Vitamin D deficiency in rheumatoid arthritis

Prevalence and association with disease activity in Western Saudi Arabia

Suzan M. Attar, FRCPC, ABIM.

ABSTRACT

الأهداف: تقدير مدى انتشار انخفاض مستوى فيتامين (د) في الدم 25D(OH) لدى المرضى الذين يعانون من التهاب المفاصل الروماتويدي (RA) مقارنةً مع الأصحاء، وتحليل العلاقة بين 25D(OH) ونشاط المرض.

الطريقة: أُجريت هذه الدراسة الاسترجاعية في قسم الطب الباطني، مستشفى جامعة الملك عبدالعزيز، جدة، المملكة العربية السعودية وذلك خلال الفترة من يناير 2010م إلى ديسمبر 2011م. شملت الدراسة 100 من مرضى التهاب المفاصل الروماتويدي، و100 من الأصحاء، وتم قياس نشاط المرض باستخدام مؤشر نشاط درجة المرض (DAS28). ولقد تم تقسيم مرضى التهاب المفاصل الروماتويدي وفقا لدرجة DAS28 إلى 3 مجموعات على النحو التالي: نشاط مرتفع أو متوسط أو منخفض. وتم قياس نسبة فيتامين (د) في المختبر المركزي.

النتائج: أشارت نتائج الدراسة إلى أن نسبة معدل فيتامين (د) في المرضى الذين يعانون من التهاب المفاصل الروماتويدي مماثل للأصحاء (14.4+32.3 نانومول/لتر) مقابل (16.4+31.4 نانومول/لتر)(p=0.41). وكان 25D(OH) عند المرضى الذين يعانون من ارتفاع نشاط المرض (18.25+8.3 نانومول/لتر) أقل من المرضى الذين يعانون من نشاط المرض المتوسط (35.13 + 15.2 نانومول/لتر) أو المنخفض (38.05+7.3 نانومول/لتر)(p<0.001). ولقد كانت هناك علاقة عكسية بين 25D(OH) ونشاط المرض (p<0.0001 ،0.42- =r).

خاتمة: أظهرت الدراسة بأن مستوى فيتامين (د) لدى المرضى الذين يعانون من التهاب المفاصل الروماتويدي مشابهة لتلك الموجودة لدى الأصحاء، ولقد تم العثور على نسبة أقل لدى المرضى ذوي النشاط المرتفع والذين لا يستجيبون للعلاج.

Objectives: To estimate the prevalence of low serum vitamin D level (25[OH]D) in patients with rheumatoid arthritis (RA) compared with healthy controls, and to analyze the association between 25(OH)D and disease activity.

Methods: This retrospective analysis included 100 RA patients (85% women) and 100 controls, not on vitamin D supplements from January 2010 to December 2011 at a tertiary care center at the Department of Internal Medicine, King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia. Disease activity was measured using the disease activity score index (DAS28). According to the DAS28 score, RA patients were divided into 3 groups as high, moderate, and low disease activity. Patients' serum 25(OH)D was measured in a centralized laboratory.

Results: The mean 25(OH)D in patients with RA was similar to the control group (32.3±14.4nmol/L) versus (31.4±16.4nmol/L) (p=0.41). Patients with high disease activity had the lowest 25(OH)D levels (18.25±8.3nmol/L) compared with patients with moderate (35.13±15.2nmol/L) and low (38.05±7.3nmol/L) disease activity (p<0.001). Serum 25(OH)D was negatively correlated with DAS28, which was statistically significant (r= -0.42, p<0.0001).

Conclusion: 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 are poorly responding to treatment, and not in a state of disease remission.

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Vitamin D is a secosteroid, which is similar to a steroid except that 2 of the 4 steroid B-ring carbon atoms are not joined.[1] In the 1980s, researchers observed high expression of vitamin D receptors (VDR) in cells other than the kidney that are capable of producing the active form of vitamin D, which is not parathyroid hormone (PTH) regulated.[2] The active form of vitamin D functions as an immune modulator, reducing activation of the acquired immune system.[3] Theoretically, vitamin D deficiency has been associated with the alteration of cellular functions and increased risk of autoimmune diseases including rheumatoid arthritis (RA).[4] Epidemiological studies implicated that low 25-hydroxyvitamin D level (25(OH)D) may be common in RA patients.[5-7] However, conflicting results have been reported on whether there is a correlation between 25(OH)D and RA disease activity.[8-10] Therefore, the aim of our study was to examined the prevalence of vitamin D deficiency among Saudi RA patients at a tertiary care center compared with a control group, and to evaluate the correlation between 25(OH)D and disease activity.

Methods. We carried out this retrospective study from January 2010 to December 2011, and the medical records of RA patients attending King Abdulaziz University Hospital (KAUH), Jeddah, Kingdom of Saudi Arabia (KSA) were studied. Approval was obtained from the Biomedical and Ethics Committee of KAUH. Rheumatoid arthritis patients not on vitamin D supplements were included in the study. They were identified according to the 1987 American College of Rheumatology (ACR) classification criteria for RA,[11] and by the 2010 ACR/European League Against Rheumatism (EULAR) classification criteria[12] for RA when the first criteria was not applicable. Patients aged less than 14 years old were excluded from the study. The following demographic features were obtained; age, gender, nationality, duration of the disease at the time of the study (in years), and weight of the patients (further divided into; underweight, normal weight, overweight and obese according to the body mass index (BMI: weight [kg] / height [m²]) medicatio used specifically disease-modifying antirheumatic drugs (DMARDs) and glucocorticoids (GC). Investigations included erythrocyte sedimentation rate (ESR: normal 0-20 mm/hr) and rheumatoid factor (RF: normal 0-20 IU/L). Activity of the disease was defined based on the disease activity score index of a 28 joint count (DAS28) using the ESR. Patients were divided into 3 groups depending on the DAS28 score: group I: high disease activity if the DAS28 was >5.1, group II: moderate disease activity if the DAS28 was between 5.1-3.2,

and group III: low disease activity if the DAS28 was <3.2.[13,14]

Concentration of 25-hydroxycholecalciferol (25(OH)D), was measured using the liquid chromatography tandem mass spectrometry (LC MS/MS) technique (Roche Diagnostic, Penzberg, Germany). Different definitions have been used to define 25(OH) status; adequacy if the serum level is >75 nmol/L (>30 ng/ml), and insufficiency if the level ranged between 75-25nmol/L (30-10 ng/ml). With regards to deficiency, 2 definitions of cutoff points were used; first the Endocrine Society definition if the serum 25(OH)D was <50 nmol/L (<30 ng/ml), second by the Canadian Osteoporosis Society if the serum was <25 nmol/L (<10 ng/ml).[15,16] Healthy age and gender matched controls were identified, and their 25(OH)D was measured.

Statistical analysis. Statistical analysis was carried out by using the Statistical Package for Social Science version 16 software (SPSS Inc., Chicago, Illinois, USA). Qualitative and ordinal (rank) data were presented as number and percentage. The quantitative data were presented as mean and standard deviation. Chi-square with a linear trend was used as a test of significance for ordinal data. Student t test was used as a test of significance for quantitative data between 2 groups, and one-way ANNOVA (F-test) was used to compare between more than 2 groups. Post hoc test (least significance difference [LSD]) was used to compare between each 2 groups if the F-test revealed a significant difference. Pearson correlation was carried out to study the correlation between the studied variables. Odds ratio and 95% confidence intervals were calculated to estimate the risk. Multivariate analysis was carried out to determine the predictors for disease activity. Receiver operating curve (ROC) was used to study validity of 25(OH)D levels to predict the activity of RA. Significance was considered when the *p*-value was less than 0.05.

Results. The RA sample was mostly female (90%) with a mean (±SD) age of 47±13 years, and disease duration of 4.7±5 years. Thirty-four RA patients (34%) had high disease activity, 54% had moderate disease activity, and 12% had low disease activity. Thirty-five patients (35%) were chronic steroids users. Ninety-eight (98%) of the patients were using DMARDs. The patients weight was classified according to the BMI as follows: underweight in 3 patients (3%), normal weight in 16 patients (16%), overweight in 40 patients (40%), and obese in 35 patients (35%). The mean values of 25(OH)D and the proportion of patients with vitamin D adequacy, insufficiency, and deficiency with different cut-off values in both RA patients and healthy matched

Table 1 - Main characteristics, means, and confidence intervals, or percentages of study groups.

Variables	RA N=100 (%)	Control N=100 (%)	*P*-value
Age in years mean (range)	47 ± 13 (14-83)	47 ± 15 (17-79)	0.81 NS
Gender, male:female	10:90	11:89	0.50 NS
Nationality, Saudi:Non-Saudi	61:39	57:43	0.33 NS
Disease duration, mean (range)	4.7 ± 5 (1-34)		
<1 year	24 (24)		
Between 1-5 years	43 (43)		
>5≤10 years	15 (15)		
>10 years	18 (18)		
Rheumatoid factor, mean (range)	81.5 ± 133 (2-698)		
RF-Positive	54 (54)		
RF-Negative	46 (46)		
DAS 28 (mean) range	4.6 ± 2 (2-13)		
High disease activity >5.1	34 (34)		
Moderate disease activity 5.1-3.2	54 (54)		
Low disease activity <3.2	12 (12)		
25(OH)D (nmol/l) (mean) range	32.3 ± 14.4 (6-64)	31.4 ± 16.4 (6-82)	
Adequate >75 nmol/L (>30 ng/ml)	0	2 (2)	0.41
Insufficiency 75-25 nmol/L (30-10 ng/ml)	57 (57)	59 (59)	0.5 NS
Deficiency			
<25 nmol/L (<10 ng/ml)	43 (43)	39 (39)	0.5 NS
<50 nmol/L (<25 ng/ml)	90 (90)	89 (89)	0.50 NS
<10 nmol/L (<4 ng/ml)	17 (17)	12 (12)	0.21 NS

P-values were obtained by t-test or Chi square test, RA - rheumatoid arthritis, DAS28 - disease activity score index, 25(OH)D - 25-hydroxyvitamin D, NS - not significant, RF - rheumatoid factor

Table 2 - Mean 25(OH)D level in 100 RA patients according to categorical variable.

Variables	N (%) N=100	Mean 25 (OH) ± SD	Test of significance
Duration of disease			
<1 year	24 (24)	27.23 ± 14.85	*p*=0.26 NS
≥1-5 years	43 (43)	26.6 ± 13.30	Between all durations
>5≤10 years	15 (15)	30.44 ± 16.98	
≥10 years	18 (18)	34.31 ± 15.81	
Rheumatoid factor			
RF-Positive	54 (54)	30.68 ± 15.02	*p*=0.125 NS
RF-Negative	46 (46)	26.44 ± 14.34	
DAS 28			*p*<0.001***Between all grade
>5.1 Group (I)	34 (34)	18.25 ± 8.31	(I) vs (II) *p*<.001*
5.1-3.2 Group (II)	54 (54)	35.13 ± 15.21	(I) vs (III) *p*<.001*
<3.2 Group(III)	12 (12)	38.05 ± 7.28	(II)vs (III) *p*<.001*
Remission			
Yes	4 (4)	40.17 ± 8.74	*p*=.056 NS
No	96 (96)	28.25 ± 14.83	
Activity			
Active	79 (79)	26.26 ± 13.31	*p*=0.006**
Inactive	21 (21)	38.01 ± 16.56	

RA - rheumatoid arthritis, DAS28 - disease activity score index, 25(OH)D - 25-hydroxyvitamin D, RF - rheumatoid factor. *P*-values obtained by ANOVA between more than 2 groups, then followed by Post hoc analysis (least significant difference (LSD). Student t-test was used for comparison between 2 groups, NS - not significant

controls are presented in (**Table 1**). The mean serum level of 25(OH)D (nmol/L) was similar between the RA patients and the matched controls, and not statistically significant (*p*=0.41). Mean 25(OH)D level in the RA patients according to different variables are presented in (**Table 2**). The mean 25(OH)D in patients with high disease activity (group I) is lower than patients with moderate and low disease activity (*p*<0.001). By one-way ANNOVA (F-test) and post hoc analysis (LSD),

there was a significant low level of 25(OH)D in group II versus group III, in group I versus group II, and in group I versus group III. The group with a high DAS28 score showed significantly lower 25(OH)D levels than the groups with moderate and low DAS28 scores. Also, the group with a moderate DAS28 showed significantly lower 25(OH)D level than the group with a low DAS28 score. The higher the DAS28 score the lower the level of 25(OH)D, which was statistically significant (*p*<0.001).
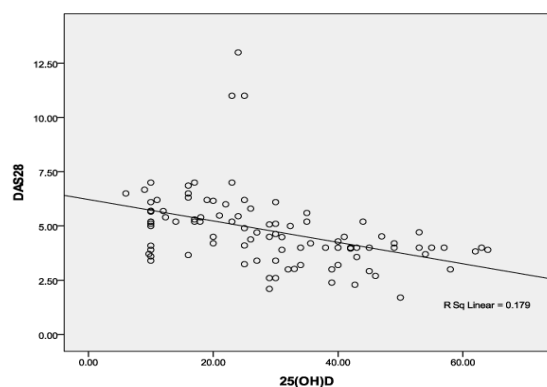
Figure 1 - Correlation between 25(OH)D and DAS28 activity score. 25(OH)D - 25-hydroxyvitamin D, DAS28 - disease activity score index
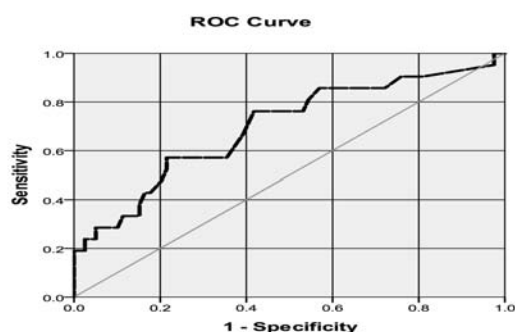

Figure 2 - Receiver operating curve of serum 25(OH)D to predict the RA disease activity assessed by DAS28. 25(OH)D - 25-hydroxyvitamin D, DAS28 - disease activity score index, ROC - receiver operating curve

By Pearson correlation, there was a significant negative correlation between 25(OH)D serum levels and disease activity measured by DAS28 (r=-0.42, *p*=0.001) (**Figure 1**), and a significant positive correlation with age (r=0.21, *p*=0.003), and rheumatoid factor (r=0.23, *p*=0.02) but not with disease duration or GC use.

The RA patients with a 25(OH)D less than 25 umol/L have a 32 times higher risk than RA patients with a 25(OH)D between 25-75umol/L to have a higher DAS28 score (OR=32.1, 95% CI: 9.35-118, *p*<0.001). Binary logistic regression analysis to differentiate patients with high DAS28 score versus moderate and low DAS28 scores, showed that the most significant predictor for high DAS28 was low 25(OH)D (B-coefficient =-0.052, OR=1.05, 95% CI: 1.02-1.09, *p*=0.002). There was no statistically significant association between the DAS28 and the age of the patients, disease duration, RF, or glucocorticoid use. By ROC curve analysis, sensitivity and specificity for different cut off points of 25(OH)D to predict active RA was obtained. Among RA patients, at a cut off point of 25 nmol/L the sensitivity of 25(OH)D

levels to detect active disease was 87%, and the specificity was 70% (**Figure 2**). If we depend on 25(OH)D levels, we could accurately differentiate between RA disease activity who would be active through high DAS28 or not (95% CI: 72-86, *p*<0.001).

Discussion. We observed that low 25(OH)D values of <75nmol/L (<30 ng/ml) occurred in the RA patient cohort (100%) compared with the matched control (98%), which was not statistically significant. Vitamin D insufficiency between 75-25 nmol/L (30-10 ng/ml) occurred in 57% of the patients, and vitamin D deficiency whether defined as 25(OH)D <50 nmol/L (<20 mg/ml) or 25(OH)D <25 nmol/L (<10 mg/ml) occurred in 90% and 43% of the patients. Vitamin D levels are lower in patients with high disease activity. There was a significant inverse correlation between serum 25(OH)D and disease activity evaluated by DAS28. Over the past decade, vitamin D has been emerging as a major public health problem due to its role in skeletal and extra-skeletal health. No consensus exists for the optimum level of circulating serum 25(OH)D that is adequate for health. In 2007 Holik et al,[16] determined that the normal 25(OH)D level was set at more than 50 nmol/l (20 ng/ml) based on careful analysis of a large number of epidemiological studies. It is well known that replacing 25(OH)D levels may reduce secondary hyperparathyroid bone loss, and according to the meta-analysis this resulted in 23% fracture related fall prevention among the elderly (relative risk = 0.77, 95% CI: 0.65-0.90).[17] New evidence in vivo and in vitro mechanistic experiments suggests that the active form of vitamin D may play a role in the etiology of autoimmunity by inducing the T-regulatory cells and inhibiting the natural killer cells, and that 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.[18,19] Researchers observed when using parathyroid hormone (PTH) elevation as a biomarker reflecting the physiologic low levels of 25(OH)D, that the optimal level of 25(OH)D to maintain the immunomodulatory effect is more than 80 nmol/l (32 ng/ml).[15,17,20] In 2010, Canadian osteoporosis guidelines[15] considered 25(OH)D deficiency as <25nmol/L based on non randomized controlled trials or cohort studies, while the Endocrine Society defined deficiency as <50nmol/L,[21] which was in agreement with the Institute Of Medicine (IOM). Accordingly, in this paper different cutoff points were used when defining 25(OH) deficiency for the accuracy of the results. However, it is agreed that the 25(OH)D assay is the recommended method of screening over the serum 1,25-dihydroxyvitamin D (1,25(OH)2D)

assay due to its longer circulating half-life of 2-3 weeks as well as not being PTH regulated.[21] The reported prevalence of low vitamin D <75 nmol/L (<30 ng/ml) is a worldwide predicament in various populations, ranging from 20% to over 80%.[15,22,23] Because of this wide variation, an individual has to be careful when interpreting low vitamin D results as such a discrepancy depends upon many factors. First; the definition used, as the prevalence rises considerably if the threshold of 25(OH)D levels are set at a higher level, such as <75nmol/L (<30 ng/mL) rather than <50 nmol/L (<20 ng/mL). Second is the variability between methods of assay and laboratories. There have been numerous reports of discrepancies between the results of the LC-MS/MS and immunoassays, as with the LC-MS/MS assay the 25(OH)D values could be 40% higher than the value reported using immunoassay.[24] Efforts are ongoing to standardize the testing protocol to improve the testing accuracy. Finally, different genetic background, that leads to vitamin D receptor (VDR) polymorphism.[25] However, recent studies showed no difference in the VDR genotype frequencies obtained among a Saudi population and other reported studies in western society.[26]

Most of the studies of the non-skeletal effects of vitamin D are often observational. A recent systematic review published in 2010, showed there have been 7 cases control studies evaluating the frequency of vitamin D deficiency in RA patients compared with controls.[8] The studies showed that the frequency of vitamin D deficiency in RA patients ranged between 30-63%. Two studies revealed lower levels of 25(OH)D in RA patients compared with healthy matched controls, but 5 studies did not find any difference between RA and controls.[10,27-29] Our study showed a higher frequency of vitamin D deficiency in the RA population than any other study (90%) when using 25(OH)D <50 nmol/L (<20 mg/ml), which is higher than the reported prevalence in Italy (52%),[28] the USA (43%),[29] and Iran (41%).[30] None of our patients achieved the optimum level required for the immunoregulatory effect, which is >80 nmol/L. The mean 25(OH)D in patients with RA was similar to that observed in the control group that represents the general population (32.3+14.4nmol/L) versus (31.4+16.4nmol/L) (*p*=0.41), which has been documented previously.[30] This confirms that low 25(OH)D is a general problem in Saudi Arabia, which was observed in a recent large study of 1,172 healthy women in which (80%) were vitamin D deficient (serum 25(OH)D <50 nmol/L).[26] With respect to RA, a systematic review conducted in 2008 observed that older studies had no association between 25(OH)D and RA disease activity, however, those studies were

using a single measure (blood work - ESR or CRP) as a guide for disease activity.[31] Since the introduction of DAS28 as a marker for RA disease activity, results have been conflicting as some studies showed a lower level of 25(OH)D was associated with high DAS28,[32,33] but others did not.[10,34] The present study shows a strong negative correlation between 25(OH)D and DAS28. Out of the 43 patient with 25(OH)D less than 25umol/L, most had a high disease activity (35/43 [81%] patients), 9/43 (21%) patients showed moderate disease activity, but none had low disease activity. We assessed the disease activity using the DAS28 score, which includes a symptomatic evaluation of the patients, physical examination, and blood work. This is in agreement with the British cohort by Patel and colleagues[35] who evaluated 206 patients with early polyarthritis in which (45%) were classified as RA after one year. They found a strong inverse association between serum 25(OH)D and baseline disease activity, assessed by tender joint counts, DAS28, and health assessment questionnaire scores (HAQ) only at disease onset, but not in patients with a disease duration longer than 1-2 years. Interestingly, they observed that an increase in 25(OH)D of 10 ng/ml (25 nmol/l) would be needed to reduce the DAS28 score by 0.3 point. They also observed that an increase in 25(OH)D of ~30 ng/ml (75 nmol/l) would be needed to reduce the DAS28 score by one point.

Based on our analysis, a physician could predict an 87% chance for active disease if RA patients presented with vitamin D level of 25umol/L. We evaluated the hypothesis concerning the immunomodulatory effect of 25(OH)D, by detecting the correlation between 25(OH)D and RF, which was not significant. This lessens the theory that links between low 25(OH)D in RA patients is autoimmune in nature and could be related to another mechanism which is not known, this has been also observed by Feser et al.[36] The advantages of this study include that it is case control, and that there are limited studies in the region evaluating the relationship between RA and disease activity. The study was limited by its retrospective nature, which limits us from inquiring regarding further personal details such as diet and sun exposure. We suggest carrying out a prospective study to evaluate whether or not maintaining 25(OH)D level at 80 nmol/L in patients with RA will help in inducing remission.

In conclusion, we found that vitamin D deficiency is a common problem in RA patients, but not more common than the age matched control group from the general population. Serum vitamin D level was inversely related to disease activity. Thus, special attention should be given to checking 25(OH)D levels in patients with active disease as its deficiency affects health status.

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