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Vitamin D deficiency Associates with Disease Severity in Rheumatoid Arthritis Patients

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ABSTRACT

Vitamin D has been considered as a key player in various autoimmune disorders, such as rheumatoid arthritis, due to its immunological modulatory effect. Together with Anti-double stranded DNA (Anti-dsDNA), Anti-cyclic citrullinated peptide (Anti-CCP) is considered among the indicators of the severity of the disease in rheumatoid arthritis. The present study aimed to measure the level of vitamin D in RA patients besides measuring anti-dsDNA, anti-CCP, RF, complete blood count (CBC) and Erythrocyte Sedimentation Rate (ESR) for comparing it with its corresponding level in the healthy control group. This study was a descriptive cross-sectional study involving sequential RA patients who visited the Rheumatology clinic for outpatients during a three-month period starting from July 2018, at the Baghdad Teaching Hospital. Sixty individuals (30 RA patients and 30 age and gender matched healthy control) were enrolled in this study. Blood samples were collected and serum levels of anti-dsDNA, 25-hydroxy vitamin D (25[OH]), anti-CCP and RF besides C3, C4, CBC and ESR were measured. A highly significant decrease was displayed in the mean level of vitamin D in RA patients (18.67±17.70 ng/mL) when compared to its level in the control group (35.07 ±3.71 ng/mL), (normal value: 20 and 40 ng/mL), whereas, anti-dsDNA level normal value: (<30.0 IU/mL) was significantly increased in RA patients (122.27±65.89 IU/ml) as compared to its mean level in the control group (17.77±3.56 IU/ml) with an inverse relationship between anti-dsDNA levels vitamin D levels in RA patients. No statistical difference was noted in C3 and C4 levels between patients and control groups. Rheumatoid arthritis patients with positive RF had statistically higher anti-CCP (normal value: less than 20 U/ml) than those with negative RF (39.83±11.449 vs 21.67±4.658 u/ml). As expected, a highly significant increase was observed in ESR of patients when compared to that of the control. In addition, an alteration was noted in some circulating blood cells count.

KEYWORDS: RA; vitamin D; anti-CCP; anti-dsDNA.

الخلاصة

نظرًا لدوره في تنظيم الجهاز المناعي ، فقد تم اقتراح فيتامين د كلاعب رئيسي في بعض أمراض المناعة الذاتية ، مثل التهاب المفاصل الرثوي. جنبا إلى جنب مع الحمض النووي المضاد المزدوج (Anti-dsDNA)، يعتبر Anti-cyclic citrullinated peptide (Anti-CCP) من بين مؤشرات شدة المرض في التهاب المفاصل الرثوي. هدفت الدراسة الحالية إلى قياس مستوى فيتامين (د) في مرضى التهاب المفاصل الرثوي بالإضافة إلى قياس مضاد dsDNA ، ومضاد CCP ، و RF ، وتعداد الدم الكامل (CBC) ومعدل ترسيب كرات الدم الحمراء (ESR) لمقارنته بمستواه المقابل في مجموعة السيطرة. كانت هذه الدراسة عبارة عن دراسة مقطعية وصفية شملت مرضى التهاب المفاصل الرثوي المتسلسل الذين زاروا عيادة الروماتيزم للمرضى الخارجيين خلال فترة ثلاثة أشهر تبدأ من تموز 2018 ، في مستشفى بغداد التعليمي. تم تسجيل ستين فردًا (30 مريضًا بالتهاب المفاصل الرثوي و 30 من الاشخاص الاصحاء والمطابقين بالعمر والجنس كمجموعة سيطرة). تم جمع عينات الدم وقياس مستويات المصل من مضادات dsDNA و 25-هيدروكسي فيتامين D (25 [OH]) ومضاد CCP و RF بالإضافة إلى C3 و C4 و CBC و ESR. تم عرض انخفاض كبير للغاية في المستوى المتوسط لفيتامين د في مرضى التهاب المفاصل الرثوي (18.67 ± 17.70 نانوغرام / مل) عند مقارنته بمستواه في مجموعة التحكم (35.07 ± 3.71 نانوغرام / مل) ، (القيمة الطبيعية: 20 و 40 نانوغرام) / مل). في حين أن القيمة العادية لمستوى مضاد dsDNA: (<30.0 IU / mL) زادت بشكل كبير في مرضى التهاب المفاصل الرثوي (122.27 ± 65.89 IU / ml)مقارنة بمستوى متوسطها في مجموعة التحكم (17.77 ± 3.56) IU / ml مع علاقة عكسية بين مستويات فيتامين (د) في مضادات dsDNA في مرضى التهاب المفاصل الرثوي. لم يلاحظ أي فرق إحصائي في مستويات C3 و C4 بين المرضى مجموعة السيطرة. مرضى التهاب المفاصل الروماتويدي الذين لديهم نتائج موجبة RF لديهم مضادات CCP أعلى إحصائيًا (القيمة العادية: أقل من 20 وحدة / مل) من أولئك الذين لديهم RF سلبي (39.83 ± 11.449 مقابل 21.67 ± 4.658 ش / مل). كما هو متوقع ، لوحظت زيادة كبيرة للغاية في ESR عند المرضى بالمقارنة مع المجموعة الضابطة. بالإضافة إلى ذلك ، لوحظ تغيير في عدد خلايا الدم المنتشرة.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory, systemic autoimmune disease that deteriorates joints by eroding bone and destroying cartilage [1]. With a female:male ratio of 3 to 1, rheumatoid arthritis tends to develop later in life [2]. A symmetrical polyarthritis that primarily affects the tiny joints is the key clinical characteristic. Almost every organ in the body as well as other musculoskeletal system parts (bursitis, tendinopathy, muscular atrophy, osteoporosis) can be impacted by rheumatoid arthritis. Skeletal, haematological, neurological, pulmonary, cardiac, renal, and ophthalmic symptoms of RA are possible [3]. Pain, joint swelling, and stiffness are signs of active disease along with elevated levels of inflammatory markers [2].

The pathogenesis of RA results from an interaction of environmental, and genetic risks which increase a break in immunologic tolerance besides a gradual increase of pathologic immunologic processes which lately manifests in the phenotype of RA [3]. Therefore, it is interesting to note that during infliximab therapy, both a rise in antinuclear antibodies and the development of antibodies to double-stranded DNA, notably of the IgM type, have been reported. [4] . Vitamin D regulates the metabolism of calcium and phosphate and keeps the skeleton properly calcified.

Immunomodulatory hormone is another name for it [5]. Low serum 25(OH)D levels are linked to an increased risk of RA [6].

Based on the immunologic actions of 1,25(OH)2D that decrease TH1 and TH17 responses and stimulate Treg activity, vitamin D and its metabolites are thought to have a therapeutic effect against RA. A critical factor in the development of symmetrical polyarthritis and the persistent synovial inflammation seen in RA is the over-activation of TH1 and TH17, as well as malfunctioning Treg [7].

Rheumatoid factor, one of the earliest serological markers for the diagnosis of RA is an autoantibody that targets the Fc region of IgG. It's taken RF as nonspecific because it is also present in other collagen vascular disorders such as SLE and Sjögren's syndrome in addition to healthy individuals [8]. The presence of RF in blood often proceed any signs of joint inflammation, which suggests that locations other than the joints may be involved in the induction of autoimmunity. Measuring different RF isotypes are thought to be useful in the management of RA patients [9, 10]. Other markers used in the diagnosis of RA is the anti-cyclic citrullinated peptide antibodies (anti-CCP). The studies imply that these antibodies are linked to more severe and erosive disease with a proposed relationship with disease activity and functional capacity. The combination of these two markers can be helpful in deciding the therapeutic strategy [11]. Anti-dsDNA is classically used as a marker in patients with systemic lupus erythematosus. Nevertheless, studies have shown that it can be also elevated in other autoimmune diseases such as RA. This autoantibody has been linked with an aberrant state of autoimmunity and systemic inflammation in RA patients [12].

The present study was aimed at measuring the level of vitamin D in RA patients besides measuring anti-dsDNA, anti-CCP, RF, CBC and ESR for comparing it with its corresponding level in the healthy control group.

MATERIALS AND METHODS

Sample collection

This study, included RA patients (28 females and 2 males) who visited the rheumatology department of Baghdad Teaching hospital during three months starting from July 2018. Inclusion criteria was clinical and laboratory diagnosis of RA, while patients were excluded if they were already taking vitamin D supplements. Sera were separated from patient blood samples and kept at -20 °C until needed. Additionally, serum was taken from 30 completely healthy, gender- and age matched individuals.

Determination of Anti-dsDNA and anti-CCP levels

Serum Anti-dsDNA and anti-CCP levels were measured by ELISA (Euroimmun, EU) according to the manufacturer's instructions.

Determination of 25-hydroxy vitamin D (25[OH]D), C3 and C4

25-hydroxy vitamin D (25[OH]D), C3 and C4 serum levels were measured using abbott c4000 (Abbott, USA). According to consensus, 25(OH)D concentrations between 20 and 30 ng/mL are regarded as insufficient, whereas those below 20 ng/mL are called vitamin D deficiency [13].

Determination of Rheumatoid factor

RF was measured qualitatively (Rose-Waaler kit). In addition, a blood autoanalyzer (cell Dyn, USA) was used to measure hematological parameters.

Statistical analysis

Statistical analysis was done with Graph Pad Prism 5.0. (USA) and SPSS (Statistical Package for Social Sciences v. 24). The data were represented as means ±SD. T-Test was used to identify the effect of parameters between study groups and simple linear regression was used to assess the relation between parameters under study. When the results had * P<0.05, ** P<0.01 and *** P<.001, they were classified as statistically significant.

RESULTS AND DISCUSSION

Out of the 30 patients with RA, 0% had normal vitamin D level, 6.66% had insufficient vitamin D level, 53.33% suffered from vitamin D deficiency and 40.01% had sever deficient vitamin D levels. On the other hand, all of the control group had normal vitamin D levels. Statistical analysis shows the significant statistical difference between RA patients and control in Mean Vitamin D level (18.67± 17.70 vs 35.07 ±3.71 ng/ml).

As expected, the mean level of anti-dsDNA was higher in RA patients (122.27±65.89 IU/ml) than the control group (17.77±3.56 IU/ml). When Complement protein 3 (C3) levels in patients' and controls' sera were measured, it was found that the mean level of C3 was not different between RA (103.30 ± 13.60 mg/dl) patients and control group (105.80±16.13 mg/dl). In addition, measurement of complement protein 4 (C4) in the sera of patients and control showed that the mean serum level of C4 was not different between RA (23.80 ±6.70 mg/dl) patients and control group (26.27 ±9.36 mg/dl) as shown in Table 1.

Table 1. Immunological parameters and vitamin D concentration among studied groups.

Parameters	RA (n=30)	Control (n=30)
Mean Vitamin D (ng/ml)	18.67± 17.70	35.07 ±3.71*
Normal vitamin D 30-40 (ng/ml)	0.00 %	100%
Insufficient vitamin D 20-30 (ng/ml)	6.66 %	0.0 %
Deficiency vitamin D 8.0-20 (ng/ml)	53.33 %	0.0 %
Sever deficiency Less than 8.0 (ng/ml)	40.01 %	0.0 %
dsDNA (IU/ml)	122.27±65.89	17.77±3.56**
C3 (mg/dl)	103.30 ± 13.60	105.80±16.13
C4 (mg/dl)	23.80 ±6.70	26.27 ±9.36

*denotes (P ≤.05), ** denotes (P ≤.01).

The findings of this study support those of earlier research, which had shown that RA patients had lower levels of 25-OH Vitamin D than healthy controls [14]. Low levels of 25-OH vitamin D, which is known to stimulate immunological tolerance, have been found in RA patients. [15]. Due to its immunological functions and the fact that immune tolerance is affected by vitamin D deficiency, autoimmune illnesses (like RA) might develop. As a result, vitamin D regulates the immune response through a variety of pathways (e.g., stimulate regulatory T cells, decrease antigen presentation, and inhibit the pro-inflammatory T helper type 1 profile) [16]. Thus, autoimmune diseases, which contributed to the low level of Vitamin D, and Vitamin D deficiency was linked to musculoskeletal pain, as well as finding that RA patients treated with corticosteroids had a low level of Vitamin D, even though the use of corticosteroids has a minimal impact on the level of Vitamin D, corticosteroids have decreased the level of Vitamin D in RA patients [17].

Regression analysis showed an inverse highly significant relationship between anti-dsDNA and vitamin D. Increased anti-dsDNA level associated with low vitamin D level as shown in Figure 1.

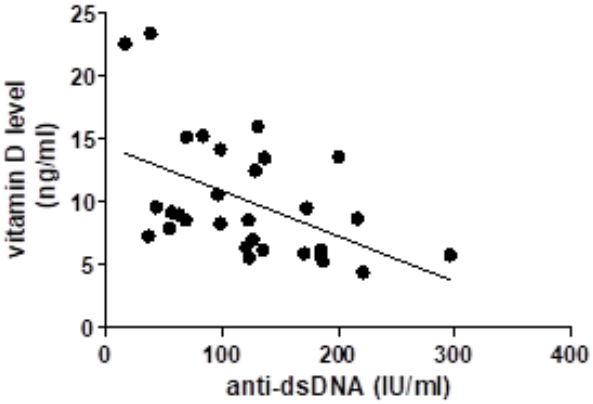

Figure 1. Simple Liner Regression between Vitamin D and anti-dsDNA levels.

Anti-CCP (u/ml) and RF levels in RA were assessed using ELISA. Statistical analysis performed by independent T test showed highly significant difference in anti-CCP between RA patients with negative RF and those with positive RF as seen in Table 2 which shows that RA patients with positive RF had significantly higher anti-CCP level.

The finding of anti-CCP IgG antibody is significant since it is a particular biomarker for RA. Strong evidence of their pathophysiologic involvement is produced when the anti-CCP IgG antibody stimulates the complement system through both classical and alternative pathways. It has been demonstrated that apoptosis and inflammation-induced cellular death are the causes of the increased CCP production (necroinflammation). Inflammation rather than a sickness is what causes this process [18].

Table 2. Distribution of anti-CCP in RA patients according to RF

	RF	N	Mean± Std.	T-Test	P-Value
ANTI–CCP (u/ml)	-Ve	12	21.67±4.658	5.193	.000
	+Ve	18	39.83±11.449		

The Fc region of IgG antibodies is the target of the autoantibody rheumatoid factor. The most common blood test for rheumatoid factor is used to diagnose rheumatoid arthritis. [19]. Traditionally, bacteria or immunological complexes have been thought to be the primary triggers of complement activation. The latter have been connected to the activation of the complement system in RA. Circulating immune complexes from RA patients, however, don't seem to have much of an impact on complement system activation. Other possible causes for complement activation in RA should be taken into consideration as there is insufficient evidence that immune complexes are the primary cause of the condition, CRP could be one of these causes. The current study showed that complement protein 3 and 4 had comparable levels in both patients and control which could be attributed to corticosteroids treatment in RA patients [20].

Data displayed in table 3 demonstrated an alteration in complete blood count (hemoglobin level, platelets, lymphocyte, neutrophil, eosinophil and basophil count), besides an increase in ESR when comparing RA patients with the control group ($p<0.01$).

Anemia is a prevalent hematological condition among RA patients. This characteristic may be caused by a variety of conditions, including an iron deficit, an erythropoietin production defect, a decrease in the bone marrow's ability to respond to erythropoietin, and a problem with the way that iron is released from the reticulo-endothelial system.[21].

Table 3. Distribution of anti-CCP in RA patients according to RF

studied groups		WBC count (10^9 /L)	Hemoglobin (g/dL)	platelet count (10^9 /L)	Lymphocyte count (%)	Monocyte count (%)	Neutrophils (%)	Eosinophil (%)	Basophil (%)	ESR (mm/hr)
RA	M	5.06	10.68**	412.57**	24.88**	3.89	63.85**	1.54**	0.91**	100.70**
	Std.	2.45	1.27	124.62	8.13	1.38	7.53	0.77	0.37	39.89
Control	M	6.05	13.95	315.70	39.57	4.05	52.86	2.49	0.56	7.86
	Std.	1.96	0.62	73.78	5.82	0.72	10.29	1.32	0.21	1.97

An RA diagnosis is supported by an increased ESR, CRP, and/or positive RF test. For many RA clinical studies, the inclusion criteria include an ESR of less than ≥28 mm/h and/or aberrant CRP. Remission criteria for RA set by the American College of Rheumatology (ACR) include ESR values of less than <20 mm/h for males and <30 mm/h for women [22]. Regression analysis proved that there is an inverse relationship between vitamin D level and anti-dsDNA in RA patients. Both of anti-CCP and anti-dsDNA levels give an indication of disease progression. A previous study has concluded that providing RA patients with supplements of the aforementioned vitamin, reduced the progression of the disease which highlights an association between this vitamin and disease activity in RA patients [23]. Complete blood counts are usually done in RA patients to spot any side effects associated with the treatment/or disease related alterations. Common alteration includes anemia and thrombocytosis [24].

CONCLUSIONS

it seems that vitamin D deficiency is very common in RA patients, and that vitamin D deficiency may be related to the severity of the condition. This decrease in vitamin D level was accompanied with an increase in both of anti-CCP and anti-dsDNA

which may imply that vitamin D deficiency associates with the severity of rheumatoid arthritis. Further large-scale studies are warranted to confirm such conclusion.

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REFERNCES

[1] L. L. W Ishikawa, P. M. Colavite, T. F de Campos Fraga-Silva, L. A. N Mimura, T. G. D França, S. F. G. Zorzella-Pezavento, F. Chiuso-Minicucci, L. D. Marcolino, M. Penitenti, M. R. V. Ikoma. Vitamin D deficiency and rheumatoid arthritis. Clinical reviews in allergy & immunology. 2017;52(3): PP373-88.

[2] A. Silman, L. Klareskog, F. Breedveld,B. Bresnihan,R. Maini, P. Van Riel,D. Symmons. Proposal to establish a register for the long-term surveillance of adverse events in patients with rheumatic diseases exposed to biological agents: the EULAR Surveillance Register for Biological Compounds. Annals of the rheumatic diseases. 2000;59(6): PP419-20.

[3] J.Sokolove. Rheumatoid Arthritis Pathogenesis and Pathophysiology. Lung Disease in Rheumatoid Arthritis: Springer; 2018. PP. 19-30.

[4] G. Steiner, J. Smolen Autoantibodies in rheumatoid arthritis and their clinical significance. Arthritis research & therapy. 2002;4(2): PP1-5.

[5] N. Charoenngam, M. F. Holick. Immunologic effects of vitamin D on human health and disease. Nutrients. 2020;12(7): PP 2097.

[6] Y. H. Lee, S. C. Bae. Vitamin D level in rheumatoid arthritis and its correlation with the disease activity: a meta-analysis. Clinical and experimental rheumatology. 2016;34(5): PP 827-33.

[7] M. M Aslam, P. John, A. Bhatti, S. Jahangir, M. I. Kamboh. Vitamin D as a Principal Factor in Mediating Rheumatoid Arthritis-Derived Immune Response. BioMed research international. 2019;2019: PP 3494937. 10.1155/2019/3494937

[8] U. Singh, A. Vishwanath, P. K. Verma, N. K. Singh, R. C. Shukla, S. Singh, S. Singh, G. K. Sonkar. Is rheumatoid factor still a superior test for the diagnosis of rheumatoid arthritis? Rheumatology international. 2010;30(8): PP1115-9.

[9] V. Malmström, A.I.Catrina, L. Klareskog L. The immunopathogenesis of seropositive rheumatoid arthritis: from triggering to targeting. Nature Reviews Immunology. 2017;17(1): PP60.

[10] R. H. Hussein, I. A. MezherAl-Rayahi, K.Taha. Rheumatoid factor isotypes in a sample of Iraqi rheumatoid arthritis patients. Journal of Global Pharma Technology. 2018;10(8): PP141-5.

[11] D. Sieghart, A. Platzer, P. Studenic F. Alasti, M. Grundhuber, S. Swiniarski, T.Horn, H. Haslacher,S. Blüml,J. Smolen. Determination of autoantibody isotypes increases the sensitivity of serodiagnostics in rheumatoid arthritis. Frontiers in immunology. 2018; PP 9:876.

[12] T. G. Nguyen, K. J. McKelvey, L. M. March, D. J. Hunter, M. Xue, C. J. Jackson, J. M. Morris. Aberrant levels of natural IgM antibodies in osteoarthritis and rheumatoid arthritis patients in comparison to healthy controls. Immunology letters. 2016;170: PP27-36.

[13] M. F. Holick. Vitamin D status: measurement, interpretation, and clinical application. Annals of epidemiology. 2009;19(2): PP73-8.

[14] B. A. Hamad. Relationship between vitamin D3 level and rheumatoid arthritis patients attending Rizgary Teaching Hospital in Erbil city. Zanco Journal of Medical Sciences (Zanco J Med Sci). 2017;21(2): PP1736-42.

[15] S. T. Weiss. Bacterial components plus vitamin D: the ultimate solution to the asthma (autoimmune disease) epidemic? Journal of Allergy and Clinical Immunology. 2011;127(5): PP1128-30.

[16] L. E. Bartels, C. L. Hvas, J. Agnholt, J.F. Dahlerup,R. Agger. Human dendritic cell antigen presentation and chemotaxis are inhibited by intrinsic 25-hydroxy vitamin D activation. International immunopharmacology. 2010;10(8): PP 922-8.

[17] A.M.Al-Gebori,M.H.M Alosami., N.H. Al-Hashimi. Prevalence Of 25-Hydroxy Vitamin D Deficiency and Some Biochemical Parameters in Iraqi Patients with Rheumatoid Arthritis and Their Associations with Disease Activity. Prevalence. 2020;13(4).

[18] D. M. Ahmed, D. F. Salloom. The Association between Toll-like Receptor 7 and Hepatitis C Virus in a Sample of Iraqi Rheumatoid Arthritis Patients. Journal of Global Pharma Technology. 2018;1: PP1-8.

[19] S. F. Nielsen, S. E. Bojesen, P. Schnohr., B. G. Nordestgaard. Elevated rheumatoid factor and long-term risk of rheumatoid arthritis: a prospective cohort study. BMJ. 2012; PP345.

[20] E. T. Molenaar, A. E. Voskuyl,A. Familian., G.J.van Mierlo,B.A. Dijkmans,C.E. Hack. Complement activation in patients with rheumatoid arthritis mediated in part by C-reactive protein. Arthritis & Rheumatism:

Official Journal of the American College of Rheumatology. 2001;44(5): PP997-1002.

[21] E. T. Ali, A. S. Jabbar, A. N. Mohammed. A comparative study of interleukin 6, inflammatory markers, ferritin, and hematological profile in rheumatoid arthritis patients with anemia of chronic disease and iron deficiency anemia. Anemia. 2019.

[22] T. Sokka, T. Pincus. Erythrocyte sedimentation rate, C-reactive protein, or rheumatoid factor are normal at presentation in 35%–45% of patients with rheumatoid arthritis seen between 1980 and 2004: analyses from Finland and the United States. The Journal of rheumatology. 2009;36(7):PP1387-90.

[23] L. E. Jeffery, K. Raza, M. Hewison, Vitamin D in rheumatoid arthritis-towards clinical application. Nature reviews Rheumatology. 2016;12(4):201-10.

[24] S. I. Taha, S. F. Samaan, R. A. Ibrahim, N. M. Moustafa, E. M. El-Sehsah, M. K Youssef M. K. Can Complete Blood Count Picture Tell Us More About the Activity of Rheumatological Diseases? Clinical Medicine Insights: Arthritis and Musculoskeletal Disorders. 2022; 15: 1179 5441221089182. 10.1177/

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