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SERUM VITAMIN-D INVERSELY ASSOCIATES WITH THE DURATION OF DISEASE IN MALE PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Rheumatoid arthritis (RA), primarily a chronic inflammatory debilitating autoimmune disease of the synovial joint linings has prevalence of about 0.5% in the population world over. Role of serum vitamin (vit-D) (25-hydroxyvitamin D (25(OH)D) has been found quite potential and promising for preventing certain manifestations in general population. Serum vit-D (ng/ml) was measured by ELISA technique for assessing its association with age (years) and duration of disease (DD) in male patients (n: 71, age 55-59 years, DD: 1-15 years) with RA (subgroups: RA-DD 1-5 years, RA-DD 6-10 years, RA-DD 11-15 years. Healthy male subjects served as controls (n: 40, age: 55-59) for comparing the vitamin D levels in male patients with RA. Highly significant decrease in vit-D was found in the RA patients with DD of 6-10 and 11-15 years ($P<0.0001$). In general, the RA patients with DD of 1-15 showed a highly significant decrease in vit-D levels. The DD 1-5 vs. DD 6-10 and DD 1-5 vs. DD 11-15 gave significant variations. Age against vit-D gave highly significant correlation for control subjects (R^2: 0.554, $P <0.0001$). Patients with RA-DD 1-15 years showed highly significant correlation (R^2: 0.408, $P <0.0001$) for the DD and vit-D. Subgroups of RA did not show significant correlations due to lowered levels of vit-D except RA-DD 11-15 that showed significant correlation (R^2: 0.223, P:0.015). Conclusively, the interventional approaches to supplement vit-D for improving the serum levels of vit-D in patients with RA of better controlled larger sample size seem potentially beneficial for future studies.

Key words: Rheumatoid arthritis, vitamin D, vitamin D deficiency/insufficiency, age of patients, duration of disease

INTRODUCTION

Deficiency and even insufficiency of vitamin D (vit-D) might be linked to rheumatoid arthritis (RA) and other autoimmune disorders (Marques *et al*., 2010). The vit-D is involved in regulating the innate and adaptive immune system and also boosting immune system that may lead to cause decrease in its levels in autoimmune disorders (Aranow, 2011). This occurs due to the expression of vit-D receptors by the immune cells.

Rheumatoid arthritis (RA), primarily a chronic inflammatory debilitating autoimmune disease of the synovial joint linings (Holick, 2006; Kramer *et al*., 2023) has prevalence of about 0.5% in the population world over (Silman and Pearson, 2002). It affects the joints, and its progression influences the multisystem processes. There are several approaches for the pathogenesis and management of RA. It was revealed that bone, cartilage and tendons are damaged in result of disordered activation of osteoclasts, B and T-cells, chondrocytes, fibroblasts, dendritic cells and the proteolytic enzymes, and it is considered as the pathogenesis of RA (Anić and Mayer, 2014; Kramer *et al*., 2023). However, role of serum vitamin vit-D (25-hydroxyvitamin D (25(OH)D) has been found quite potential and promising for preventing certain manifestations in general population (Wicherts *et al*., 2007; Dzik and Kaczor, 2019; Wang *et al*., 2019). One report shows that 70% of RA patients had vit-D insufficiency (Furuya *et al*., 2013).

Several studies document and reviews explain the significant role of vit-D in RA (Yong *et al*., 2020; Zhang *et al*., 2020; Charoenngam, 2021; Cutolo *et al*., 2021; Hu *et al*., 2021; Punceviciene *et al*., 2021; Vyas *et al*., 2021). Low serum levels of vit-D are involved in the pathogenesis of RA that provide evidence of the impact vit-D on clinical manifestations in RA (Cutolo *et al*., 2021). RA patients are more prone and show bone loss earlier than controls (Hu *et al*., 2021). A link between vit-D levels and RA was, hence, revealed (Punceviciene *et al*., 2021).

Association of vit-D (25(OH)D) variations with the disease activity in RA patients predicted significant value in clinical applications (Yong *et al*., 2020). It was found that Low serum levels of vitamin D3 negatively associated with increased serum levels of IL-9 (interleukin-9) and TLR2 expression and disease severity in patients with RA (Vyas *et al*., 2021). Decreased serum levels of vit-D and increased serum

levels of IL-17 were obtained as risk factors for RA, though the mechanisms involved require further investigations (Zhang *et al*., 2020). However, one study investigated no obvious association existing between serum 25OHD and RA disease activity or progression (Harrison *et al*., 2020).

Quite little work has been carried out in Saudi Arabia for elucidating the association of serum vit-D and RA, and specially the association of vit-D with the duration of RA. The present study was hence, planned to be conducted to have insights about the influence of the duration of disease (DD) on serum levels of vitamin D in male patients with RA.

METHODS AND MATERIALS

A total of 40 healthy male control subjects (age: 55-59 years) were included for comparing the data of RA male patients. All RA patients (n: 71, age 55-59 years) studied in the present report were in the range of 1-15 years of duration of disease (DD). The patients were subdivided on the basis of the DD. Three subgroups made were: RA-DD 1-5 years (n: 22), RA-DD 6-10 years (n:23), and RA-DD 11-15 years (n: 26).

The subjects (control or RA) not interested in participating in the present study were not included. Only those subjects were included who showed their personal willingness. History of the patients was taken, and proper diagnosis and differential diagnosis were performed to obtain the patients with moderate RA.

Diagnosis was carried out considering the clinical response determined by following the instructions of the European League Against Rheumatism response criteria, and disease- activity score-28 (DAS28) erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) (DAS28-ESR and DAS28-CRP). Serum vitamin-D (25-hydroxyvitamin D (25(OH)D) (vit-D, ng/ml) was determined by ELISA technique. The ESR and CRP levels were determined by kit methods.

The data of the present work was analysed statistically using mean ± SD. Unpaired t-test was applied for analyzing two tailed P values. The $P \leq 0{,}05$ was considered as the significant level. The values of coefficient of determination (R^2) and P were obtained for various associations. Linear regression and correlations were obtained for the associations of age and DD with the levels of vit-D. The data was recorded properly and analyzed using SPSS software version 23.

RESULTS

Serum vitamin D levels (ng/mL) in healthy male subjects were used as controls for comparing the vitamin D levels in male patients with RA (Table 1). Age (years) in both control and RA subjects was quite similar and did not differ significantly. Highly significant decrease in vitamin D levels was found in the RA patients with the DD as 6-10 years and 11-15 years ($P < 0.0001$) (Table 1). In general, the RA patients with DD of 1-15 showed a highly significant decrease in vitamin-D levels. The DD 1-5 vs. DD 6-10 and DD 1-5 vs. DD 11-15 gave significant variations.

Table 1. Age and vitamin D levels in healthy male control subjects and patients with RA.

Variables	Control (N: 40)	RA (n: 71)			
		DD (1-5) N: 22)	DD (6-10) (N: 23)	DD (11-15) (N: 26)	DD (1-15) (N: 71)
Age (years)	54.53 ± 2.97	54.32 ± 2.90	54.87 ± 3.03	54.96 ± 3.09	54.73 ± 2.99
Vit-D (ng/mL)	31.88 ± 8.73	30.68 ± 13.32	15.98 ± 7.11 *, $	13.41 ± 5.17 *, $	19.59 ± 11.66 *

*: $P < 0.0001$ (C vs. RA), $: $P<0.0001$ (DD 1-5 vs. DD 6-10 and DD 1-5 vs. DD 11-15)

Tukey-Kramer test verified the results for two-tailed P values obtained by two sample unpaired t-test. One way ANOVA showed highly significant variations among the groups (F: 33.09; $P< 0.0001$).

The age against vitamin D regressions gave highly significant correlation for control subjects (R^2: 0.554, $P <0.0001$, Fig.1).

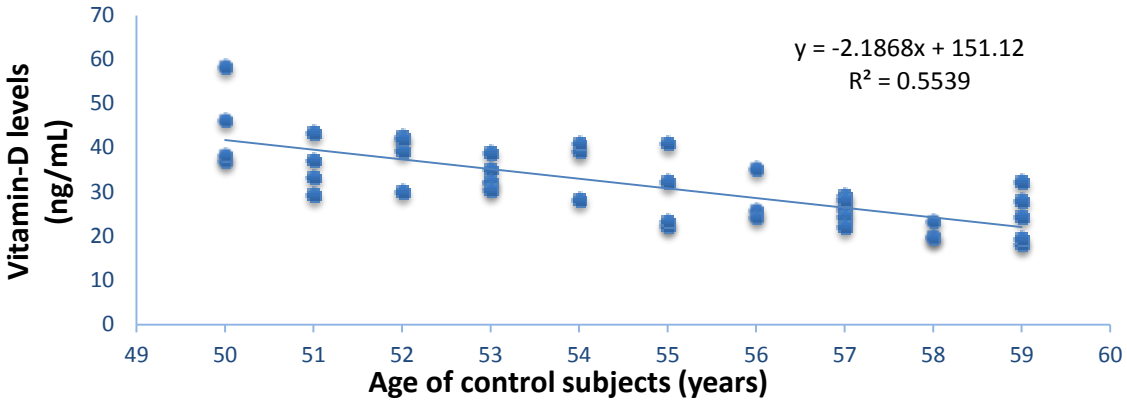

Fig. 1. Correlation of age and vitamin D levels in healthy controls.

However, RA DD 1-15 and all subgroups (RA DD 1-5, RA DD 6-10, RA DD 11-15) showed highly decreased levels of vitamin D (Fig.2) compared to that as shown in Fig 1 for normal control subjects, and hence no significant correlation of vit-D with the age of patients.

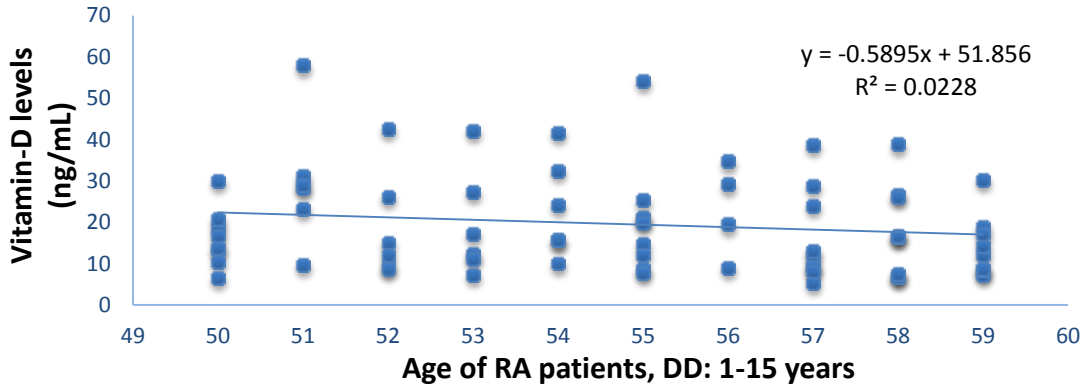

Fig 2. Correlation of age and vitamin D levels in patients with RA.

The regression lines drawn for assessing the correlation of duration of disease with vitamin D levels in patients with RA DD 1-15 years showed highly significant correlation (R^2: 0.408, $P < 0.0001$, Fig.3).

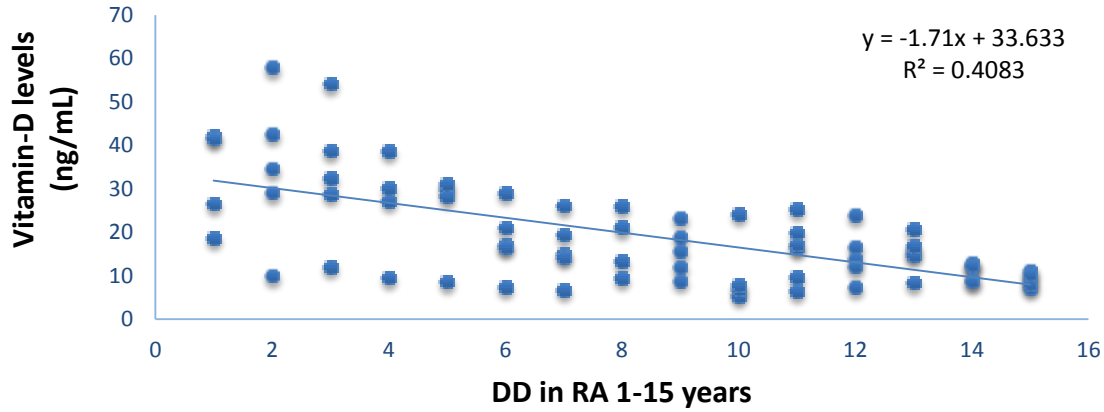

Fig. 3. Correlation of the duration (DD) of disease and vitamin D levels in patients with RA.

The subgroup of RA did not show significant correlations due to lowered levels of vitamin-D except RA DD 11-15 that showed significant correlation (R2: 0.223, p : 0.01).

DISCUSSION

Present study investigates deficiency of vit-D in association with the duration in male subjects with RA of well-controlled age (age range: 55-59 years) group. Vit-D was found to be an independent risk factor for patients with RA, that is quite similar to other reports (Tofanello *et al*., 2012; Vaes *et al*., 2019).

These results can be interpreted on basis of several reports indicating the involvement of inflammatory processes (Bischof-Ferrari *et al*., 2004; Jain *et al*., 2018; Dzik and Kaczor, 2019) as vit-D itself has been attributed to its anti-inflammatory and immunomodulatory properties (Boonstra *et al*., 2001; Gregori *et al*., 2002; McInnes and Schett, 2007; Tang *et al*., 2009; Villaggio *et al*., 2012; Charoenngam and Holick, 2020; Mouterde *et al*., 2020). The role of serum vit-D in the pathogenesis of RA is evident in the present study similar to a previous report (Jeffery *et al*., 2016).

Low levels of serum vit-D were revealed as associated with RA (Wicherts *et al*., 2007; Atwa *et al*., 2013; Wang *et al*., 2019). Serum levels of vit-D were found to decrease in patients with RA with an average duration of about 9.4 ± 6.2 years, though the vitamin D receptor (VDR) gene polymorphisms were not found linked to RA (Senosi *et al*., 2022). Hence, the present study provides evidence that low levels (insufficiency) and deficiency of vit-D seem necessary while considering the therapeutic target for better management.

Several studies provide evidence of the significant role of vit-D in RA (Yong *et al*., 2020; Zhang *et al*., 2020; Charoenngam, 2021; Cutolo *et al*., 2021; Hu *et al*., 2021; Punceviciene *et al*., 2021; Vyas *et al*., 2021), as we investigated, though no clear association existing between serum 25OHD and RA disease activity or progression was also investigated (Harrison *et al*., 2020).

It was suggested that the progression or duration of disease causes Vit-D deficiency in RA, and hence, larger populations with various disease activity duration are required to be studied for better understanding of RA (Sedrakyan *et al*., 2020).

Though the present study is a cross-sectional as well as case-controlled and this is the merit of the present study, it still has limitations. Sample size was limited, and it comprised only male patients with age range of 55-59 years. Furthermore, this study did not have the vit-D supplementation as a confounding factor. Some important factors that could not be included in the present study were the supplementation of diet, exercise, socioeconomic status, seasonal variations, and other interventional approaches, to correlate and provide further evidence of the involvement of vit-D in RA. Conclusively, interventional approaches to supplement vit-D for improving the serum levels of 25(OH) D in patients with RA of better controlled larger sample size seem potentially beneficial for future studies.

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