

Evaluation Of Vitamin D Level In Serum Blood Of Rheumatoid Arthritis Patients In Babylon Province

Mustafa Jawad Abed Al Imari*¹; Laith A.I.K. Al-Kaif¹; Fuad Ghazi Hassan¹; Saif Anwar Jaafar¹; Hayder A. A. Al-Asady¹; Marwa fadhil Alsaffar¹; Wathiq Essam Hameed²; and Ziyad Trik²

¹ Department of Medical Laboratory Technique, Al-Mustaqbal University College, Babylon, Hilla, Iraq

² M.B.Ch.B. Imam Sadiq Hospital, MOH, Babylon, Hilla, Iraq

Corresponding Author: mustafaalammary85@gmail.com

ABSTRACT

Recent studies proved vitamin D deficiency or insufficiency had been involved in the emerging of many autoimmune diseases like rheumatoid arthritis. The study aim was to evaluate vitamin D₃ serum level in RA Babylon province patients and the relationship with BMI and progressive of this disease. A case-control study was conducted in a private OZONE medical center of rheumatology and physical treatment, Babylon province, Iraq. A total of 30 serum samples of Rheumatoid arthritis (RA) cases were enrolled and compared with 30 serum samples of apparently healthy control. The serum level of vitamin D was detected for all study samples by VIDAS® system from bio Mérieux, French. A total of 30 include 11 males and 19(63.4%) females divided into three groups of deficiency sufficiency and insufficiency. The age (years) mean \pm SD were 47.7 \pm 12.06, 47.03 \pm 12.39 for RA patients and control respectively. The BMI (kg/m²) mean \pm SD was 37.08 \pm 4.6714, 33.506 \pm 4.6059 for RA and control respectively, and the obesity was significant in RA patients (P=0.004). The number frequency of the two study groups was appeared significant (P< 0.0001, P=0.0003) in both categories; deficiency and normal vitamin D respectively, while insufficient vitamin D did not reveal significant differences between both RA and control. The overall serum level means \pm SD was 17.63 \pm 10.61, 33.3 \pm 12.73 for RA patient (n=30) and healthy control (n=30) respectively, and appeared high significance (P<0.0001). The serum level of vitamin D was noticed significantly decreased in RA patients compared to control. The study concluded in presence of a positive relationship between BMI and RA onset, and a negative relationship between vitamin D and developing RA is almost obese females over forty years and consider one of the predisposing factors for developing RA disease.

Keywords: Vitamin D, autoimmune disease, rheumatoid arthritis RA, BMI.

Correspondence:

Mustafa Jawad Abed Al Imari

Department of Medical Laboratory Technique, Al-Mustaqbal University College, Babylon, Hilla, Iraq

*Corresponding author: Mustafa Jawad Abed Al Imari email-address: mustafaalammary85@gmail.com

INTRODUCTION

Vitamin D₃ is found in several types of foods, and most of it is made from dehydrocholesterol-7 in the dermis of the skin when exposed to ultraviolet (UVB) sunlight, then the enzyme 25-hydroxylase (CYP2R1) located in the liver convert vitamin D to 25-hydroxycholecalciferol (25-OHD₃). Vitamin D has a role in bone mineralization and calcium hemostasis by mineralization of the collagen matrix and many effects outside the skeleton¹. Autoimmune diseases arise from the abnormal activation of the immune system by losing immune tolerance and producing autoantibodies such as anti-glutamic acid decarboxylase autoantibody in diabetes mellitus type 1^{2,3}. Vitamin D plays in regulating several immune activities, such as anti-oxidant, anti-inflammatory, and anti-fibrosis, many studies in the last decade have increased interest in finding a relationship between the level of vitamin D and the emergence of autoimmune diseases. Studies have found an inverse relationship between vitamin D levels and the extent of development of these diseases in several countries around the world, such as lupus erythematosus, multiple sclerosis, ulcerative colitis, common psoriasis, and RA^{4,5}. The RA is considering an autoimmune disease with an age-related incidence that affects 1% of adults and the prevalence increasing with age to reach 2% of the population characterized by a chronic systemic disease involving joints and other vital organ morbidity and mortality significantly reported^{6,7}.

Vitamin D or 25 (OH) D may have an immunological role in the development or emergence of RA from its effect on the gene expression process of pro-inflammatory cytokines^{8,9}.

Although several studies confirm the role of vitamin D as a factor in the emergence of autoimmune diseases, additional data is needed to support these findings², so the present study aimed to partially fill the gap of data related to vitamin D abnormality (either deficiency or insufficiency) in RA individuals in Babylon province.

MATERIAL AND METHODS STUDY DESIGN AND POPULATION

All standard cases had been studied in the center in a private OZONE medical center of rheumatology and physical treatment, Babylon province, Iraq. The ethical approval of all patients was obtained to use their demographic and clinical properties data in the present study. According to inclusion/exclusion criteria of this study only 30 RA patients (19 females, 11 males) matched demographically with 30 healthy individuals, all of them were subjected to the study between December 2018 and March 2019. The venous blood samples were obtained for all participants in the present study and centrifuged serum was collected and stored at -20°C for the immunological detection of vitamin D (25OHD) analyses.

IMMUNOLOGICAL DETECTION OF VITAMIN D

The serum samples of all study groups were subjected to an automated quantitative test kit (60 test) for determination of 25-hydroxyvitamin D Total from VIDAS® (bio Mérieux, French) depending on the principle of ELFA (Enzyme-Linked Fluorescent Assay) technique¹⁰. All study groups, were categorized according to age into three subgroups 16-35, 36-55, 56-75; and categorized according to vitamin D

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serum level; deficiency (<20 ng/mL), insufficiency (20–30 ng/mL), and normal (>30 ng/mL) ¹¹.

STATISTICAL ANALYSIS

The statistical analyses had been carrying out by using SPSS Software for Windows version 16.0 (SPSS Inc., Chicago USA, IL, USA). The associations between RA patients and control according to demographic data and four categories of vitamin D serum levels were calculated and the differences were considered significant when p-value <0.5.

THE RESULTS

Table (1): The demographic characteristics RA patients and control

Item	RA patients n=30	Control n=30	Chi-square	P-value
Age, years, mean ±SD (range)	47.7±12.06 (52)	47.03±12.39 (44)	–	0.83
Sex, Male (%) / female (%)	11(36.6)/19(63.4)	12(40)/18(60)	0.0686	0.7934
BMI, kg/m ² mean ±SD	37.08±4.6714	33.506±4.6059	–	0.004

VITAMIN D STATUS IN THE STUDY POPULATION

The serum levels of vitamin D were evaluated for RA and control, the level of vitamin D means ±SD for the age group (16-35) was 9.25±1.89, 22.83±9.78 for RA patients and control group respectively, and show significant differences (P=0.014) between both study groups (table.2). While for the age group (36-55) The serum level of vitamin D₃ mean ±SD was 19.77±11.98, 35.5±13.67 for RA patients and control group respectively, it revealed highly significance (P=0.0016) between both study groups. In the age group (56-75) The serum level of vitamin D mean ±SD was 17±8, 36.5±10.34 for RA patients and control group respectively, there was high significance (P<0.001) between both study

CHARACTERISTICS OF THE STUDY POPULATION

The demographic properties (age, sex, and BMI) of the study population (RA patients and control) are shown in table (1). The age (years) mean ±SD were 47.7 ±12.06, 47.03 ±12.39 for RA patients and control respectively. The sex of most RA patients was female 19(63.4%). In this study, age and sex had been matched between two study groups, and no significant differences (P=0.83). The BMI (kg/m²) mean ±SD was 37.08 ±4.6714, 33.506 ±4.6059 for RA and control respectively. The RA patients were highly obese and different significantly from the control group (P=0.004).

groups. The present study does not reveal any significances between RA patients and control according to mean ±SD of serum level vitamin D in three categories deficiency, insufficiency, and normal level as reported in (table.2). The number frequency of the two study groups was appeared significant (P< 0.0001, P=0.0003) in both categories; deficiency and normal vitamin D₃ respectively, while insufficient vitamin D did not reveal significant differences between both RA and control. The overall serum level means ±SD was 17.63± 10.61, 33.3±12.73 for RA patient (n=30) and healthy control (n=30) respectively, and appeared high significance (P<0.0001).

Table (2): Evaluation of vitamin D in RA and control according to three categories of serum level value; Deficiency, Insufficient and Normal

Age group (year)	Study group	Deficiency of Vitamin D ng/mL	Insufficient of Vitamin D ng/mL	Normal of Vitamin D ng/MI	P-value
16-35 N(mean±SD)	RA	4(9.25±1.89)	--	--	0.014
	Control	2(11.5±4.94)	2(25±4.24)	2(32±1.41)	
	P-value	0.43	--	--	
36-55 N(mean±SD)	RA	10(11.1±3.24)	4(23.75±2.36)	4(37.5±9.25)	0.0016
	Control	2(15.5±4.94)	2(27.5±2.12)	10(41.1±11.35)	
	P-value	0.13	0.133	0.585	
56-75 N(mean±SD)	RA	6(12.83±2.48)	--	--	<0.001
	Control	--	3(26±3)	7(41±8.86)	
	P-value	--	--	--	
Total N=60(%)	RA N=30(%)	20(66.6)	5(16.6)	5(16.6)	--
	Control N=30(%)	4(13.3)	7(23.3)	19(63.3)	
P-value*		< 0.0001	0.5196	0.0003	--

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DISCUSSION

The current study showed that patients with rheumatoid arthritis had been lower vitamin D serum values than healthy controls, and indicated a negative correlation between vitamin D serum values and activity of RA disease, this evidence supported by many meta-analyses studies worldwide⁷. The anti-inflammatory action for the vitamin D in synovial fluid and reverse serum level with C- reactive proteins may explain the importance of this vitamin in the progress of RA disease in those that suffering deficiency in vitamin D¹². The demographic characteristic; age, sex, and BMI RA and control matched between two study groups according to inclusion to minimize the differences (no significance) between RA patients and healthy control and reveal accepted results. It was found a positive relationship between age and onset of RA disease which improved with previous studies⁶. It was reported in (table.1) the mean age of 47.7 of all RA participants and the onset of disease activity for most RA patients were over 40 years. The change related to disease activity registered in Twenty-eight joints DAS28 over age may return to different activation of markers, CD25, CD69, CD95, and HLA-DR, on the CD4 T cell surface^{13,14,15}. The sex ratio (2:1) between female and male explain the activity of RA in female (63.3%) more than male (36.6) as shown in table (table.1), this fact variable between many studies but most of them insure the high prevalence of female other than male and some studies include only female sex, this difference may return to activity of sex hormones such as estrogen, progesterone, and androgens which influencing the preclinical phases and onset of RA in the female by affecting on induction or subversion of many immune cells^{16,17}. The BMI of RA patients in the current study (table.1) A positive association has been demonstrated between obesity and most rheumatoid arthritis cases as many studies mentioned and explained this state which affects not only RA disease onset, also effect on the activity of biological therapy (antitumor necrosis factor alfa Anti-TNF) of RA patients¹⁸. The previous worldwide studies by^{19,20,21}, in addition to recent local studies in Iraq by²² reported the association between BMI and female sex in decreasing vitamin D value in the onset of many autoimmune diseases which support findings of the present study. The current study found out significant differences between patients who are suffering from RA and healthy control number frequency according to deficiency of vitamin D serum level (table.2), also, low serum level of RA significantly suggests the potential role for the vitamin D in the maintenance of immune homeostasis according to the expression of vitamin D receptors on immune cells²³. Also, the Utilization of vitamin D supplements appeared in the improvement of rheumatic therapy^{24,25,26,27}. Although several studies confirm the present lack of vitamin D in certain patients who suffered from rheumatoid arthritis, this deficiency may vary according to seasons²⁸.

CONCLUSIONS

The present study concluded that lack of vitamin D is one of the predisposing factors related to increasing RA incidence especially in female forty-year age and obesity BMI and many studies required to understand vitamin D mechanisms on genetically, demographically, and environmentally onset of RA.

ACKNOWLEDGMENTS

We thank OZONE center of rheumatology and physical treatment for their collaboration and the use of study data especially the physiotherapist; Fatin.

REFERENCES

1. Harrison, S. R., et al. (2019). Vitamin D, autoimmune disease, and rheumatoid arthritis. *Calcified tissue international*, 1-18.
2. Rosen, Y., et al. (2016). Vitamin D, and autoimmunity. *Scandinavian journal of rheumatology*, 45(6): 439-447.
3. Hassan, F. G., & Al-Imari, M. J. A. (2019). Anti-Glutamic Acid Decarboxylase Autoantibody as Biomarker for Diagnosis of Type 1DM Patients in Iraq. *Indian Journal of Public Health Research & Development*, 10(2): 778-783.
4. Agmon-Levin, N., et al. Vitamin D in systemic and organ-specific autoimmune diseases. *Clinical reviews in allergy & immunology*. 2013; 45(2): 256-266.
5. Murdaca, G., et al. (2019). The emerging role of vitamin D in autoimmune diseases: an update on evidence and therapeutic implications. *Autoimmunity reviews*, 102350.
6. Inala, L., et al. (2014). Age at onset determines severity and choice of treatment in early rheumatoid arthritis: a prospective study. *Arthritis research & therapy*, 16(2): R94.
7. Lin, J., et al. (2016). Serum vitamin D level and rheumatoid arthritis disease activity: review and meta-analysis. *PloS one*, 11(1): e0146351.
8. Jarrah, M. I., et al. (2018). The association between the serum level of vitamin D and ischemic heart disease: a study from Jordan. *Vascular health and risk management*, 14: 119.
9. Gopal, K., et al. (2019). Effects of vitamin D on disease activity and serum interleukin-6 in rheumatoid arthritis. *International journal of rheumatic diseases*, 22(5): 834-841.
10. Moreau, E., et al. (2016). Performance characteristics of the VIDAS® 25-OH Vitamin D Total assay—comparison with four immunoassays and two liquid chromatography-tandem mass spectrometry methods in a multicentric study. *Clinical Chemistry and Laboratory Medicine (CCLM)*, 54(1): 45-53.
11. Thacher, T. D., & Clarke, B. L. (2011). Vitamin D insufficiency. In *Mayo Clinic Proceedings*, Elsevier, 86(1): 50-60
12. Li, D., et al. (2019). Serum and synovial fluid vitamin D metabolites and rheumatoid arthritis. *The Journal of steroid biochemistry and molecular biology*, 187: 1-8.
13. Matsui, T., et al. (2007). Disease Activity Score 28 (DAS28) using C-reactive protein underestimates disease activity and overestimates EULAR response criteria compared with DAS28 using erythrocyte sedimentation rate in a large observational cohort of rheumatoid arthritis patients in Japan. *Annals of the rheumatic diseases*, 66(9): 1221-1226.
14. Wells, G., et al. (2009). Validation of the 28-joint Disease Activity Score (DAS28) and European League Against Rheumatism response criteria based on C-reactive protein against disease progression in patients with rheumatoid arthritis, and comparison with the DAS28 based on erythrocyte sedimentation rate. *Annals of the rheumatic diseases*, 68(6): 954-960.
15. Pawłowska, J., et al. (2011). Older age of rheumatoid arthritis onset is associated with the higher activation status of peripheral blood CD4+ T cells and disease activity. *Clinical & Experimental Immunology*, 163(2): 157-164.
16. Alpizar-Rodríguez, D., et al. (2017). The role of female hormonal factors in the development of rheumatoid arthritis. *Rheumatology*, 56(8): 1254-1263.
17. Favalli, E. G., et al. (2019). Sex and management of rheumatoid arthritis. *Clinical reviews in allergy & immunology*, 56(3): 333-345.

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18. Yllan, A. V., et al. (2017). SAT0145 Influence of body mass index (BMI) on the disease inflammatory activity and treatment response in patients with rheumatoid arthritis.
19. Dressler, N., et al. (2016). BMI and season are associated with vitamin D deficiency in women with impaired fertility: a two-center analysis. *Archives of gynecology and obstetrics*, 293(4): 907-914.
20. Gianfrancesco, M. A., et al. (2017). Evidence for a causal relationship between low vitamin D, high BMI, and pediatric-onset MS. *Neurology*, 88(17): 1623-1629.
21. Pallav, K., et al. Predictors of vitamin D deficiency in inflammatory bowel disease and health: A Mississippi perspective. *World journal of gastroenterology*. 2017; 23(4): 638.
22. Zarzoor, A. M., Hamza, W. S., & Al-aqbi, Z. T. (2020). Evaluation of the Correlation between Vitamin D3 Serum Level, Age, gender, and BMI in Rheumatoid Arthritis Patients in Al-Kut City/Iraq. *Indian Journal of Forensic Medicine & Toxicology*, 14(1): 564-568.
23. Wen, H., & Baker, J. F. (2011). Vitamin D, immunoregulation, and rheumatoid arthritis. *JCR: Journal of Clinical Rheumatology*, 17(2): 102-107.
24. Baker, J. F., et al. (2012). Associations between vitamin D, disease activity, and clinical response to therapy in rheumatoid arthritis. *Clinical and Experimental Rheumatology-Incl Supplements*, 30(5): 658.
25. Mukherjee, D., et al. (2019). Effect of 1, 25 dihydroxy vitamin D3 supplementation on pain relief in early rheumatoid arthritis. *Journal of family medicine and primary care*, 8(2): 517.
26. Mouterde, G., et al., (2019). Association between Vitamin D deficiency and disease activity, disability, and radiographic progression in early rheumatoid arthritis. The ESPOIR cohort. *The Journal of Rheumatology*.
27. Heidari, B., Hajian-Tilaki, K., & Babaei, M. (2019). Vitamin D deficiency and rheumatoid arthritis: epidemiological, immunological, clinical, and therapeutic aspects. *Mediterranean Journal of Rheumatology*, 30(2): 94.
28. Herly, M., et al. (2020). Impact of season on the association between vitamin D levels at diagnosis and one-year remission in early Rheumatoid Arthritis. *Scientific Reports*, 10(1): 1-9.