Bioengineering Research

Evaluation of the relationship between vitamin D levels and related serum markers as well as disease activity in patients with rheumatoid arthritis: A cross-sectional study

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Abstract

Introduction: Many studies have reported the role of vitamin D in autoimmune disease including rheumatoid arthritis (RA) which is related to several serum autoantibodies such as RF and Anti CCP. It also has been shown that vitamin D deficiency affect the DAS28 and VAS score inversely. Therefore, the aim of this study was to assess the relationship between vitamin D deficiency and RA-related autoantibodies including Anti CCP and RF levels and also evaluate the association between these parameters and the severity of rheumatoid arthritis.

Methods: In this cross-sectional study, RA patients over 16 years of age who refer to the clinic and rheumatology ward were enrolled. The severity of diseases was assessed via the DAS28 scoring system. Serum levels of 25(OH) vitamin D with other rheumatoid related laboratory evaluations including Anti CCP, RF and CRP were determined.

Results: A total of 100 patients with a mean age of 45.27± 14.14 were included and evaluated; of them, 75% were female and 77% lived in the city. Most of the patients (66%) had moderate DAS28 levels; however, no substantial relationship was observed between DAS28 and vitamin D levels. A significant inverse relation between serum 25(OH)D level and disease duration, as well as a significant positive correlation with the level of education, was observed. There was also no significant correlation between RA-related autoantibodies (e.g. anti CCP, and RF) and inflammatory serum marker (e.g. CRP) with 25(OH)D.

Conclusion: Due to vitamin deficiency in the majority of cases (73%) and the relationship between serum 25(OH)D levels and the duration of disease, it can be concluded that vitamin D levels should be checked in those patients.

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1. Introduction

Rheumatoid arthritis (RA), as a chronic and systemic polyarticular arthritis, affects approximately 1% of the population and predominantly females. Furthermore, the prevalence increases with age and is more common in the fourth and fifth decades of life [1,2]. The disease presents itself with systemic polyarthritis as well as extra-articular manifestation including rheumatoid nodules, pulmonary fibrosis, vasculitis, and serositis. Moreover, the course of RA is highly variable and unpredictable for each patient. Mostly, the disease activity is continuous and alters at the same time, with varying degrees of joint deformities and functional disorders [3].

Antibodies against cyclic citrullinated peptides (CCP) and rheumatoid factor (RF) have been identified in studies based on the serological examination of RA patients; while they have about the same susceptibility, they have been documented to have higher specificity for anti-CCP. These RA-related autoantibodies are also specifically associated with the prognosis of the disorder [4,5].

The pathogenesis of autoimmune diseases including RA depends on the environmental and genetic factors. In this regard, vitamin D has been identified as a pathogenetic factor for autoimmune disorders such as multiple sclerosis and type 1 diabetes [6]. Specifically, after the discovery of its receptors in the cells linked to the immune system, the regulatory function of vitamin D was proposed. Activated dendritic cells have also been proven to develop hormones linked to vitamin D which inhibits the proliferation of T cells, especially Th1 and the production as well as secretion of antibodies, inflammatory cytokines, and B cells. It also differentiates the monocytes from dendritic cells, interacting in vitro with the activity of T cells [7,8]. Higher doses of vitamin D are associated with a reduced incidence of rheumatoid arthritis, and lower vitamin D levels are associated with a greater severity of the condition, according to the literature. In addition, lower levels of 25-Hydroxy Vitamin D (25(OH)D), as a prehormone formed in the liver and as a metabolite evaluated by physicians worldwide to ascertain a patient's vitamin D status, have been found in RA patients who have not been in the recovery process or who have not reacted to treatment; also, the activity of the disease, usually assessed by joint swelling and tenderness, inflammation, and Hypovitaminosis D will also increase the number of swollen and sore joints as well as the amount of CRP, thus aggravating the self-assessment of the patient's health[9-12].

Based on the literature, the disease activity is associated with serum RA-related autoantibodies levels including RF and anti-CCP [13]. on the other hand, hypovitaminosis D may elevate the disease activity. Therefore, hypovitaminosis D may be in association with the level of the mentioned autoantibodies. Furthermore, studies in this regard are limited. Therefore, this study was conducted to evaluate the relationship between vitamin D insufficiency and serum RF and anti-CCP levels and also its association with the severity of rheumatoid arthritis to provide better evidence in the management of these patients.

2. Material and Method

In this cross-sectional study, patients aged 16 years and above referring to the rheumatological clinic from April to September 2015 with a diagnosis of RA based on the guidelines of the American College of Rheumatology (ACR) were included [14]. Patients who had a history of recent vitamin D supplement use in the previous 3 months were excluded from our study. The sample size was calculated based on a study by Soubrier et al [15] By considering a standard deviation of 0.45, power of 90% to detect a difference in HAQ (primary outcome) of at least 0.24, we reached a sample size of 74 patients. Also, by considering a two-sided type-I error at 0.05, a total sample size of 82 patients was calculated. The obtained data consisted of the patient's demographic information and comorbid diseases along with their RA disease features such as duration of the disease and drugs used, serum levels of RF, and CRP.

The patients were then examined by a single expert rheumatologist who was blinded about the patients' paraclinical data. Evaluation of patients was based on DAS28 and a cut-off point of above 2.6 for active RA

disease, while less than 2.6 was considered as remission and responsive to RA treatment.

For the assessment of the pain severity in the patients, the Visual Analogue Scale system (VAS), a 10 cm scaled ruler was used to show the degree of pain in these patients, such that 0 and 10 were its two extremes representing no pain and worst pain, respectively. Furthermore, 1-3 score denoted relative pain, 4-6 moderate pain, and 7-10 severe pain overall [16]. Several internationally published papers have confirmed the reliability and durability of the VAS scale, [17,18] and many Iranian national publications confirmed its durability with a correlation coefficient of R: 0.88 [19].

Additionally, 2cc blood was obtained and sent to the laboratory. The samples were evaluated by a single laboratory technician who was blinded about the patient's data. For determining the vitamin D's serum levels, an ELISA kit (Euroimmun Kit, Nima Pooyesh Teb Company, Tehran, Iran) was utilized [20]. Also, Anti CCP, RF, and CRP levels of the samples were measured.

The patient's demographic, clinical, and paraclinical data were analyzed using SPSS version 22.0 software. Spearman coefficient was used to evaluate the relationship between the variables. A P value of lower than 0.05 was assigned as significant.

3. Results

A total of 100 participants fulfilled the inclusion criteria and were followed till the end of our study. Males constituted 25% of RA cases, while 75% of the cases were female. The mean age of the participants was 45.27 years (SD: 14.14, range 20 – 87). Among them, the majority (77%) were from urban areas, while most of the cases were also illiterate (43%). Table 1 demonstrates the demographic features of the patients in our study.

As Table 1 demonstrates, the majority (73%) had either insufficient or deficient serum levels of vitamin D. The average level of 25(OH)D was 24.17 ng/mL. Based on the spearman coefficient analysis between the serum levels of 25(OH)D and DAS28 score, there was no significant correlation among these two groups (Table 2). Also, 66% of the patients had a moderate level of DAS28 (2.6- 3.2), which were not in remission. The paraclinical data of the patients in our study is displayed in Table 3.

The results of Table 2 demonstrate a significant correlation among education levels and 25(OH)D (P<0.01) which shows an increase in the serum levels of this vitamin with the increase of educational levels. Also, based on the assessment of the relationship between 25(OH) D's serum levels and the duration of the disease, a significant correlation was obtained (P value: 0.0001, correlation coefficient: -.0408); therefore, with an increase in the duration of the disease, 25(OH) D's serum levels decreased.

As to the association of laboratory data and 25(OH)D, no significant correlation was obtained (Table 3).

Our variables were evaluated based on gender and the data are shown in Table 4.

As demonstrated in Table 4, there was only a significant relationship between RF levels and gender (P<0.05). The relationship between the 25(OH)D's serum levels based on the location of residence was also evaluated using T-test. For urban areas, an average of 23.20 (SD=21.2) was obtained, while for rural areas an average of 27.41 (SD:17.57) was obtained. No substantial association was seen between the location of residence and serum levels of 25(OH)D (T= 0.866, P=0.724).

4. Discussion

Hypovitaminosis D has been associated to autoimmune disease including RA in various studies [6]. Moreover, it was considered as a prognostic and reflective factor of disease activity. Anti-CCP and RF, on the other hand, were confirmed as diagnostic and also prognostic serum markers in RA patients. Therefore, the correlation between RA and vitamin D as well as its relationship with the mentioned autoantibodies separately prompted us to investigate if there is any significant correlation between the RA-related autoantibodies and 25(OH)D. Interestingly, we found no substantial association between Hypovitaminosis D and the foregoing serum markers. Thus, the lack of an appropriate amount of vit D may not affect autoantibodies-dependent prognosis.

In this study, 73% of our sample population were classified as vit D deficient. Haque et al. reported a favorable result which was 63%. Therefore, the levels below optimal vit D can be considered as a predisposing factor in developing RA features in

Table 1: Demographic and clinical features of rheumatoid arthritis patients and their correlation with 25(OH)D levels

Variable	Frequency *n=100*
Gender	
Male	25 (25%)
Female	75 (75%)
Residence	
Urban	77 (77%)
Rural	23 (23%)
Occupation	
Housewife	60 (60%)
Public employee	17 (17%)
Unemployed	8 (8%)
Manual worker	4 (4%)
Freelance	5 (5%)
Farmer	3 (3%)
Retired	3 (3%)
Education	
Illiterate	43 (43%)
Under-Diploma	28 (28%)
Diploma	16 (16%)
Bachelors and masters	13 (13%)
25(OH)vitamin D	
Deficient	29 (29%)
Insufficient	44 (44%)
Optimal	27 (27%)
DAS28[a] score	
Mild	21 (21%)
Moderate	66 (66%)
Severe	13 (13%)
[a]: disease activity score	

Table 2 Correlation of DAS28, age, education, hospital stay, and drug used in RA with 25(OH) D.

Variable	r	P.value
DAS28[a] score		
Mild	0.122	0.671
Moderate	-0.132	0.432
Severe	0.222	0.98
Age	-0.181	0.07
Education	0.277	0.005
Disease duration	-.0408	0.0001
Drug used	-0.372	0.743
[a]: disease activity score		

susceptible patients, and routine administration of vit D as a cheap and safe supplement should be well-thought-out. Rennie et al. also recommended that vit D supplement intake reduces the severity of RA symptoms.

This study showed no significant relationship between DAS28 score and vit D levels which indicate the disease activity VAS score; in other words, the number of swollen and tender joints, inflammatory blood markers (e.g., ESR and CRP) and patient's assessment of healthiness as well as pain self-assessment were not dependent on vit D metabolites level.

Several experiments have shown that there is no correlation between the level of 25(OH)D and the activity of the disease [21]. Findings suggesting an opposite correlation between vitamin D metabolite levels and disease activity are contentious [22, 23]. In a cross-sectional sample of 206 patients, Patel et al. found that each 10-ng / ml rise in the 25(OH)D level was correlated with a 0.3 decrease in the DAS28 score [24]. Despite lack of general association between vitamin D levels and DAS28, Higgins et al. claimed that, in the presence of vitamin D deficiency, patients could view themselves or be viewed by assessors as having reacted poorly to disease adjustment. He also offered evidence that the VAS factor is inversely linked to vitamin D, measuring the patient's understanding of symptoms, with lower levels delivering higher VAS values [25].

Based on our findings, we detected an inverse relationship between RA disease duration and vit D levels. Chandrashekara et al. reported in a parallel study that patients with vit D deficiency had a prolonged duration of disease, [26] while Haque et al. and Higgins et al. did not find any relationship between the disease duration and vit D metabolites [23,25]. Lower levels of 25(OH)D over time may be related to functional and physical disability in RA patients followed by low exposure to sunlight and, therefore, a decrease in the level of vit D metabolites. Patients with a greater period of sickness could also have had a worse diet or reduced exposure to sunshine.

In this report, we also revealed no significant correlation of vit D metabolites level with inflammatory factors including CRP. Conversely, Kerr, et al. polemically, introduced the anti-CCP antibody positivity and higher CRP concentrations, as the associated factor for the higher risk of vit D deficiency [27]. Every 10-ng / ml improvement in the 25(OH)D level was correlated with a 25 percent reduction in the CRP level, as reported by Patel et al [24] In a similar study among 55 patients with RA, serum 25(OH)D levels were significantly negatively correlated with CRP levels [28]. Furthermore, Chandrashekar et al. found a significant improvement in CRP levels following vit D supplement consumption [26]. Nevertheless, there are controversial results on investigating the relationship between 25(OH)D and some inflammatory serum factors; further evaluation of RA patients considering various stages should be performed.

There are few studies that assess the relationship of plasma 25(OH)D levels with autoantibodies which are related to RA such as anti-CCP and RF in patients diagnosed with RA. We found no association between vit D levels with anti-CCP and RF, regardless of the severity and duration of disease. Feser et al. also indicated the absence of a substantial relationship between vit D metabolites level and the mentioned autoantibodies which are in accordance with our results [29]. Anaparti et al. and Yanan et al. described an inverse relationship between vit D and anti-CCP.[30,31] Controversially, Herly et al. declared a significant positive association between vit D and anti-CCP [32]. A positive association was also reported by Anaparti et al. between RF and vit D levels [30] It seems that vit D deficiency does not affect the higher titer of RA autoantibodies, but further studies with larger samples are required to confirm this result.

Many studies have focused on the association of vit D and demographic variables; however, in our study, no significant correlation was obtained between vit D levels and variables including age, gender, and living location. Haque et al. confirmed the lack of correlation between sex and vit D levels [23], whereas there are several studies which proved significant

Table 3: Paraclinical data of rheumatoid arthritis patients and their correlation with 25(OH)D serum levels.

Laboratory test	Mean	correlation coefficient	P. value
Anti CCP[a] (U/ml)	174	0.008	0.940
VAS[b] (0-100)	64.3	0.110	0.275
DAS28[c]	3.48	0.588	0.383
RF[d] (IU/ml)	11.18	0.139	0.169
CRP[e] (mg/L)	7	0.394	0.246

[a]: anti-cyclic citrullinated peptide; [b]: visual analog scale; [c]: disease activity score; [d]: rheumatoid factor; [e]: C-reactive protein

Table 4: Association of variables based on gender (n=100; male=25; female=75)

Variable	Gender	Mean	Standard deviation	P
25(OH)D[a] (ng/mL)	*Male*	25.27	15.71	0.292
	Female	23.80	21.85	
Anti-CCP[b] (U/ml)	*Male*	208.20	570.11	0.667
	Female	163.42	441.31	
RF[c] (IU/ml)	*Male*	16.56	28.17	0.02
	Female	9.38	6.99	
VAS[d]	*Male*	66.80	30.51	0.903
	Female	63.46	29.88	
DAS28[e]	*Male*	3.39	1.36	0.420
	Female	3.51	1.16	
CRP[f] (mg/L)	*Male*	6.24	5.86	0.686
	Female	7.25	6.56	

[a]: 25-hydroxyvitamin D; [b]: anti-cyclic citrullinated peptide; [c]: rheumatoid factor [d]: visual analog scale; [e]: disease activity score; [f]: C-reactive protein

differences in vitamin D levels concerning gender. The difference in 25(OH)D levels according to gender was attributed to sex-related hormones [33,34]. Other studies also did not report any significant correlation as per age [35,23]. Furthermore, there was no defined relationship between hypovitaminosis D and living location [36]. Further studies with larger sample size are needed to assess the mentioned variable

It has been shown that there is an inverse association between vit D level and educational status. On this account, the higher the educational level, the lower the vit D level. Forrest et al. and Patel et al. also confirmed the negative correlation of the vit D level with educational status, as a marker of low socioeconomic status [37,24]. Lower socioeconomic status can also impact food choices among a minority of the populations and subsequently result in choosing less vit D rich foods such as fish. It is suggested that informing the society can reduce the incidence of vit D deficiency.

There were some limitations in our study. It is possible that patients with longer disease duration had a worse diet or less sunlight exposure, so lower levels of 25(OH)D are reported by them. The second limitation was difficulty to precisely explain the causal relationships due to the fact that the study was cross-sectional.

5. Conclusion
Vitamin D associated metabolites levels do not seem to be associated with the existence of RA-related autoantibodies such as RF and Anti CCP in RA patients. Furthermore, no significant correlation between serum inflammatory markers including CRP and vit D levels was found. Although there are no definitive results determining the exact ratio of 25(OH)D to disease activity or RA-related serum markers, evidence was found about the association of 25(OH)D and disease duration. Considering the levels below normal of 25(OH)D in a large number of cases (73%), 25(OH)D should be checked in those patients.

List of abbreviation
25(OH)D: 25-hydroxyvitamin D; Anti-CCP: anti-cyclic citrullinated peptide; RF: rheumatoid factor; VAS: visual analog scale; DAS28: disease activity score; CRP: C-reactive protein; RA: rheumatoid arthritis

Declarations

Ethics approval and consent to participate
Written informed consent was obtained from the patients in our study. The purpose of this research was completely explained to the patients and were assured that their information will be kept confidential by the researcher. This research was approved by the ethical committee of Kurdistan University of Medical Sciences.

Consent for publication
Written informed consent was obtained from the patients regarding the publication of this study.

Availability of data and materials
SPSS data of the participant can be requested from the authors. Please write to the corresponding author if you are interested in such data.

Competing interests
The authors declare that they have no competing interests.

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