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Regular paper

Vitamin D status in patients with rheumatoid arthritis: a correlation analysis with disease activity and progression, as well as serum IL-6 levels

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Objectives. Recent epidemiological studies suggested an association between a poor vitamin D [25(OH)D] status, inflammatory mediators, and rheumatoid arthritis (RA). We have recently proposed that pro-inflammatory interleukin 6 (IL-6) may represent a good marker for disease activity of RA. The aim of this study was to investigate the relationship between serum 25(OH)D levels and disease activity, joint damage, as well as serum IL-6 levels in a Polish RA population. Materials and Methods. Serum 25(OH)D levels were measured in 35 female RA patients and 38 age- and gender-matched healthy controls. Statistical correlations between 25(OH)D levels and the disease activity score 28 (DAS 28), joint damage based on the Steinbrocker criteria, as well as serum IL-6 levels were performed. Results. There was no statistically significant difference between levels of 25(OH)D in RA (16.89±8.57 ng/ml) and healthy controls (14.12±7.51 ng/ml), and the vitamin D deficiency (<20 ng/ml) was found in 71.43% of RA patients and 73.68 % of healthy controls. While vitamin D status did not correlate with DAS 28 (r=0.265, *p*=0.149) and joint damage based on the Steinbrocker criteria (r=0.367, *p*=0.065), a positive correlation between 25(OH)D and IL-6 (r=0.537, *p*=0.002) was observed in RA. Conclusion. Although further studies on a larger group of patients will be needed to confirm the data presented here, it seems that hypovitaminosis D is common in the RA patients and middle-aged non-RA healthy women in the Polish population. 25(OH)D levels were similar in the RA patients and age- and gender-matched healthy controls, and were not associated with joint damage and disease activity in patients.

Key word: rheumatoid arthritis, vitamin D deficiency, 25(OH)D, IL-6

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Abbreviations: DMARDs, disease-modifying anti-rheumatic drugs; 25(OH)D, non-active 25-hydroxyvitamin D; RA, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA), a systemic autoimmune disease of the connective tissue, is characterized by inflammation of synovial joints that can lead to cartilage destruction and bone erosion (Jeffery *et al.,* 2016). The etiology of the disease remains unclear, however, the interactions between genetic and environmental factors have been demonstrated in RA. The disease affects 0.3–1.0% of the general population and is more prevalent among women and in developed countries (Chaudhari *et al.,* 2016).

Although much progress has been made in revealing key players in pathophysiology of RA, its therapy remains challenging and in most cases still consists of conventional immunosuppressive treatment with corticosteroids and disease-modifying anti-rheumatic drugs (DMARDs) (Singh *et al.,* 2016). The introduction of biological DMARDs, including IL-6 blocking agents, has led to improved management of RA, nevertheless, the economic costs associated with this disease are still high (Yusof & Emery, 2013; Chaudhari *et al.,* 2016).

There is an increasing interest in the role of vitamin D as a potential treatment for a number of inflammatory diseases (Jeffery *et al.,* 2016). Vitamin D is a crucial secosteroid (pro)-hormone with a broad range of biological effects ranging from the classical role as a mediator of calcium and phosphorus metabolism promoting the healthy mineralization, growth and remodeling of the bone, to anti-microbial activity, and modulation of cellular differentiation (Hall & Juckett, 2013). In addition, vitamin D exerts suppressive functions on cells of the adaptive immune response, i.e. those which are directly involved in the RA development (Alluno *et al.,* 2015; Chaudhari *et al*., 2016; Jeffery *et al.,* 2016).

The majority of vitamin D pool in the body (~90%) is endogenous. Upon UV radiation, pro-vitamin D is synthesized in the skin, and subsequently converted to non-active 25-hydroxyvitamin D [25(OH)D] in the liver, and then to the active 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] in the kidney. Because 25(OH)D has a long half-life (2-3 weeks), it is the best serological biomarker for assessing the status of vitamin D in the body (Holick *et al.,* 2011). Interestingly, several epidemiological studies demonstrated an association between a poor 25(OH)D status and RA, however studies contesting these observations are also found (Braun-Muscovici *et al.,* 2011; Craig *et al.,* 2010; Hong *et al.,* 2014; Kostoglou-Athenassiou *et al.,* 2012; Racovan *et al.,* 2012; Rossini *et al.,* 2010; Sahebari *et al.,* 2014; Turhanoflu *et al.,* 2011). Since the evidence from epidemiological studies concerning the relationship between serum 25(OH)D concentrations and RA is inconsistent (Jeffery *et al.,* 2016; Lin *et al.,* 2016), the aim of this study was to verify this assumption by assessing patients from northern Poland.

Table 1. General characteristics of RA patients.

	RA (n=35)
Disease duration (years)	8.92±8.75
Disease activity score (DAS 28)	4.18±1.01
Steinbrocker's criteria (stage)	
I	n=5
II	n=6
III	n=2
IV	n=13
undefined	n=9

MATERIALS AND METHODS

Patients and controls. Thirty-five female patients with RA (mean age 47.54±14.78) fulfilling ACR criteria for the classification of rheumatoid arthritis and 38 female healthy controls (mean age 51.66±6.79) were included in this study. The disease activity was assessed according to the Disease Activity Score including 28 joint counts (DAS 28-ESR=DAS 28), and joint damage was evaluated based on the Steinbrocker radiographic criteria (I-IV). Selected characteristics of the RA patients are presented in Table 1. Serum samples of the patients were collected throughout the whole year and some insignificant fluctuations in the 25(OH)D levels over a specific period of a year have been observed (Supplementary Data 1 at www.actabp.pl). The use of human biological material was approved by the Ethics Committee of the Medical University of Gdańsk, Poland, and written informed consent was obtained according to the Declaration of Helsinki.

Detection of serum 25(OH)D. Serum levels of vitamin D were measured by a 25(OH) vitamin D ELISA kit (Sigma–Aldrich) according to the manufacturer's instructions.

Cytokine measurements. Serum levels of IL-6 were measured by a flow cytometric bead array (Becton Dickinson).

Statistical analysis. All statistical analyses were performed using GraphPad Prism 5 (San Diego, California). The Shapiro-Wilk test was used to verify whether the data had normal distribution. Non-normal distributed data was analyzed by Mann Whitney U test and Spearman's rank correlation test. *P* values less than 0.05 were considered as significant.

RESULTS

Inadequate serum levels of vitamin D are common in the RA patients and healthy controls

In this study, we examined serum levels of the major circulating form of vitamin D – 25(OH)D in the RA patients, as well as age- and gender-matched healthy controls in the Polish population. The mean serum level of 25(OH)D in the RA patients was not significantly different from that of the controls (16.89±8.57 ng/ml vs. 14.12±7.51 ng/ml, respectively; *P*=0.13) (Fig. 1). Vitamin D deficiency (<20 ng/ml) and insufficiency (21–29 ng/ml) were found in 71.43% (n=25) and 17.14% (n=6) of the RA patients and 73.68% (n=28) and 23.68% (n=9) of the healthy controls, respectively.

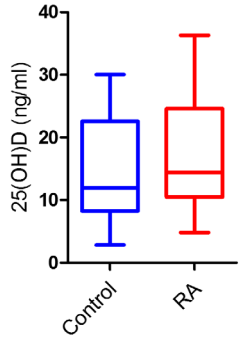

Figure 1. Vitamin D status in patients with rheumatoid arthritis is similar to that of healthy controls.
Serum levels of 25-hydroxyvitamin D [25(OH)D] in rheumatoid arthritis (RA) patients (n=35) and age- and gender-matched healthy controls (n=38), measured by ELISA. The box-plot presents the median value, the inter-quartile range and the Min–Max values.

Vitamin D status does not correlate with disease activity and joint damage in RA

There was no significant relationships between the vitamin D levels and DAS 28 (r=0.265, *p*=0.149) (Fig. 2A), as well as joint damage based on the Steinbrocker criteria (r=0.367, p=0.065) in the RA patients (Fig. 2B). The lack of significant differences in the above-mentioned analysis has been confirmed by using one-way ANOVA test (Supplementary data 1). We found, however, that levels of serum IL-6 positively correlated (r=0.537, *p*=0.002) with the levels of 25(OH)D

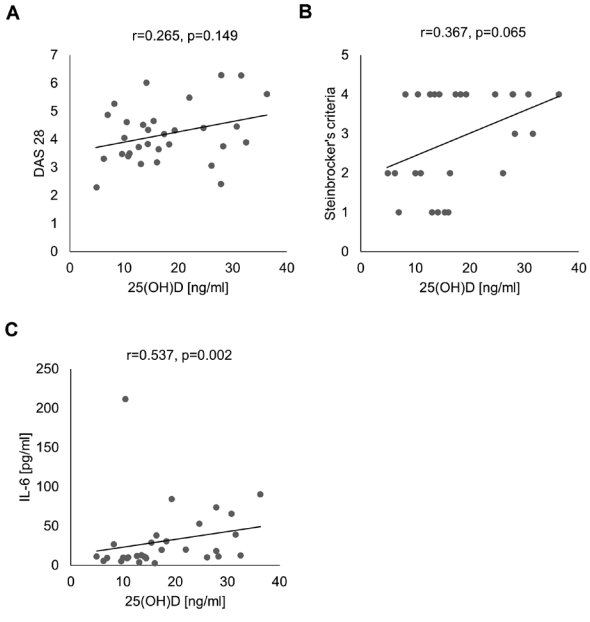

Figure 2. Vitamin D status is not associated with disease activity and joint damage in RA patients.
Analysis of a relationship between levels of 25-hydroxyvitamin D [25(OH)D] and disease activity score 28 (DAS 28) (**A**), joint damage based on the Steinbrocker radiographic criteria (**B**), as well as serum IL-6 levels (**C**) in the RA patients.

in the RA patients (Fig. 2C). There was no such correlation in a control group (r=–0.044, p=0.792).

DISCUSSION

Previous studies have demonstrated that vitamin D deficiency can impair immune function, resulting in an increased prevalence of autoimmune diseases such as multiple sclerosis, type 1 diabetes mellitus, and systemic lupus erythematosus (Yang *et al.,* 2013). Vitamin D deficiency has been also implicated in the pathogenesis of RA, a systemic autoimmune disease of the connective tissue characterized by inflammation of synovial joints (Jeffery *et al.,* 2016). Our results suggest that the vitamin D status is generally not altered in patients with RA in northern Poland when compared with age- and gender-matched healthy individuals (16.89±8.57 ng/ml *vs.* 14.12±7.51 ng/ml, respectively). Our findings are consistent with regard to the level of vitamin D in other cohorts of patients with RA. For instance, Kostoglou-Athanassiou and coworkers (2012) and Hong and coworkers (2014) found that the mean serum levels of 25(OH)D in the RA patients were 15.26 ng/ml and 17.25 ng/ml, respectively. Both authors, however, observed that the mean levels of vitamin D were significantly lower in the RA groups than in respective controls (Hong *et al.* 2014; Kostoglou-Athanassiou *et al.* 2012). This discrepancy might be due to the difference in the level of vitamin D in populations of healthy people inhabiting different latitudes and the fact that in Poland vitamin D deficiency is observed in most of the healthy people (Kmieć *et al.,* 2014, 2015; Pludowski *et al.,* 2016). Recent epidemiological study based on 5775 adult volunteers in the general Polish population revealed that the mean 25(OH)D concentration in the studied population was 18.0±9.6 ng/ml (Pludowski *et al.,* 2016). Another study based on 448 healthy volunteers from northern Poland showed that the mean level of vitamin D was 14.3±6.6 ng/ml (Kmieć *et al.,* 2014), and was significantly dependent on seasonal sun exposure in a follow-up study (Kmieć *et al.,* 2015). It has been generally accepted that 25(OH)D serum concentration below 20 ng/ml should be defined as vitamin D deficiency (Holick *et al.,* 2011). Here, vitamin D deficiency (<20 ng/ml) was found in the majority of both, the RA patients (71.43%) and healthy controls (73.68%). Our results are consistent with the findings of the previous study since vitamin D deficiency was found in 76.3% of RA patients from central part of Poland and in 84.4% of healthy adults from northern Poland (Kmieć *et al.,* 2014; Raczkiewicz *et al.,* 2015). Several studies have found inverse association between inadequate vitamin D status and disease activity in RA (Cutolo *et al.,* 2006; Haque *et al.,* 2010; Hong *et al.,* 2014; Kerr *et al.,* 2011; Kostoglou-Athenassiou *et al.,* 2012; Raczkiewicz *et al.,* 2015; Turhanoflu *et al.,* 2011; Welsh *et al.,* 2011). There are also reports indicating negative correlations between vitamin D levels and pro-inflammatory cytokines, including TNF-α, IL-1β, IL-17, as well as IL-6 in RA (Mateen *et al.,* 2017). By contrast, no correlations between vitamin D deficiency and disease activity in RA have been reported by other authors (Baker *et al.,* 2012; Braun-Muscovici *et al.,* 2011; Craig *et al.,* 2010; Racovan *et al.,* 2012; Rossini *et al.,* 2010; Sahebari *et al.,* 2014). In this study, no significant relationships were found between vitamin D levels and either disease activity and joint damage based on the Steinbrocker radiographic criteria in the RA patients. We have recently found that in the RA patients, the serum levels of pro-inflammatory IL-6 were significantly elevated (about 7-fold compared to healthy control) and positively correlated with disease activity and joint damage based on the Steinbrocker criteria (Tukaj *et al.,* 2010). In the study presented here, a positive correlation between the vitamin D status and IL-6 levels has been observed in the RA patients. It is unclear why the results of our study differ from those obtained by Mateen and coworkers (2017). In addition to the different cohorts living in distinct geographical latitudes, the heterogeneity in disease activity and therapeutic status may have contributed to these discrepant outcomes. This unexpected observation indicates that the mechanisms of the role of vitamin D in expression of IL-6 are not completely elucidated. Naghavi and coworkers (2015), for instance, had found that vitamin D supplementation of patients with multiple sclerosis led to a significant up-regulation of IL-6 in peripheral blood mononuclear cells (PBMCs). We hypothesize that higher levels of pro-inflammatory IL-6 in patients with RA may stimulate the synthesis of 25(OH)D as a counterbalancing mechanism for inflammatory niche and the non-exclusive explanation for this assumption might be supported by the fact that UVB radiation, being the key factor for cholecalciferol (vitamin D_3) synthesis in the skin, may also trigger cutaneous inflammatory response mediated by induction of IL-6 expression in an IL-1α-depended way (Chung *et al.,* 1996). The presence of such correlation, as well as lack of correlations between vitamin D and disease progression or activity, do not simply reflect the hypothesis of the contribution of hypovitaminosis D in RA. While the link between IL-6 and RA is quite well established (Yusof & Emery, 2013), the impact of vitamin D deficiency on disease activity, joint damage, and soluble inflammatory mediators remains in need of further elucidation. The essential question arises whether poor 25(OH) D status is a cause or consequence of the RA development. Further, it is still unclear whether the association of vitamin D deficiency with RA severity, observed in some studies, supports the hypothesis of a role for vitamin D in the initiation or rather progression of the disease. To address this question, further analyses in prospective vitamin D supplementation trials are required. Future studies on a larger and better characterized group of patients (e.g. information on vitamin D supplementation, sun exposition or treatment) are needed to confirm the data presented here and to further clarify whether RA progression and/or activity is related to the vitamin D status.

CONCLUSIONS

Our results suggest that the vitamin D status is generally not altered in Polish patients with RA when compared with age- and gender-matched healthy controls. The levels of 25(OH)D examined in both, the RA and non-RA adults showed that a majority of the Polish population are vitamin D deficient. The lack of associations between the vitamin D levels and disease activity or progression, as well as presence of a positive correlation between 25(OH)D and IL-6 levels in patients suggest the need to verify the hypothesis of vitamin D involvement in RA.

Conflict of interest

The authors state no conflict of interest.

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