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Evaluation of Vitamin D and Trace Element Level in Sera of Patients with Rheumatoid Arthritis

Parween Abdulsamad Ismail[1*], Akam Jasim Mustafa[2], Dlzhin Bahram Waissi[3], Yusra Sirwan Kareem[4]

[1]Department of Chemistry, College of Education, University of Salahaddin, Erbil, Iraq.
[2,3,4]Department of Chemistry, Faculty of Science, Soran University, Erbil, Iraq.
*Correspondence to: Parween Abdulsamad Ismail (E-mail: parween7abdulsamad@yahoo.com)
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Abstract
Objectives: The purpose of this research was to assess the serum concentration of (1,25-(OH)2D3) and trace elements in patients with rheumatoid arthritis as compared to healthy individuals.
Methods: This research was performed on 72 participants, 37 cases of RA and 35 healthy individuals, with age range 39–67 years. Serum (1,25-(OH)2D3) levels was determined and compared in cases and healthy controls.
Results: The serum (1,25-(OH)2D3) levels were significantly lower in the RA patients (13.24 ± 1.06 ng/ml), when compared to the healthy individuals (26.9 ± 1.7 ng/ml). The examination of data revealed that the levels of (Magnesium, Manganese, Nickel and Rubidium) were remarkably elevated in RA patients when compared to healthy control, serum levels of (Chromium, Cobalt and Germanium) were remarkably decreased in RA patients when compared to healthy control.
Conclusion: (1,25-(OH)2D3) insufficiency is more widespread among patients with RA and may be one of the reasons leading to evolution of rheumatoid arthritis. Supplementation with (1,25-(OH)2D3) may be required both for the avoidance of bone loss besides for painkilling in patients suffering from rheumatoid arthritis. Current results propose that alterations in the serum concentration of trace elements may yield good indication to their role in the pathogenesis of rheumatoid arthritis.
Keywords: (1,25-(OH)2D3), trace elements, Rheumatoid arthritis

Introduction

Rheumatoid arthritis, or RA, is an autoimmune and inflammatory disease of unknown causation.[1] T cells and B cells play a key role in the progression of the Rheumatoid arthritis.[2] The action of T cells beside that of B cells in the development of RA has been further confirmed by the effectiveness treatment of methods influencing both T cells and B cells.[3,4] (1,25-(OH)2D3) insufficiency may elevate the hazard for the progression of RA.[5] (1,25-(OH)2D3) has been reveal to change the of genes expressions that influence activity of cells such as, multiplication Distinction, cell death program, and maturation.[6] (1,25-(OH)2D3) is participated in the inhibition of interleukin-2, generation of antibody, and in lymphocyte multiplication, and, thus, is considered as controlling factor for the immune system[7] critical in the maturation and function of both T-regulatory cells[8,9] and dendritic cells.[10,11] It has been proposed that physiologically active formula of (1,25-(OH)2D3) affects the operation by which immune cells receive A molecular messenger that making them to emigrate to normal sites of extra Tissue making up the lymphatic system, besides sites of inflammation.[12] Ii is known that (1,25-(OH)2D3) blocks secretion of interferon gamma and negatively control IL-12 generation by negative-acting nuclear factor kappa-light-chain-enhancer of activated B cells.[13] The impression of (1,25-(OH)2D3) receptor following stimulation, presenting on antigen cells, dendritic cells, T cells and B cells, further propose a regulation of immune system by (1,25-(OH)2D3).[14–16] Given the influences of (1,25-(OH)2D3) as immune system suppressor and the possible link between (1,25-(OH)2D3) insufficiency and autoimmune disorder, (1,25-(OH)2D3) has been examined as possible participant in the mechanism of action of various autoimmune diseases. Epidemiologic study shows low (1,25-(OH)2D3) levels in autoimmune diseases such as RA.[17–20] However, it is ambiguous whether low intake of (1,25-(OH)2D3) is hazard factor of progression of Rheumatoid arthritis, and the relationship between low serum (1,25-(OH)2D3) concentrations and elevated activity in RA patients remains arguable.[21]

The great interest of trace metallic elements chronic inflammatory disorder due to their role as a co-factor in various metabolic pathway including function of immune system.[22] Many studies indicate that there was a link between concentration of trace elements and duration rheumatoid arthritis diseases. Serum trace elements as well as transport proteins such as ceruloplasmin and albumin, play a key role in the antioxidant protection mechanism of cell. Microelement status hence may be participated in the pathogenesis of Rheumatoid arthritis or be influenced by chronic inflammatory condition of RA.[23] The goal of the current study was to evaluate the potential variation in the concentrations of vitamin D and trace elements in Rheumatoid arthritis patients.

Materials and Methods

The research was performed on 87 (51 females & 36 males) RA patients without treatment for RA who were referred to the consultant clinic at the department of Rheumatology, outpatient clinic in Erbil city. The diagnosis of those patients has been performed under supervision of a specialist physician in rheumatology department. Excluded criteria of the current research include those patients with a history of gastrectomy, chronic diarrhea and patients who were under the treatment with of (1,25-(OH)2D3) and/or calcium supplementation.

The patients age who involved in this study ranged from 39–67 years. Sixty-five healthy individuals matched in age and

gender were also participated as controls. 3–5 ml of blood was obtained by venipuncture. The collected blood was transferred to a plain tube and left to clot at room temperature (20–25°C) for 15 minutes. The clotted blood was centrifuged at 2000 rpm for 15 minutes; and by then, serum was collected and distributed into aliquots of (200 µl) in Eppendorf tubes, which was stored in freezer, after completion collection of all samples the serum samples were defrosted to be ready for estimation of (1,25-(OH)2D3). The concentration of (1,25-(OH)2D3) in serum was estimated by ELISA technique using a kit manufactured by CUSABIO company. Serum trace elements (Cr, Co, Mg, Mn, Ni and Rb) were estimated by using 1275 A Varian, atomic absorption spectrophotometer.

Results and Discussion

Serum Levels of Vitamin D

Figure 1 & Table 1 reveal the results of (1,25-(OH)2D3) concentrations in (serum a) samples of control and Rheumatoid arthritis (RA) patients. The results reflect a remarkable decrease ($P < 0.001$) in the serum level of vitamin D of Rheumatoid arthritis (RA) groups in comparison to that of the control.

(1,25-(OH)2D3) is considered as one of principal environmental elements that involved to Rheumatoid arthritis, there is a high prevalence of bones weakens in Rheumatoid arthritis patients.[24] Studies have showed that cytokines and T cells play a key role in the development of RA.[25] Vitamin D appears to interact with the immune system through its roles on the controlling and distinction of cells such as lymphocytes, macrophages, and natural killer cells, as well as interfering in the production of cytokines. 25(OH)D is one of principal steroid hormones, which is implicated in the metabolic pathways of bone and modulation of immune system. Receptors of (1,25-(OH)2D3) are present on the T cell and B cell of lymphocytes, dendritic cells and macrophages. The control of cell proliferation and differentiation with inhibition the release of inflammatory elements were attribute to the binding of 25(OH)D with their receptors. Consequently, they will play major roles in controlling of immune reactions.[26] Thus, (1,25-(OH)2D3) may not only ameliorate symptom of osteoporosis in patients with RA but also play a key role in the controlling of immune system.

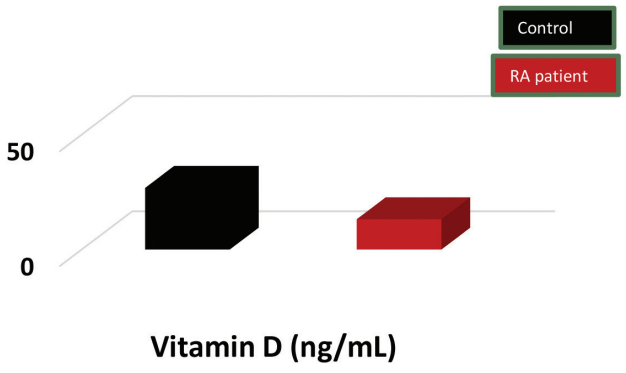

Fig. 1 **Mean values of serum vitamin D in control and RA patients groups.**

Table 1. **Mean values of serum vitamin D in control and RA patients groups**

Parameter	RA patient Mean ± SD	Control Mean ± SD	*P*-value
Vitamin D (ng/mL)	13.24 ± 1.06	26.9 ± 1.7	$P < 0.001$

Insufficiency of (1,25-(OH)2D3) may be linked with an elevated hazard for the progression of Rheumatoid arthritis. (1,25-(OH)2D3 is known to produce regulated response of lymphocytes to self-antigens.[12] Thus, vitamin D insufficiency may disarrange immune tolerance and produce the progression of autoimmune disorder, such as rheumatoid arthritis. Immunomodulatory features of Vitamin D on the immune system taking place both in an endocrine and in a paracrine mode.[27] It seems to control the immune reactions by a various of mechanisms, such as lowering expression of antigen molecules,[28] inhibiting the proinflammatory T helper type 1 profile [and promoting regulatory T cells. 1,25(OH)2D3 repress proliferation and immunoglobulin generation and delay differentiation of B-cell precursors into plasma cells.[29] These finding brace a role for (1,25-(OH)2D3) insufficiency in the pathogenesis of autoimmune inflammatory conditions in including RA. Results propose that (1,25-(OH)2D3) receptor agonists may inhibit and repress established collagen promoted arthritis. It has been reported that (1,25-(OH)2D3) may be negatively affected in acute reaction, that is, its concentrations may reduce in the position of inflammation, such as in active Rheumatoid arthritis. Despite that, therapy with rituximab in Rheumatoid arthritis did not influence (1,25-(OH)2D3) concentrations, although it reduced indicator of inflammation.[30]

Supplementation with (1,25-(OH)2D3) It has been supposed as a tool to stimulate unresponsiveness of the immune system and thus inhibit the progression of autoimmune disorder. It has been described that amalgamation of antirheumatic drugs with vitamin D has been proposed for RA. Patients with Rheumatoid arthritis are susceptible to osteoporosis. Merlino et al.[5] indicated a negative association between higher intake of (1,25-(OH)2D3) and Rheumatoid arthritis risk. They examined data from a study of 29,368 women without a history of Rheumatoid arthritis at study baseline, and through 11 years of follow-up, 152 patients with RA were diagnosed. Higher intake of (1,25-(OH)2D3) was negatively correlated with risk of Rheumatoid arthritis. Another study with 100 RA patients and 100 controls, not on (1,25-(OH)2D3) supplements, noticed that patients with high rate of RA activity had the reduced (1,25-(OH)2D3) concentrations. Remarkably lower (1,25-(OH)2D3) level were found in patients who were imperfectly responding to therapy and those patients suffering from disappearance of signs and symptoms of RA.[31] Sabbagh et al.[32] also found insufficient (1,25-(OH)2D3) condition in patients with systemic autoimmune rheumatic diseases (SARDs), accompanying significantly strong correlation with disease activity in Rheumatoid arthritis patients. This study demonstrated the requirement for appropriate assessment of (1,25-(OH)2D3) status in these patients to ensure the intake of the recommended quantity of (1,25-(OH)2D3). Studies conducted by Kareem et al.[33] and Yagiz et al.[34] found remarkably lower Vitamin D concentrations in patients with RA as compared to control population, thus supporting the potential role of (1,25-(OH)2D3) in the progression, development, activity and treatment of various kinds of autoimmune diseases.

Table 2 shows the results of trace elements (Cr, CO, Ni, Mg, Mn and Rh in serum samples of control and Rheumatoid arthritis patients. The results reveal a remarkable decline ($P < 0.001$) in the levels of Chromium and Cobalt of Rheumatoid arthritis patients groups in comparison to that of the control as shown in Figure 2.

The role of trace metallic elements in inflammatory condition is of great interest because various trace elements act as of co-factors in metabolic pathways including articular tissues and function of immune system.[22] Chromium is one of the principal trace elements. Have a key role in preserving proper health; Chromium involved in the regulation metabolism of glucose and lipid.[35]

The reasons that Chromium may be powerful in preventing rheumatoid arthritis is that post-menopausal women under the treatment with Chromium supplement displayed elevated plasma dehydroepiandrosterone, a precursor compound of estrogen synthesis which block bone loss, and declined excretion of urinary calcium and hydroxy proline, which are indicators of bone loss.[36] These exasperating results need to be validated, and the prevention of bone loss needs to be confirmed by the use of ways that can directly detect alterations in bone contents with Chromium supplementation.[37,38] Figure 2 shows the mean values of serum Chromium level in control and patients groups, Chromium deficiency in patients with rheumatoid arthritis disease, the results demonstrated low level in patients group as compared with those in control group. The results are in line with the data recorded by Cerhan et al.[39]

Table 2. **Mean values of serum trace elements in control and RA patients groups**

Trace elements	Mean (ng/ml) ± SD (Control)	Mean (ng/ml) ± SD (RA patients)	*P*-value
Cr (ng/ml)	47.1 ± 2.02	39.1 ± 2.32	0.001
Co (ng/ml)	40.5 ± 5.1	34.23 ± 3.15	0.001
Mg (ng/ml)	17523.67 ± 1044	21974.82 ± 4980	0.0001
Mn (ng/ml)	25.5 ± 5.43	34.21 ± 8.54	0.001
Ni (ng/ml)	22.5 ± 4.5	35.41 ± 9.07	0.001
Rb (ng/ml)	0.892 ± 0.53	1.71 ± 0.52	0.001

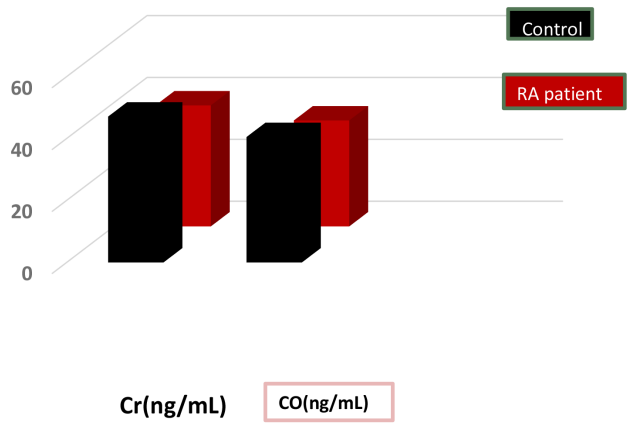

Fig. 2 **Mean values of serum Chromium in control and RA patients groups.**

Table 2 include the results acquired for the Magnesium content in the sera of rheumatoid arthritis and control group. The mean serum Magnesium concentration between the two groups is highly significant elevated ($P < 0.0001$). These results are in line with the previous work.[40] Figure 3 shows the mean values of serum Magnesium level in control and patients groups. Manganese is one of several trace elements that are essential for health of bone. One study[41] record that taking amalgamation of Calcium, Zinc, Copper and Manganese involved in lessen spinal bone loss in a post-menopausal women group. Patients with arthritis tend to possess low concentration of super oxide dismutase (SOD). Manganese is an essential mineral in the protein, fat metabolism, healthy immune, normal bone growth and energy production. The measured results of serum Manganese are recorded in Table 2 & Figure 4, these results revealed significant elevation ($P < 0.001$) in RA patients when compared with control group, in the literature there was very little information about this point. Manganese is needed for the usage of vitamin B1 and vitamin E, it is required in the synthesis of cartilage and synovial fluid of the joints. Manganese insufficiency can cause inadequate bone production. An excess of Manganese can cause deficient Iron metabolism.[42]

Table 2 and Figure 4 show the mean values of serum Nickel concentration in control and patients groups. The results reflect a significant decrease ($P < 0.001$) in the levels of Nickel of RA patients groups in comparison to that of the

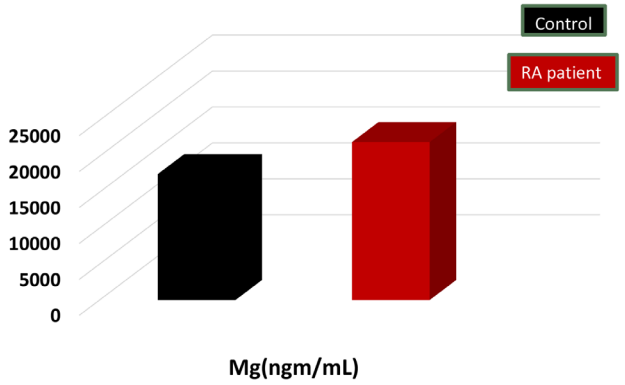

Fig. 3 **Mean values of serum Magnesium in control and RA patients groups.**

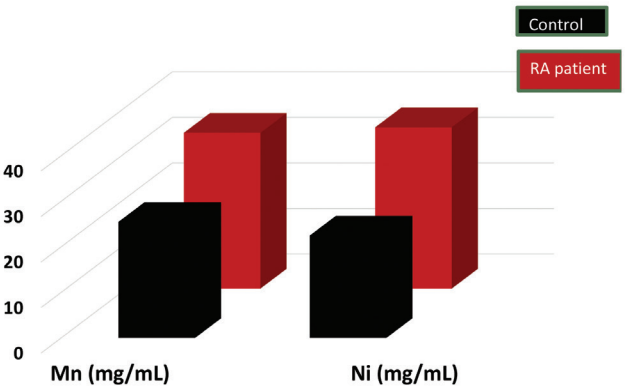

Fig. 4 **Mean values of serum Manganese in control and RA patients groups.**

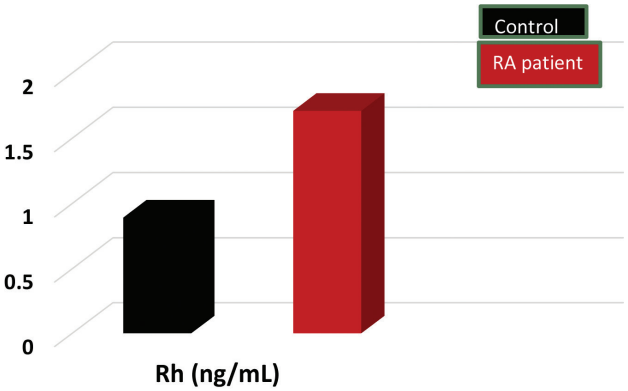

Fig. 5 **Mean values of serum Rubidium in control and RA patients groups.**

control, Nickel was to be found in blood and tissue at quite consistent physiological significance.

Nickel is needed for normal growth and proliferation in animals and presumably in human being as well it seem to have action in the adjustment of the immune system. Nickel plays very important role in biological system such as hormonal control in, enzyme activity, also in structure or function of nucleic acid compounds such as RNA, DNA and protein.[43,44] Metabolism of other nutrients like Calcium and vitamin B12 is also changed because of Nickel inadequacy. Bone evolution resistance to infection and immune function are some of the difficulty linked with Nickel inadequacy.

Serum trace elements in rheumatoid arthritis patients and control groups in Figure 5 indicated a remarkable increase (P = 0.001) in serum Rubidium. The difference in the serum concentrations of trace elements has been studied by many researchers to discover the potential association between manifestation, clinical features, and response to the system of therapy with the trace element concentrations.[23,45,46]

Conclusion

Insufficiency of (1,25-(OH)2D3) is highly widespread in patients with rheumatoid arthritis, and that inadequacy in (1,25-(OH)2D3) may be associated with disease seriousness in RA. (1,25-(OH)2D3) augmentation in daily diet may be required for the stave off of osteoporosis and for pain reassurance in patients with RA. The alteration of trace elements in sera of rheumatoid arthritis patient groups indicated specialty of various trace elements in the physiological and pathological status of rheumatoid arthritis disease. Trace elements are not a remarkable predictor test for RA disease. Early and accurate prognostication of trace elements alterations may be more important when the rheumatoid arthritis patients due to the possible release of the trace elements from the injured tissues which is critical for performing an ideal therapy consequence in RA patients.

Conflicts of Interest

None.

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