 1).

Table 1: Statistical comparison between patients and control groups, as regards Vit D, RF and CRP

<table>
<thead>
<tr>
<th></th>
<th>Patients (n = 60)</th>
<th>Controls (n = 40)</th>
<th>t*/z*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vit.D (nmol/L)</td>
<td>47.65 ± 21.80</td>
<td>93.60 ± 61.82</td>
<td>5.29*</td>
<td>0.000(HS)</td>
</tr>
<tr>
<td>RF (IU/L)</td>
<td>30 (28 – 69.25)</td>
<td>26 (22.25 – 28)</td>
<td>-3.036*</td>
<td>0.003(HS)</td>
</tr>
<tr>
<td>CRP (mg/L)</td>
<td>8.15 (5.6 – 16)</td>
<td>3.1 (2.75 – 4.38)</td>
<td>-3.572*</td>
<td>0.001(HS)</td>
</tr>
</tbody>
</table>

* Data were presented as mean±SD and compared together using Independent t-test  
z*: Data were presented as Median and IQR and compared together using Mann-Whitney test  
HS: Highly statistical significant

Clinical measures available included HAQ score (median = 0.5, interquartile range 0.125-1), swollen joint count (median = 0, interquartile range 0-2), tender joint count (median = 5, interquartile range 2-11), and VAS (median = 30, interquartile range 10-60).

Roc curve showing that the cut off point between patients and controls regarding to serum vitamin D level ≤ 60 nmol/L is the best cut off point with area under the curve (AUC) 82%, sensitivity 76.67%, specificity 75%, positive predictive value 82.1% and negative predictive value 68.2%. The prevalence of vitamin D insufficiency (25(OH)-D level ≤ 60.0 nmol/L, equivalent to ≤ 24 ng/ml) was examined in the patients group and it was found to be 76.7%. Ninety-five percent confidence intervals (95% CIs) were generated (Figure 1).

Figure (1): Roc curve between patients and controls regarding to the level of serum vitamin D

There was a highly statistically significant difference between patient with vit D insufficiency and patients without vit D insufficiency as regards ESR,
number of tender joints, number of swollen joints, VAS, DAS score and HAQ (Table 2).

Table (2): Comparison between patients with and without vit D insufficiency

<table>
<thead>
<tr>
<th></th>
<th>With insufficiency (&lt; 60)</th>
<th>Without insufficiency (≥ 60)</th>
<th>t/z*</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR</td>
<td>40 (30 – 50)</td>
<td>23 (11.5 – 33.5)</td>
<td>2.556*</td>
<td>0.011</td>
</tr>
<tr>
<td>No. of Tender Joints</td>
<td>6 (4 – 14)</td>
<td>2 (1.75 – 4)</td>
<td>3.385*</td>
<td>0.001</td>
</tr>
<tr>
<td>No. of Swollen Joints</td>
<td>1 (0 – 2)</td>
<td>0 (0 – 0)</td>
<td>3.212*</td>
<td>0.002</td>
</tr>
<tr>
<td>VAS</td>
<td>40 (20 – 60)</td>
<td>10 (0 – 13.75)</td>
<td>3.821*</td>
<td>0.000</td>
</tr>
<tr>
<td>DAS Score</td>
<td>3.83±1.34</td>
<td>2.42±1.04</td>
<td>3.631*</td>
<td>0.001</td>
</tr>
<tr>
<td>HAQ</td>
<td>0.63 (0.38 – 1)</td>
<td>0 (0 – 0.16)</td>
<td>3.045*</td>
<td>0.004</td>
</tr>
</tbody>
</table>

* Data were presented as mean±SD and compared together using Independent t-test
z*: Data were presented as Median and IQR and compared together using Mann-Whitney test

Additionally, we examined the prevalence of vitamin D deficiency and insufficiency defined as a 25(OH)-D level < 20 ng/ml (< 50 nmoles/liter) and < less than 30 ng/ml (< than 75 nmoles/liter) (Table 3).

Table (3): Prevalence of vitamin D deficiency and insufficiency in patients and controls group.

<table>
<thead>
<tr>
<th>Vit. D Groups</th>
<th>Patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
</tr>
<tr>
<td>Deficiency (&lt; 50 nm)</td>
<td>32</td>
<td>53.30%</td>
</tr>
<tr>
<td>Insufficiency (50 – 75 nm)</td>
<td>21</td>
<td>35.00%</td>
</tr>
<tr>
<td>Sufficiency (&gt; 75 nm)</td>
<td>7</td>
<td>11.70%</td>
</tr>
<tr>
<td>Total</td>
<td>60</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

We divided patients with RA into four groups according to DAS28 as RA patients in remission (Group 1, n = 18), low activity (Group 2, n = 12), moderate activity (Group 3, n = 17), and high activity (group 4, n = 13). The mean of the 25-OH D vitamin level was 63.39±18.32 nmol/l in group 1, 51.00±7.90 nmol/l in group 2, 48.41 ±20.95 nmol/l in group 3 and 21.77 ±10.49 in group 4. 25-OH vitamin D levels of the patients in the high activity group (group 4) were the lowest (p < 0.01). The demographic, clinical features of the groups are shown in Table 4.

Table (4): Demographic and clinical features of RA patients with different disease activity.

<table>
<thead>
<tr>
<th></th>
<th>Patients in remission (n=18)</th>
<th>Low activity (n=12)</th>
<th>Moderate activity (n=17)</th>
<th>High activity (n=13)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>27.44±4.4</td>
<td>31.01±4.7</td>
<td>35.51±5.7</td>
<td>30.16±6.7</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>Duration of disease</td>
<td>3.67±1.75</td>
<td>7.11±4.5</td>
<td>5.61±4.4</td>
<td>4.21±2.3</td>
<td>&gt;0.05*</td>
</tr>
<tr>
<td>HAQ</td>
<td>0 (0–0.13)</td>
<td>0.56 (0.38–0.69)</td>
<td>0.38 (0.38–0.88)</td>
<td>1.25 (1–1.4)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>DAS28</td>
<td>2.02 (1.56–2.1)</td>
<td>2.85 (2.78–2.98)</td>
<td>4.14 (3.83–4.46)</td>
<td>5.39 (5.24–5.63)</td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>25(OH) vitamin D (nmol/L)</td>
<td>63.39±18.32</td>
<td>51.00±7.9</td>
<td>48.41±20.95</td>
<td>48.71±10.49</td>
<td>&lt;0.01*</td>
</tr>
</tbody>
</table>

Data were presented as mean±SD and compared using One Way ANOVA test
* Data were presented as median and IQR and compared using Kruskall-Wallis test

In unadjusted analyses, 25(OH)-D levels were significantly inversely associated with VAS score, swollen joint counts, tender joint counts, RF, CRP, HAQ, and DAS-28 with p value <0.01 (Table 4). None of these results were significantly changed after multivariate adjustments for age, gender, glucocorticoid and DMARD use.

Table (4): Correlation between vitamin D levels and the different studied parameters.

<table>
<thead>
<tr>
<th></th>
<th>Vitamin D</th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>-0.379</td>
<td>0.003 (HS)</td>
<td></td>
</tr>
<tr>
<td>CRP</td>
<td>-0.162</td>
<td>0.217 (NS)</td>
<td></td>
</tr>
<tr>
<td>No. of Tender Joints</td>
<td>-0.664</td>
<td>0.000 (HS)</td>
<td></td>
</tr>
<tr>
<td>No. of Swollen Joints</td>
<td>-0.670</td>
<td>0.000 (HS)</td>
<td></td>
</tr>
<tr>
<td>VAS</td>
<td>-0.665</td>
<td>0.000 (HS)</td>
<td></td>
</tr>
<tr>
<td>DAS Score</td>
<td>-0.663</td>
<td>0.000 (HS)</td>
<td></td>
</tr>
<tr>
<td>HAQ</td>
<td>-0.629</td>
<td>0.000 (HS)</td>
<td></td>
</tr>
</tbody>
</table>

NS: Non significant   HS: Highly statistical significant
Statistical Analysis

Statistical analysis was performed utilizing statistical analysis for social science (SPSS) USA version 17. Means (SD) were calculated for variables showing normal distribution and median and 25–75th percentiles (interquartile range [IQR]) for those deviating from it. When transformations were insufficient to obtain normal distribution, we applied nonparametric Spearman’s correlation coefficient. When appropriate, multiple regression analysis was carried out. The comparison between two groups with quantitative parametric data was done using Independent t-test, while the comparison between more than two groups with quantitative non-parametric data was done using Mann-Whitney test. The comparison between more than two groups with quantitative parametric data was done using one way analysis of variance (ANOVA), while the comparison between more than two groups with quantitative non-parametric data was done using Kruskall-Wallis test. p value < 0.05 was considered significant.

III. DISCUSSION

Vitamin D is known to induce immunologic tolerance [14]. Thus, vitamin D deficiency may induce the development of autoimmune diseases, such as RA. Vitamin D has immunomodulatory properties[15,16]. It regulates the immune response by a variety of mechanisms, such as decreasing antigen presentation[17], inhibiting the pro-inflammatory T helper type 1 profile[18] and inducing regulatory T cells [19]. 1,25(OH)2D3 suppresses proliferation and immunoglobulin production and retards differentiation of B-cell precursors into plasma cells [20]. These data support a role for vitamin D deficiency in the development and progression of RA and other autoimmune diseases.

Our results show that 25-OH vitamin D levels are significantly lower in patients with RA compared to healthy controls. Additionally, vitamin D levels are lower in the patients with RA with high activity than those with low activity. Likewise, we found that there was significantly inverse correlation between serum 25-OH vitamin D levels and Rheumatoid arthritis disease activity as assessed by DAS28 score. Several studies have evaluated the association between vitamin D levels and RA activity. In a study involving 1191 patients with RA and 1019 controls, Rossini and colleagues [21] found an inverse association between vitamin D levels and disease activity in RA. Also, our results were in agreement with Welsh et al.; Kerr et al.; Cutolo et al.and Haque and Bartlett [22,23,24,25]. Additionally, Orbach et al.[26] study demonstrated that patients with various autoimmune diseases had lower levels of 25-OH vitamin D level than healthy adults. By contrast, others did not find a relationship between vitamin D deficiency and disease activity in RA [27,28,29]. In the study done by Braun-Moscovici and colleagues [28], their subjects had high disease activity and low 25(OH) D3 levels, accounting for a high vitamin D deficiency rate, which might have influenced the study outcome and the lack of correlation with disease activity.

The present study shows not only the relationship between disease activity and vitamin D level, but also the negative correlation between vitamin D level and HAQ, CRP, VAS, number of tender joints and number of swollen joints. This may be the result of lower vitamin D levels in patients with high disease activity levels because of poor exposure to direct sunlight. We did not have any information about sun exposure in this study. However, this association remains despite adjusting for age, gender and medication use, demonstrating a robust relationship. Our results were in agreement with Diaz et al[30] who studied a group of patients with lupus nephritis.

HAQ score is thought to be a disease specific tool for the assessment of inflammatory joint disorders [31]. In our study, those with higher vitamin D level had lower HAQ scores. These data provide further support that vitamin D plays an immunomodulatory role in inflammatory arthritis.

The present study demonstrated an association between vitamin D status and self-reported pain. Previous studies have suggested a link between low vitamin D and musculoskeletal pain [32], and some have suggested that patients with chronic pain should be screened for vitamin D level [33]. More recently, the notion of an association between pain and vitamin D status has been refuted. Furthermore, treatment of hypovitaminosis D does not appear to be effective in management of patients with chronic pain [34].

In our study, hypovitaminosis cut-off for serum 25-OH vitamin D concentration of 24 ng/ml or < 60 nmol/l was chosen according to ROC curve. Vitamin D deficiency was defined as serum 25OHD below 10 ng/ml (15 nmol/liter) because both serum 1,25(OH)2D and calcium absorption declined significantly at this level [35]. The World Health Organization (WHO) defined vitamin D insufficiency as serum 25OHD below 20 ng/ml (50 nmol/liter). However, others started to define vitamin D deficiency as serum 25OHD level below 20 ng/ml and vitamin D insufficiency as less than 30 ng/ml (75 nmol/liter) [36]. The primary argument for this change in definition is based on the finding that serum PTH, which is inversely related to serum 25OHD, decreases as serum 25OHD increases and reaches a plateau at a serum 25OHD of approximately 30 ng/ml (75 nmol/liter). This is certainly controversial because numerous studies show a large variation in the plateau level of PTH ranging from a serum 25OHD of 18 ng/ml (45 nmol/liter) to 30 ng/ml (75 nmol/liter). This change in definition of vitamin D insufficiency actually has major significance because the dose of vitamin D required to increase people to a minimum serum 25OHD of 20 ng/ml (50 nmol/liter) is
approximately 800 IU daily, whereas increasing people to a minimum level of 30 ng/ml (75 nmol/liter) would require approximately 4,000 IU daily [37].

In the present study, we examined the prevalence of vitamin D deficiency and insufficiency defined as a 25(OH)-D level $< 20$ ng/ml (< 50 nmol/liter) and $< 30$ ng/ml (< 75 nmol/liter) and we found that vit d deficiency is more prevalent in patients than control groups while no difference was found between patients and control groups as regards vit D insufficiency. We have found that vitamin D deficiency (25(OH)D values $<20$ ng/ml) is common in RA patients affecting 53.3% of the entire cohort. Our results are similar to those reported by Rossini et al. and Craig et al. [21,27], with a prevalence of vitamin D deficiency ranging from 30 to 63%.

There are limitations to this study that should be considered. The month of the year should be identified as seasonal variations (increase) of 25(OH)D levels between winter and summer were found to be significant. Additionally, sun exposure time should be calculated, as this may be the result of lower vitamin D levels in winter and summer were found to be significant. Further studies on larger scales on adult and juvenile RA patients are recommended. Additionally, a long follow up study is recommended in order to assess the level of serum vitamin D from the beginning of the course of disease, also before and after therapy in order to monitor the effect of the type and intensity of treatment on the level of vitamin D.

V. REFERENCES


