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Evaluation of Vitamin D3 and Vitamin E levels in Rheumatoid Arthritis Patients

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Abstract

Rheumatoid Arthritis (RA) is characterized by both systemic and local inflammation. It is considered to be among the most common systemic inflammatory illnesses, which include synovial swelling that can lead to joint degeneration and extra-articular complaints if not solved.

Vitamin D may be linked to an increased likelihood of getting rheumatoid arthritis (RA), as well as symptoms in individuals. People with RA had considerably greater symptoms of drying (ocular, cutaneous, and mouth), which might have been due to subsequent Rheumatoid Arthritis (RA) itself.

The study sought to investigate vitamin D3 and E levels in RA individuals as well as determine their relationship to disease activity. A doctor working at Baqubah medical center and a private practice reported 100 cases (80 individuals infected with rheumatoid arthritis and 20 controls). From each scenario, samples of blood were taken.

The findings regarding determining vitamin D and E levels significantly dissimilar. The Patients' sample registered arithmetic mean levels for vitamins D3 (21.18 ± 1.10), whereas the control sample had a mathematical mean (24.35 ± 2.01).

There was no substantial difference between the two groups. In terms of vitamin E, the sick group had a lower arithmetic mean (23.52±1.25) compared to the control group (30.54±2.28), indicating a significant distinction within the groups.

Patients with RA had significantly lower vitamin E and D levels, which suggests that their antioxidant defense systems are weaker and oxidative stress is higher.

Keywords: Rheumatoid Arthritis, vitamin D3, vitamin E, Sjögren's Syndrome.

Introduction

Rheumatoid arthritis (RA), is a complex autoimmune condition characterized the immune system mistakenly attacks the body's own tissues, primarily the lining of the joints (synovium) and systemic inflammation (1). One of the most frequent systemic autoimmune illnesses is defined by rheumatic autoimmune diseases, which can lead to joint destruction and extra-articular symptoms if not managed (2). The first recorded instance of rheumatoid arthritis (RA) may date back to Antiquity in the Greek and Roman Empires, when the Latin phrase "rheuma" or "rheumatism" was used to describe arthralgia caused by the humors instability (3).

Oily eggs, seafood, milk and yogurt, and enriched foods contain vitamin D3 (VD3), which acts as a secosteroid. In humans, the skin produces the majority of vitamin D from 7-dehydrocholesterol, that is then modified by UV radiation to form parental vitamin D3 (VD3). Vitamin D insufficiency was first identified as a cause of rickets due to its role in the mineralization of bone and calcium control. Recent research has connected vitamin D levels to a variety of health conditions, including extra skeletal ramifications. Vitamin D may influence an autoimmune disorder pathogenesis (4-6).

Vitamin D3 has been shown in studies to block antigen expression as well as increase and control T cell activity, indicating that it is a critical regulator of different immune system genes. VD primarily reduces the onset and progression of RA by lowering cytokines levels (7,8). Dietary antioxidants micronutrients operate as scavenger of reactive oxygen radicals that are potentially protecting against free radical- mediated harm to tissue in an inflammatory join Antioxidants may potentially prevent the appearance of RA. Vitamin E (α-tocopherol) is the primary lipid-soluble, chain-breaking antioxidant present in biological membranes. Lipids are essential components of normal fluid in synovial spaces, and synovial lipid changes can occur in RA (9). Low vitamin E levels may therefore be deleterious in inflammatory arthritis. Individuals with inflammatory arthritis had significantly reduced mean synovial tissue fluid concentrations of a substance called compared to controls, indicating that it gets absorbed inside an inflamed join (10).

A shortage of vitamin D3 could increase the chance of developing RA. Recent research has focused on the significance of vitamin D insufficiency in the pathophysiology of RA, in addition to the link between vitamins D deficit and RA activity. RA is an inflammation illness that involves flaring and remissions, with flares accompanied with pain. Vitamin D insufficiency has also been connected with generalized pain in the muscles and bones (11).

Sjögren's Syndrome (SS) constitutes a recurrent inflammatory disorder that mostly affects exocrine glands. It is more common in middle-aged women and can manifest as primary Sjögren's Syndrome (pSS) or secondary Sjögren's Syndrome (sSS) in combination with additional arthritic illnesses such as autoimmune illnesses arthritis (RA), systematically lupus erythematosus (SLE), systematic sclerosis, poly mixed cryoglobulinemia, and chronic arthritis nodosa.

Rheumatoid arthritis (RA) is the predominant connective tissue disease linked to Sjögren's syndrome (SS) and can cause further signs known as sicca signs and symptoms, which include redness of the optic nerve (keratoconjunctivitis sicca) and parched throat (xerostomia) (12).

Aim of the Study: Assessment of Vitamin D3 and Vitamin E Levels in Patients with Rheumatoid Arthritis.

Subjects and methods:

Collection of Samples

A total of 80 patients' sufferers diseased with rheumatoid arthritis, and 20 control from a consultant at Baqubah General Hospital and private clinics from each case. Blood samples were collected from October 2024 to April 2025 from the laboratory at Baqubah General Hospital and private clinics. Written informed consent was obtained from all participants. Blood samples from each case were taken for levels of vitamin D3 and E is measured using an assay.

Estimation of Vit E in serum

To estimate vitamin E in serum using ELISA, prepared samples and standards, add them to a pre-coated antibody plate, then incubated with a detection reagent. Washed the plate to remove unbound components, add a second detection reagent (antibody conjugate), and washed again. Finally, add the TMB substrate solution, incubated to develop color, added a stop solution, and measured the color intensity at 450 nm using a spectrophotometer.

Estimation of Vitamin D3

The Roche Diagnostics Vitamin D total assay quantifies the total 25-OH vitamin D in human

blood and plasma using competitive electrochemiluminescence protein binding.
The assay captures 25-OH D3 and 25-OH D2 with a vitamin D3 binding protein (VDBP) (Roche Diagnostics, Mannheim, Germany).
The assay requires 27 minutes of 3-step incubation. Pretreatment reagent releases VDBP-bound 25-OH vitamin D in step 1.
the sample is mixed with a special VDBP that has a ruthenium label to create a compound with 25-OH vitamin D. Streptavidin-coated microparticles and biotin-labelled 25-OH vitamin D are added in the third incubation step. The free sites of ruthenium-labeled VDBP become occupied, forming a complex. Vitamin D binding protein and biotinylated 25-OH vitamin D form the complex. Biotin-streptavidin interaction binds the complex to the solid phase. With Roche Diagnostics' quality control materials, between-day precision was CV = 4.9% and 1.9% at mean concentrations of 43.3 and 105105 L/L. Our lab verified both tests for precision, linearity, and accuracy using CLSI methods.
The study determined reference ranges as follows, based on the recommendations from the American Society for Bone and Mineral Research's 28th Annual Meeting in 2006 and the Canadian consensus conference on osteoporosis in 2006: Deficiency: <25 nmol/L, Optimal/Sufficiency: 75-200, Insufficiency: 25-75, and Toxicity: >250. This study was considered. The newest IOM dietary reference consumption guidelines the study examined vitamin D3.
At least 50 nmol/L of serum 25(OH)D is sufficient, according to the latest IOM

guidelines. Spectrophotometric techniques using redox reactions to produce colored

compounds, can also be used to determine of Vitamin D3 levels.

Statistical Analysis

The SPSS (2019) (24) package was deployed to analyze the impact of different groups on study measures. The T-test was performed to detect significant differences between means. In this study, the Chi-Square test was utilized to determine the significance of proportion differences.

Results

The findings regarding determining vitamin D3 as well as E concentrations were variable. The participants with illness group reported a numerical average for vitamins D3 (21.18 ± 1.10), whereas the other group had an arithmetic mean (24.35 ± 2.01). There was no statistically significant difference comparing the two categories. In terms of vitamin E, the sick group had a lower arithmetic mean (23.52±1.25) compared to the control group (30.54±2.28), indicating a significant distinction amongst the group's tables (1).

The investigation documented arithmetic mean values for dry eye syndrome in patients and the control the group (1.85±0.045 and 1.04±0.040, respectively). Patients had a higher mathematics mean than those in the control group, indicating an important distinction. The sick group had a greater numerical average for skin condition (1.70±0.05) compared to the control group (1.40±0.10). This difference was highly significant. The arithmetic mean for dry mouth was 1.68±0.05 in patients and 1.44±0.101 in the control group, indicating a significant distinction amongst the two groups. The results showed no a significant difference among the inherited. and cigarette smoking factors, with the mathematical mean in patients (1.32±0.054) and (1.53±0.057), accordingly, compared to the control group (1.32±0.09), (1.44±0.10) as shown in table (2).

Table 1: Descriptive Statistics for (Vitamin D3 and Vitamin E) in patients/rheumatoid arthritis as well as control groups

Study groups	No.	Mean±SD (Vit D3)	Mean±SD (Vit E)
control	**20**	24.35± 2.01[a]	30.54±2.28[a]
Patients	**80**	21.18± 1.10[a]	23.52±1.25[b]
P-value		** **0.01**	* **0.04**

*a, b: Different letters within the same row indicate significant differences between study groups at the significance level (P≤0.05).

** Highly significant differences

* Significant differences

Table 2: Descriptive Statistics between individuals with autoimmune arthritis with the control groups according to symptoms

Study groups	No.	Mean±SD				
		(dry eyes)	(dry skin)	(dry mouth)	(genetic factor)	(smoker)
control	**20**	1.04± 0.040[b]	1.40± 0.10[b]	1.44± 0.101[b]	1.32± 0.09[a]	1.44± 0.10[b]
Patients	**80**	1.81± 0.045[a]	1.70± 0.05[a]	1.68± 0.05[a]	1.32± 0.054[a]	1.53± 0.057[b]
P-value		** **0.01**	** **0.01**	* **0.04**	**n.s.** **0.07**	**n.s.** **0.07**

*a, b: Different letters within the same row indicate significant differences between study groups at the significance level (P≤0.05).

** Highly significant differences
* Significant differences

A comparison of symptoms between individuals with in patients/rheumatoid arthritis and healthy controls was conducted, with the results presented in Table (2).

Discussion

In order to lower serum vitamin D3 levels in people with rheumatoid arthritis and compare them to normal, Qadir et al. (2024) conducted this study (13). A prior investigation according to Yassin et al. (2014), vitamin D3 deficiency has been more widespread among people who had RA than in healthy controls, while the mean blood vitamins D3 level in those suffering from RA was found to be relatively low when compared to healthy volunteers (14). Low D3 levels have been recognized as a risk factor for RA progression. Vitamin D3 deficiency or inadequacy was substantially more common in RA patients than in healthy controls, with mean blood vitamin D concentrations significantly lower (15). Vitamin D3 plays a regulating position, and located on many immune system cells. Immunomodulation occurs by triggering T and B cells. This could be attributed to lower D3 vitamin levels reported in patients with high disease activity levels due to insufficient exposure to direct sunlight. This study includes no information on exposures to the sun. However, this correlation maintains even after correcting for age, gender, and use of medicines, suggesting a robust connection (16).

Previous investigations by Ketfi et al. (2021) found that inadequate vitamin E levels may constitute an indicator for RA, with higher risks associated with inadequate amounts of α-tocopherol and the pigment beta-car (17). In the study by Zamudio-Cuevas et al. (2022), high-dose vitamin E therapy reduced the progression

of the disease in RA patients. In addition, Jean et al. (2017) proposed that intake of vitamin E or an antioxidant called beta-car was inversely connected with the likelihood of getting RA in individuals compared to control groups (18). The current study aimed to investigate the connection among eye dryness, skin that is dry, and dry swallowing and the disease activity in patients with RA. The findings of this study indicate that those diagnosed with RA displayed dryness with an extremely significant difference P-value=0.01 in the signs of dryness in individuals with RA compared with control groups. Kang et al., (2018) ruptured drop of water height has been noticed as a significant marker of a dry eye test, the uncovered configuration needed by the frequently employed interventional optical surfaces comprehensive analyzer may alter lacrimal height measurements due to impulse tearing fluids (19). Although participants in the study by Lai, Shih-Chung, et al. (2023) had subjectively dry eye symptoms and indicators, reflex production of tears was near to its regular level, which could explain such outcomes. RA patients continue in the condition of reflexive production of tears for a long time; hence, the tear secretion continues to lay in the normal range; RA participants may not notice a decreased tear production, and symptoms associated with psychologically dry eye may have been caused by other sources (20).

Protudjer et al. (2022) showed that people with a condition called rheumatoid arthritis (RA) have considerably greater mouth dryness symptoms than those in the control group. Furthermore, one study found that dryness in the mouth was the least common oral symptom among those using the medicine (21).

Genetic markers associated with auto immune disorders play a significant role in the development of persistent inflammation. The majority of traits are decided by a person's genotype as well as their surroundings. External factors encompass any details outside of DNA that contribute to a patient's traits. They are highly broad and can affect in various ways, both alone and in connection with heredity (22). Rheumatoid arthritis was a complex illness that affects around 1% of the global population. HLA-DR4 is a key gene underlying the onset of rheumatoid arthritis. In rheumatoid arthritis individuals of European origin, up to 60-70% have the HLA-DR4 gene, compared to 30% of the general population (23).

In addition, Schäfer this study discovered no statistically significant difference (P-value=0.07) in the genetic cause of patients with RA compared to control groups. Smokers have higher levels of pro-inflammatory proteins known as inflammatory mediators in their own bodies. These factors contribute to both the joint and bodily harm associated with RA. Tobacco smoke causes the body to release a variety of peptides connected to activation in RA (24). Marshall et al., reported that tobacco smoke does trigger the release of certain peptides that contribute to the activation of rheumatoid arthritis (RA). Specifically, smoking can lead to the production of citrullinated proteins, which then become targets for the immune system, triggering, inflammation and potentially leading to the development or exacerbation of RA (25).

Conclusion:

The vitamin E levels were considerably lower in RA patients, indicating higher oxidative stress and reduced antioxidant defense systems.

The levels of vitamin D3 significantly lower in those with RA, although the distinction was not statistically noteworthy.

RA patients showed a greater degree of dryness (ocular, cutaneous, and mouth), possibly due to secondary RA itself.

These manifestations have a significant impact on satisfaction existence and may be early indicators of autoimmune illnesses.

Conflict of Interest: None

Funding: Nil

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