



The Role of Vitamin D3 and CRP in Patients with Rheumatoid arthritis in Kirkuk Governorate

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ABSTRACT

The number of patients with rheumatoid arthritis in the Iraqi province of Kirkuk was investigated. One hundred blood samples were taken for the study. 60 patients suffers from RA (30 males and 30 females) and 40 healthy controls. (20 males and 20 females). The age group was 10- 65 years old. The study founded vitamin D level was considerably lower in RA patients (25.04 ± 16.12 ng/ml) in comparison to healthy control group (65.26 ± 20.43 ng/ml). also, The study founded RA with CRP titer was higher (15.18 ± 18.55 mg/dl) compared to the healthy control group (4.85 ± 9.92 mg/dl).

Keywords:

Vit.D3, CRP, Rheumatoid arthritis, Kirkuk

Introduction

Rheumatoid arthritis (RA) is a chronic, autoimmune, systemic disorder that manifests as inflammation of synovial joints, leading to joint destruction and deformity. Although RA is not a fatal disease in general, but the complication associated with RA such as cardiovascular, and pulmonary systems can lead to increased mortality (Yap et al., 2018). In 2015, About 24.5 million people worldwide suffer from RA diseases (Vos et al., 2016). This affects between 0.5 and 1% of people in the developed world, with a yearly incidence rate of 5 to 50 per 100,000 (Smolen JS, et al., 2016).

the treatment mainly focuses on alleviating pain, prevent or limit joint damage, improve or preserve function of the joints and optimize the quality of life (Kayanaugh and Grevich, 2018). vitamin D3 also known as cholecalciferol, is currently considered a

molecule that controls at number of functions in bone and mineral homeostasis. (Diffey, 2018; Christakos et al., 2016). In rheumatoid arthritis, C-Reactive protein is frequently examined as a measure of basic inflammation. (McInnes and Schett, 2018). test of CRP in RA its suggestions for disease and therapy response (Bombardier et al., 2012).

Aim of the Study:

This study investigates the association between vitamin D3 and CRP levels and Rheumatoid arthritis.

Statistical Analysis

The results were analyzed statistically by applying the statistical program using the Chi-square test (version 16) with a probability level ($p > 0.05$) and ($p > 0.01$).

Materials and Methods:

This study was done in the period from December 2021 and March 2022 In Kirkuk Governorate Hospitals, included 100 patient, 60 of them diagnosed with Rheumatoid arthritis and the other were healthy (control group).(The patients were questioned using a questionnaire to obtain information such as their age, gender, place of residence, number of

years suffering from Rheumatoid arthritis, blood group. Each study participant had 5 mL of venous blood drawn and divided, 2 mL going into an EDTA tube for ESR examination. The remaining 3 ml was centrifuged to separate the serum and frozen at -20C until use, Enzyme-linked immunosorbent assays were used to detect Vit.D3, and CRP.

Table (1) Kit contents HS CRP:

No.	Material	Specification	Quantity
2	Enzyme conjugate	6.0 ml	1 vial
3	Standard A	10 µg ml	1 vial
4	Standard B	0.5 µg ml	1 vial
5	Standard C	1.0 µg ml	1 vial
6	Standard D	2.5 µg ml	1 vial
7	Standard E	5.0 µg ml	1 vial
8	Standard F	10 µg ml	1 vial
9	Substrate A	6 ml	1 vial
10	Substrate B	6 ml	1 vial
11	Stop Solution	6 ml	1 vial
12	Wash Solution 100x	10 ml	1 vial
13	Balance Solution	3 ml	1 vial

Table (2) Kit contents Vit.D3

No.	Name	96 tests	48 tests
1	Antibody percolated plate	8x12	8x6
2	Human Vit.D ₃ standard	2 vial	1 vial
3	Biotiny loated antibody (1:100)	1 vial	1 vial
4	Enzyme Conjugate (1:100)	1 vial	1 vial
5	Enzyme diluent	1 vial	1 vial
6	Antibody diluent	1 vial	1 vial
7	Standard diluent	1 vial	1 vial
8	Sample diluent	1 vial	1 vial
9	Washing buffer (1:25)	1 vial	1 vial
10	Color reagent A	1 vial	1 vial
11	Color reagent B	1 vial	1 vial
12	Color reagent C	1 vial	1 vial
13	Instruction	1 set	1 set

Principle of the assay (C. Reactive Protein)

The quantitative sandwich enzyme immunoassay method is used in this assay. Standards and samples are pipetted into the wells of a micro plate that has been pre-coated with an antibody specific for CRP. Any CRP present is then bound by the immobilized antibody. A horseradish peroxidase (HRP) - conjugated antibody specific for CRP is added to the wells after any unbound materials have been removed. A substrate solution is then introduced to the wells after a wash to get rid of any unbound reagent, and color develops in proportion to how much CRP was initially bound. The growth of the color is halted, and the color's intensity is gauged. (Fleischmann, et al.,2017).

Principle of assay (vt.D3):

The ELISA Kit that is being used in this investigation uses the double-sandwich Elisa technique. Human VD₃ monoclonal antibody serves as the pre-coating, while polyclonal antibody with biotin labeling serves as the detecting antibody. ELISA plate wells are filled with samples and biotin-labeling antibodies before being cleaned with PBS or TBS. Following an orderly addition of avidin-peroxidase conjugates, the TMB substrate is used to color the ELISA wells after the reactant has been fully removed with TBS or PBS. TMB is catalyzed by peroxidase to turn blue, and then under the influence of acid, it turns yellow. Sample testing variables and color

depth have a favorable relationship. (Meehan, C.J., Bruce, F., 2019).

Result and Discussion :

Table (3): Mean level of vitamin D in RA patients and control group

Groups	No.	Vitamin D (ng/ml)		P. value
		Mean	SD	
RA patients	60	25.04	16.12	0.002
Control groups	40	65.26	20.43	

Table (4): Mean level of CRP in RA patients and control group

Groups	No.	CRP (mg/dl)		P. value
		Mean	SD	
RA patients	60	15.18	18.55	0.001
Control	40	4.85	9.92	

CRP is one of multiple acute phase reactants generated by hepatocytes in response to inflammation and its increase correlates with the production of inflammatory mediators (Sproston *et al.*, 2018). This study showed significance increase in blood serum C-Reactive Protein concentrations ($p \leq 0.01$) in rheumatoid arthritis group During the study. Figure (2) (15.18 ± 4.85 mg/dl) as compared with control group. These findings are agree with previous studies that demonstrated rise serum CRP in rheumatoid arthritis patients as compared to a control group (Babikir, and Gaufri, 2017; Doğan *et al.*, 2014)). High blood CRP levels in RA correspond with quick and severe development of joint destruction because it represents both systemic and local inflammatory responses. CRP generation in RA is increased by the activation of proinflammatory cytokines on hepatocytes

such as IL-6, TNF-, and IL-1- (6, 7) (Rija *et al.*, 2019; Kim *et al.*, 2015). The levels of vitamin D were low in Rheumatic patients compared with the control group as table 1. Vitamin D receptors are found on many immune system cells, and vitamin D has a regulatory effect. Immune modulation is mediated by activated T cells and active B lymphocytes. (Naji *et al.*, 2018). Peoples may have low levels of vitamin D due to inadequate exposure to direct sunlight. (Diaz *et al.*, 2009). So, it appears vitamin D deficiency is prevalent in RA patients, it appears to be related to the severity of the disease. So, vitamin D deficiency has been linked to generalized musculoskeletal pains. Vitamin D supplements may be considered a component of RA pains and the prevention of osteoporosis (Kostoglou-Athanassiou *et al.*, 2012)

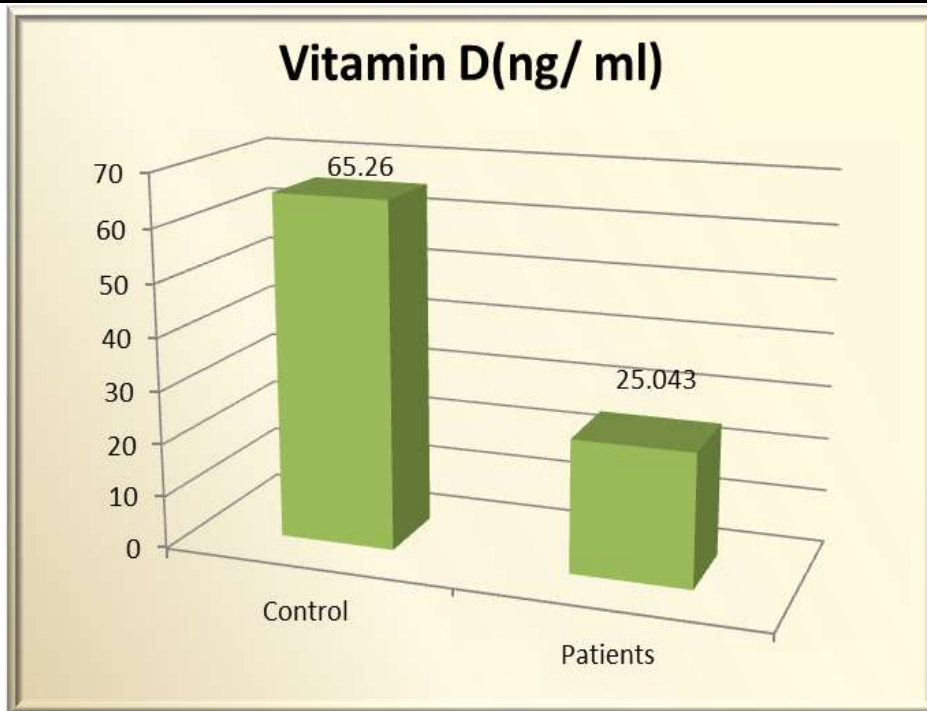


Figure (1): Mean level of vitamin D in RA patients and control group

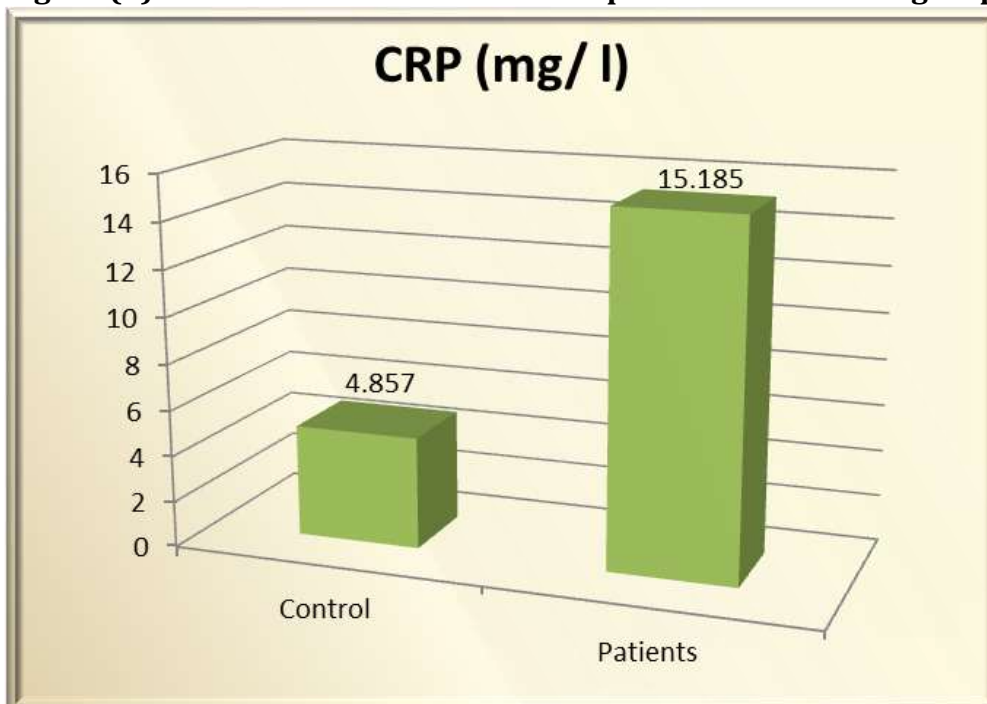


Figure (2): Mean level of CRP in RA patients and control group

Conclusion:

1. Patients with Rheumatoid arthritis showed high levels of CRP titer than control groups, and this indicates an association between CRP and RA.
2. Vitamin D levels in RA patients were lower than in healthy control group, , and this indicates an association

between Vit.D3 and Rheumatoid arthritis

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