Ministry of Higher Education
And Scientific Research
Hilla University College
Department of Medical
Laboratory Technologies

# The role of vitamin D in regulating the immune response for patients with rheumatoid arthritis

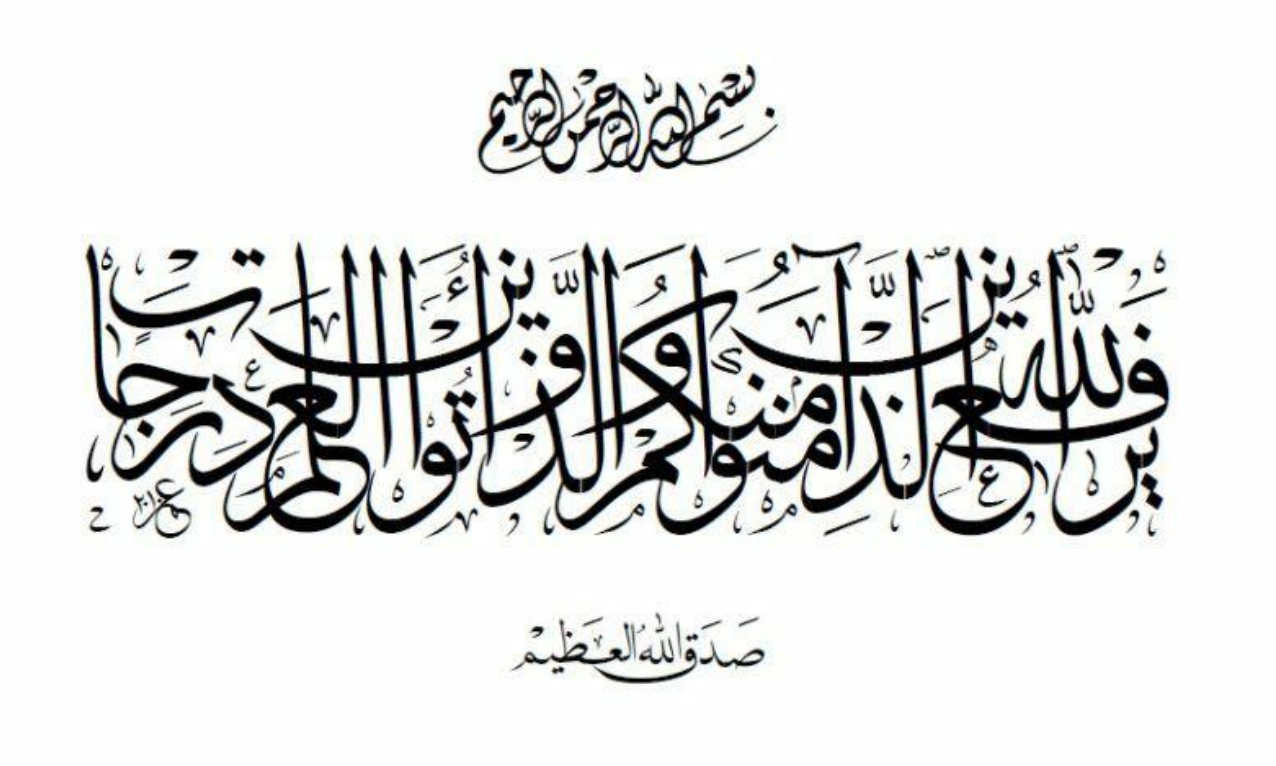
Submitted to the Hilla University College Department of Medical Laboratory Technologies
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**سورة المجادلة الأية (١١)**

## Dedication

**To....**

Everyone who, through his work, illuminated the mind of others or guided him with the correct answer to the confusion of those who asked him, demonstrated, through his grace, the humility of scholars, and with his generosity, the grace of knowledgeable people. I dedicate this humble work to my father, who never skimped on anything, and to my mother, who provided me with tenderness and love. My university journey has come to an end after fatigue and hardship, and here I am concluding my graduation thesis with all the energy and energy I would not have done without the grace of God. Praise be to God at the beginning and at the end.

## Thanks, and appreciation

I extend my sincere thanks to the Deanship of Hilla University College, represented by Professor..... and the Head of the Medical Laboratories Department, represented by the respected Professor Dr. Furqan....., and to my esteemed professors, in particular, I particularly mention my research supervisor, Ms. Maysaa Al-Qazwini. Because of the great care they showed during the study and research stages, I ask God to help them do what is best.

This research is nothing but a small effort, and I do not claim perfection in it, as imperfection is a characteristic of human beings, and I have every hope that the respected members of the discussion committee will evaluate and correct it to present it in the best light. They have my sincere thanks and gratitude for their efforts in that evaluation and correction.

## The aim of this study

Is to summarize the relationship between vitamin D and the development and outcome of disease Rheumatoid arthritis and the effect of vitamin D on the symptoms and signs of this disease. Epidemiological studies have shown that there is a possible association between low exposure to sunlight Especially ultraviolet B rays and a diet that contains a small amount of... Vitamin D and the risk of developing rheumatoid arthritis. In this study, it was found that there is a relationship between the concentration of vitamin D in the serum and the probability of infection the disease increases as the concentration of vitamin D in the serum increases through adequate exposure to sunlight Taking nutritional supplements containing vitamin D largely prevents the risk of developing inflammatory disease Rheumatoid arthritis

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# *Chapter One*

# *Introduction*

# Chapter One:

## Background:

Vitamin D, 'the sunshine vitamin', is a fat-soluble derivative of steroid 7-dehydocholesterol with autocrine, paracrine and endocrine functions. Upon exposure to sunlight, vitamin D absorbs ultraviolet (UV) light (~280 to 315 nm) and gets converted to PR calciferol in the skin. Most of the PR calciferol eventually isomerizes into cholecalciferol (vitamin D3) through thermal conversion. Ergosterol is another commonly occurring steroid in plants, which is activated by irradiation to produce ergocalciferol (vitamin D2). Both vitamin D3 formed in the skin and absorbed from digestive tract are transported to the liver where they are hydroxylated at carbon 25 to form calcidiol (also called 25 hydroxy vitamin D3 abbreviated as 25(OH)D) by liver 25-hydroxylase, CYP2R1 and CYP27A1. 25(OH)D is the major circulating vitamin D metabolite and a reliable indicator of vitamin D status (Holick et al., 1980).

Vitamin D and its prohormones have been the focus of a growing number of studies in past years, demonstrating their function not only in calcium metabolism and bone formation, but also their interaction with the immune system, which is not surprising, since vitamin D receptors are expressed in different tissues, such as brain, heart, skin, bowel, gonads, prostate, breasts, and immune cells, as well as bones, kidneys, and parathyroid glands (Jones & Twomey, 2008). Many studies have related vitamin D deficiency with several autoimmune disorders, including insulindependent diabetes mellitus (IDDM), multiple sclerosis (MS), inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), and rheumatoid arthritis (RA) (Jones & Twomey, 2008) (Lips, 2004). As the vitamin D receptor is expressed on immune cells (B cells, T cells, and antigen-presenting cells), and these immunologic cells are all capable of synthesizing the active vitamin D metabolite, vitamin D has the capability of

acting in an autocrine manner in a local immunologic milieu. Vitamin D can modulate the innate and adaptive immune responses. Deficiency in vitamin D is associated with increased autoimmunity and an increased susceptibility to infection (Aranow, 2011).

Vitamin D deficiency, which causes an imbalance in bone remodeling, is a global public health problem and its frequency is increasing. Due to the pleiotropic effects of vitamin D, its deficiency is related to a higher risk of cardiovascular diseases (Buleu et al., 2019) (Bahrami et al., 2020). infectious diseases, and autoinflammatory diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS). In addition, vitamin D taken for the treatment and prevention of disease has been debated, given its immunosuppressive effect. Anti-cancer effects of vitamin D have found application in cancer treatment (Jeon & Shin, 2018). An increased intake of vitamin D supplements by the general population and a growing number of prescriptions of therapeutic doses (including very high doses) without medical monitoring might result in a greater risk of exogenous hypervitaminosis D, with symptoms of hypercalcemia also known as vitamin D toxicity (VDT) (Marcinowska-Suchowierskaetal.,2018).

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disease that predominantly affects the synovial joints, causing significant morbidity and shortened life expectancy (Harris Jr, 1990). Although its cause is not fully understood, it has been established that genetic and environmental factors contribute to the pathogenesis of RA (Zamanpoor, 2019).The etiology or cause of RA is unknown and many cases are believed to result from an interaction between genetic factors and environmental exposures (DiBenedetti et al., 2015) (Silman & Hochberg, 2001). The incidence of RA is typically 2–3-times higher in women than men and the onset of RA, in both women and men, is highest among those in their sixties (Silman&Hochberg,2001).

The diagnosis of rheumatoid arthritis is primarily clinical. The typical presentation is polyarticular, with pain, stiffness, and swelling of multiple joints in a bilateral, symmetric pattern. A minority of patients present with oligoarticular asymmetric involvement.1 The onset is usually insidious, with joint symptoms emerging over weeks-months and often accompanied by anorexia, weakness, or fatigue. Patients usually note morning stiffness lasting more than an hour. Commonly involved joints are the wrists, proximal interphalangeal, metacarpophalangeal, and metatarsophalangeal joints, with distal interphalangeal joints and spinal joints usually spared (Harris, 2000).

# *Chapter Two*

# Chapter Two:

## 2.1. Introduction

Historically, vitamin D has been known for its role in the mineralization of teeth and bones through regulation of calcium and phosphorus homeostasis. More recently, there is emerging evidence of the role of vitamin D in protection against risk for malignant neoplasms, cardiovascular disease, and diabetes, along with osteoporosis and other bone disorders. Thus, progression of skeletal and no skeletal diseases may be influenced by circulating levels of vitamin D, based on the discovery of more than 2000 genes in the human genome that respond to vitamin D.Redefining what is considered to be a sufficient plasma level of 25-hydroxy vitamin D would potentially reclassify more people as vitamin D insufficient and trigger the need for treatment and monitoring of vitamin D levels. The relationship of blood vitamin D levels to these disorders is not entirely clear, so standardization of laboratory methods for vitamin D analysis and redefining the reference ranges that indicate health and disease are of utmost importance [Holick, 2005].

Vitamin D is a ketosteroid hormone involved in bone and calcium metabolism. It is involved in the regulation of calcium homeostasis, as it regulates calcium absorption from the gastrointestinal system [Holick, 2011]. The hormone is synthesized in the skin by the action of ultraviolet irradiation [Mason et al. 2011]. Vitamin D has extra skeletal effects as well [Fernandes de Abreu et al. 2009; Hewison, 2012]. The nonclassical actions of vitamin D are currently under discussion. Vitamin D has been found to have immunomodulatory actions [Bartley, 2010; Bikle, 2011].

Vitamin D deficiency has been shown to be correlated with the appearance of autoimmune diseases, such as diabetes mellitus type 1 and multiple sclerosis [Jankosky et al. 2012].

## 2.2. Vitamin D normal range

The normal range of 25-hydroxy vitamin D is measured as nanograms per milliliter (ng/mL). Many experts recommend a level between 20 and 40 ng/mL. Others recommend a level between 30 and 50 ng/mL.

The examples above are common measurements for results of these tests. Normal value ranges may vary slightly among different laboratories. Some laboratories use different measurements or test different samples. Talk to your doctor about the meaning of your specific test results, and whether you may need vitamin D supplements.

Many people are confused by the way these tests are reported.

- 25 hydroxy vitamin D3 (cholecalciferol) is the vitamin D that your own body has made or that you absorbed from an animal source (such as fatty fish or liver) or a cholecalciferol supplement.
- 25 hydroxy vitamin D2 (ergocalciferol) is the vitamin D that you have absorbed from foods fortified with plant vitamin D or from an ergocalciferol supplement.
- The two hormones (ergo- and cholecalciferol) work similarly in the body. The important value is the total 25 hydroxy vitamin D level in your blood (Bouillon R. Vitamin D:)

### 2.2.1. What Abnormal Results Mean

A lower-than-normal level can be due to a vitamin D deficiency, which can result from:

- Lack of skin exposure to sunlight, darkly pigmented skin, or consistent use of high-SPF sunscreen.
- Lack of enough vitamin D in the diet.
- Liver and kidney diseases.
- Poor food absorption.
- Use of certain medicines, including phenytoin, phenobarbital, and rifampin.
- Poor vitamin D absorption due to advanced age, weight-loss surgery, or conditions in which fat is not absorbed well

A low vitamin D level is more common in African American children (especially in the winter), as well as in infants who are breastfed only.

A higher-than-normal level may be due to excess vitamin D, a condition called hypervitaminosis D. This is most commonly caused by taking too much vitamin D. It can result in too much calcium in the blood (hypercalcemia). This leads to many symptoms and kidney damage (Chernecky CC, Berger BJ).

### 2.2.2. Risks

There is little risk involved with having your blood taken. Veins and arteries vary in size from one person to another and from one side of the body to the other. Taking blood from some people may be more difficult than from others.

Other risks associated with having blood drawn are slight, but may include:

- Excessive bleeding.
- Multiple punctures to locate veins.
- Fainting or feeling lightheaded.
- Hematoma (blood accumulating under the skin) .
- Infection (a slight risk any time the skin is broken) (US Preventive Services Task Force) .

## 2.3. Rheumatoid arthritis

Rheumatoid arthritis is a chronic inflammatory disorder that can affect more than just your joints. In some people, the condition can damage a wide variety of body systems, including the skin, eyes, lungs, heart and blood vessels. An autoimmune disorder, rheumatoid arthritis occurs when your immune system mistakenly attacks your own body's tissues. Unlike the wear-and-tear damage of osteoarthritis, rheumatoid arthritis affects the lining of your joints, causing a painful swelling that can eventually result in bone erosion and joint deformity. The inflammation associated with rheumatoid arthritis is what can damage other parts of the body as well. While new types of medications have improved treatment options dramatically, severe rheumatoid arthritis can still cause physical disabilities (Handout on Health: Rheumatoid Arthritis).

### 2.3.1. Symptoms

Signs and symptoms of rheumatoid arthritis may include:

- Tender, warm, swollen joints.
- Joint stiffness that is usually worse in the mornings and after inactivity.
- Fatigue, fever and loss of appetite

Early rheumatoid arthritis tends to affect your smaller joints first — particularly the joints that attach your fingers to your hands and your toes to your feet.

As the disease progresses, symptoms often spread to the wrists, knees, ankles, elbows, hips and shoulders. In most cases, symptoms occur in the same joints on both sides of your body.

About 40% of people who have rheumatoid arthritis also experience signs and symptoms that don't involve the joints. Areas that may be affected include:

- Skin
- Eyes
- Lungs
- Heart
- Kidneys
- Salivary glands
- Nerve tissue
- Bone marrow
- Blood vessels

Rheumatoid arthritis signs and symptoms may vary in severity and may even come and go. Periods of increased disease activity, called flares, alternate with periods of relative remission — when the swelling and pain fade or disappear. Over time, rheumatoid arthritis can cause joints to deform and shift out of place ( Wang H,2016).

### 2.3.2. Causes

Rheumatoid arthritis is an autoimmune disease. Normally, your immune system helps protect your body from infection and disease. In rheumatoid arthritis, your immune system attacks healthy tissue in your joints. It can also cause medical problems with your heart, lungs, nerves, eyes and skin. Doctors don't know what starts this process, although a genetic component appears likely. While your genes don't actually cause rheumatoid arthritis, they can make you more likely to react to environmental factors — such as infection with certain viruses and bacteria — that may trigger the disease (Singh JA, Wells 2020).

### 2.3.3. Risk factors

Factors that may increase your risk of rheumatoid arthritis include:

- Your sex. Women are more likely than men to develop rheumatoid arthritis.
- Age. Rheumatoid arthritis can occur at any age, but it most commonly begins in middle age.
- Family history. If a member of your family has rheumatoid arthritis, you may have an increased risk of the disease.
- Smoking. Cigarette smoking increases your risk of developing rheumatoid arthritis, particularly if you have a genetic predisposition for developing

the disease. Smoking also appears to be associated with greater disease severity.

- Excess weight. People who are overweight appear to be at a somewhat higher risk of developing rheumatoid arthritis (Majithia V, Geraci SA (November 2007).

### 2.3.4. Complications

Rheumatoid arthritis increases your risk of developing:

- Osteoporosis. Rheumatoid arthritis itself, along with some medications used for treating rheumatoid arthritis, can increase your risk of osteoporosis — a condition that weakens your bones and makes them more prone to fracture.
- Rheumatoid nodules. These firm bumps of tissue most commonly form around pressure points, such as the elbows. However, these nodules can form anywhere in the body, including the heart and lungs.
- Dry eyes and mouth. People who have rheumatoid arthritis are much more likely to develop Sjogren's syndrome, a disorder that decreases the amount of moisture in the eyes and mouth.
- Infections. Rheumatoid arthritis itself and many of the medications used to combat it can impair the immune system, leading to increased infections. Protect yourself with vaccinations to prevent diseases such as influenza, pneumonia, shingles and COVID-19.
- Abnormal body composition. The proportion of fat to lean mass is often higher in people who have rheumatoid arthritis, even in those who have a normal body mass index (BMI).

- Carpal tunnel syndrome. If rheumatoid arthritis affects your wrists, the inflammation can compress the nerve that serves most of your hand and fingers.
- Heart problems. Rheumatoid arthritis can increase your risk of hardened and blocked arteries, as well as inflammation of the sac that encloses your heart.
- Lung disease. People with rheumatoid arthritis have an increased risk of inflammation and scarring of the lung tissues, which can lead to progressive shortness of breath.
- Lymphoma. Rheumatoid arthritis increases the risk of lymphoma, a group of blood cancers that develop in the lymph system (Majithia V, Geraci SA (November 2007).

## 2.4. Erythrocyte sedimentation rate

The erythrocyte sedimentation rate (ESR or sed rate) is the rate at which red blood cells in anticoagulated whole blood descend in a standardized tube over a period of one hour. It is a common hematology test, and is a non-specific measure of inflammation. To perform the test, anticoagulated blood is traditionally placed in an upright tube, known as a Westergren tube, and the distance which the red blood cells fall is measured and reported in millimetres at the end of one hour (labtestsonline.org. Retrieved 2019-12-12).

Since the introduction of automated analyzers into the clinical laboratory, the ESR test has been automatically performed. The ESR is influenced by the aggregation of red blood cells: blood plasma proteins, mainly fibrinogen, promote the formation of red cell clusters called rouleaux or larger structures (interconnected rouleaux, irregular clusters). As according to Stokes' law the sedimentation velocity varies like the square of the object's diameter, larger

aggregates settle faster. While aggregation already takes place at normal physiological fibrinogen levels, these tend to increase when an inflammatory process is present, leading to increased ESR.The ESR is increased in inflammation, pregnancy, anemia, autoimmune disorders (such as rheumatoid arthritis and lupus), infections, some kidney diseases and some cancers (such as lymphoma and multiple myeloma). The ESR is decreased in polycythemia, hyperviscosity, sickle cell anemia, leukemia, chronic fatigue syndrome (Saha, Amit K; Schmidt, Brendan R; Wilhelmy,2019).

### 2.4.1. Normal values

Note: mm/h. = millimeters per hour.

Westergren's original normal values (men 3 mm/h and women 7 mm/h)] made no allowance for a person's age. Later studies from 1967 confirmed that ESR values tend to rise with age and to be generally higher in women. Values of the ESR also appear to be slightly higher in normal populations of African-Americans than Caucasians of both genders. Values also appear to be higher in anemic individuals than non-anemic individuals Kanfer EJ, Nicol BA (January 1997).

### Adults

The widely used rule calculating normal maximum ESR values in adults (98% confidence limit) is given by a formula devised in 1983 from a study of ≈1000 individuals over the age of 20: The normal values of ESR in men is age (in years) divided by 2; for women, the normal value is age (in years) plus 10, divided by 2.

Other studies confirm a dependence of ESR on age and gender, as seen in the following:

ESR reference ranges from a large 1996 study of 3,910 healthy adults (NB. these use 95% confidence intervals rather than the 98% intervals used in the study used to derive the formula above, and because of the skewness of the data, these values appear to be less than expected from the above formula):

| Age | 20 | 55 | 90 |
|---|---|---|---|
| Men—5% exceed | 12 | 14 | 19 |
| Women—5% exceed | 18 | 21 | 23 |

## Children

Normal values of ESR have been quoted as 1to 2 mm/h at birth, rising to 4 mm/h 8 days after delivery, and then to 17 mm/h by day 14.

Typical normal ranges quoted are:

- Newborn: 0 to 2 mm/h

Neonatal to puberty: 3 to 13 mm/h, but other laboratories place an upper limit of 20 (Pediatric Inflammatory Bowel Disease Collaborative Research Group 2007) .

# *Chapter Three*

# Chapter Three

# Materials and Methods

## 3.1. Material:

### 3.1.1. Instruments & equipment's

| No | Instruments | Company | Country |
|---|---|---|---|
| 1 | pipet | Raybiotech | U.S.A |
| 2 | syringe | Made In China Supplies | China |
| 3 | hematological automated analyzer device | sysmex | Japan |
| 4 | Pipette tip | Tipone | China |
| 5 | gloves | yellow | China |
| 6 | Biochemical automated analyzer device | VIDAS | Italy |

Table 1: Tools and equipments used in the current study

### 3.1.2. Reagents

Table 2: Reagents used in this research.

| No | Reagents | company | country |
|---|---|---|---|
| 1 | RF | LABKIT | Italy |
| 2 | ESR | Al. Malak company | Jordan |

## 3.2. Methods:

### 3.2.1. Patients (Study group):

A total number of 15 patients (subjects) were enrolled in this study during the period (7/12/2023) to (15/2/2024). These patients attended in AL-Shomali general hospital, each patient suffering from fatigue and headache, joint pain.

### 3.2.2. Sample collection:

Blood specimens were collected by venipuncture; five ml of venous were drawn by disposable syringe under sterilization technique and putting in gel and EDTA tubes then gel tube allowed to clot after that serum was separated by centrifugation 2500 rpm for 10 minute. The serum has been collected in Eppendorf tube then stored at -20 oC to be used for biochemical and immunological tests to determine concentration of Vit. D in serum and Ramotoid factor (RF). EDTA tube used for hematological purposes (WBC and ESR).

### 3.2.3. RF:

**Qualitative Method:**

Allow the reagents and samples to reach room temperature. The Sensitivity of the test may be reduced at low temperatures. Place 50 ul of the sample and one drop of each Positive and Negative control into separate circles on the slide test.

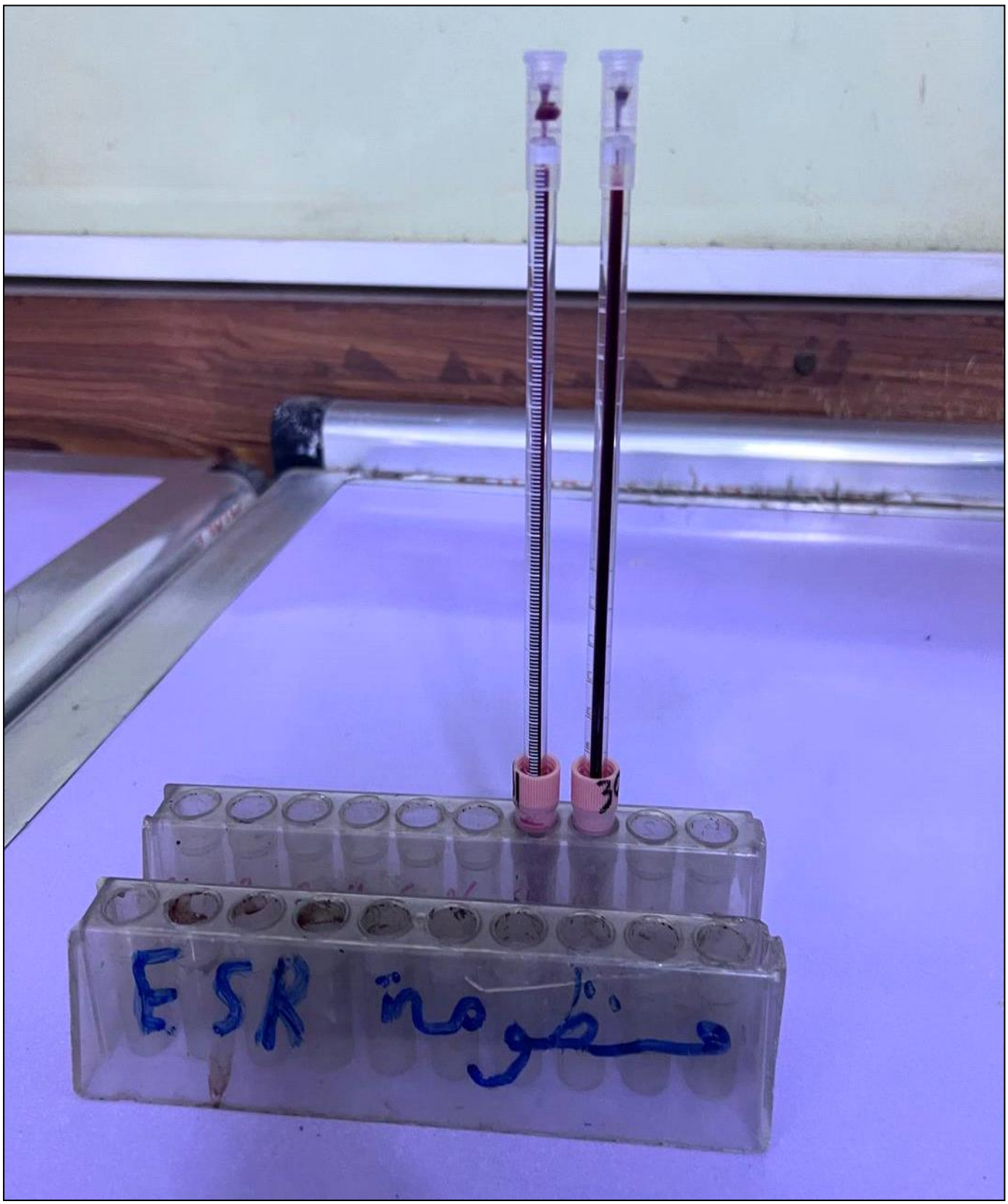
Swirl the RF Latex reagent rigorously before using and add one drop (50 ul) next to the sample to be tested. Mix the drops with a stirrer, spreading them over the entire surface of.

Place the slide on a mechanical rotator at 80 - 100 r.p.m for 2 minutes. False positive results could appear if the test is read later than 2 minutes.

### 3.2.4. ESR pippete set:

Remove the pink stopper on the prefilled vial (0.2ml of 3.8% sodium citrate is used as diluent).

1. Using a transfer pipette, fill the vial to the bottom of the indicated fill line with 0.8ml of blood to make required 4:1 dilution.
2. Replace pierceable stopper and gently invert several times to mix and shake up.
3. Place vial in its rack on a level surface. Carefully insert the pipette through the pierceable stopper until the pipette comes in contact with the bottom of the vial. The diaphragm of the pink stopper is calibrated to break under the light pressure made by inserting the pipette. The pipette will auto zero the blood and any excess will flow into the reservoir compartment.
4. To ensure proper results, it's essential that the pipette makes FIRM contact with the bottom of the vial. Let samples stand for exactly one hour and then read the numerical results of the erythrocyte sedimentation in millimeters.Dispose of the properly after use.


ESR
مضومة

## 3.3. WBC and Vitamin D3:

WBC estimated by hematological automated analyzer device (sysmex, Japan). Vit D3 measured by biochemical automated analyzer device (VIDAS, Italy).




اسم الجهاز
Vidas
الشركة المجهزة (المكتب العلمي)
اعالي الفرات
المنشأ
ايطالي
الرقم التسلسلي
IVD3003907
موقع نصب الجهاز
المختبر
سنة التجهيز
2012
سنة النصب
2012
VIDAS

# *Chapter Four*

## Chapter Four:

## 4.1. Discussion and Conclusion

| فيتامين D | RF | WBC | ESR | رقم العينة |
|---|---|---|---|---|
| 8.1 | + | 20000 | 143 | 1 |
| 8 | + | 3.7 | 65 | 2 |
| 24 | + | 11.3 | 5 | 3 |
| 8.1 | + | 25000 | 145 | 4 |
| 8.2 | + | 30000 | 141 | 5 |
| 7.1 | + | 30000 | 78 | 6 |
| 9.1 | + | 3.7 | 65 | 7 |
| 9 | + | 11.5 | 23 | 8 |
| 8 | + | 12000 | 25 | 9 |
| 10 | + | 15000 | 17 | 10 |
| 8.9 | + | 11000 | 29 | 11 |
| 7.3 | + | 16000 | 21 | 12 |
| 8.3 | + | 15000 | 27 | 13 |
| 8.7 | + | 17000 | 26 | 14 |
| 9.8 | + | 18000 | 24 | 15 |

### 4.1.1. Discussion

Rheumatoid arthritis is characterized by persistent synovitis, systemic inflammation and autoantibody production. Genetic factors and environmental factors are closely related to the incidence of rheumatoid arthritis. In developed countries, the prevalence of adult rheumatoid arthritis has increased yearly (Salaffi F, 2019).

Active rheumatoid arthritis that does not receive interventional treatment can cause joint damage and even disability. Decreased quality of life causes cardiovascular disease and other complications. Vitamin D is a fat-soluble hormone that has been extensively studied, but recent studies have found that vitamin D has a wide range of immune system activities and that it may be related to the pathogenesis of rheumatoid arthritis (Bellan M, 2017).

Lee et al. conducted a meta-analysis of the sensitivity of vitamin D levels to RA and RA activity in rheumatoid patients, indicating that vitamin D levels were negatively correlated with sensitivity to RA and RA activities. There are also studies based on rheumatoid arthritis that suggest that active vitamin D can be used as a parameter for regulating inflammation and that vitamin D has the potential to be a therapeutic biomarker and can even be used to track the disease progression and treatment effect of rheumatoid arthritis patients (Khajoei S, 2019)

In previous systematic reviews, no studies have evaluated VD as a supplement for the treatment of RA. In this study, through quantitative synthesis, we found that compared with the control group, RA patients treated with VD as complementary therapy seemed to experience more beneficial effects on the DAS28, TJC, and ESR. In contrast, the VAS, SJC, CRP and PTH showed no benefits. However, although there were no overall beneficial findings, several subgroups showed significant positive effects in terms of demographic

characteristics and interventions. Pain is a symptom that rheumatoid arthritis patients urgently need to improve. Previous studies have shown that nearly 90% of patients with rheumatoid arthritis regard pain as the first symptom requiring improvement (Heiberg T, Kvien TK. 2002).

The VAS score is a commonly used pain score in clinical work and can accurately reflect the degree of pain experienced by patients. Based on our combined research results, we found that VD supplementation did not significantly reduce the VAS in rheumatoid arthritis patients. However, in the subgroup analysis, we found that with a vitamin D intervention time> 12 w and vitamin D dose> 50,000 IU, in the VD intervention group, VAS was significantly reduced. Some experimental studies have shown that vitamin D significantly improves nociceptive thresholds and allodynia scores, supporting the analgesic effect of vitamin D on rheumatoid arthritis patients (Poisbeau P, 2019).

The DAS28 score has important significance as an evaluation indicator of the disease activity of rheumatoid arthritis patients and whether vitamin D is effective after drug treatment. Research analysis found that the DAS28 score of patients with rheumatoid arthritis was effectively reduced after vitamin D supplementation. In contrast, when the vitamin D dose was ≤50,000 IU or the duration was ≤12 w, there was no difference between the vitamin D supplement group and the control group. This may be because large-dose and long-term vitamin D supplementation can reduce the inflammatory factor response (Tabatabaeizadeh SA, Avan A, 2017).

Mainly through the differentiation of Toll-like receptors and T cells (mainly Th17 cells) to control the innate and adaptive immune system. It can even be used as an important regulator of various genes in the immune system. The SJC, TJC, and ESR are closely related to the DAS28 score (Prevoo ML, 1995).

Through comprehensive analysis, it was found that the TJC and ESR were significantly reduced in the vitamin D supplement group, which was consistent with the study that found that the DAS28 score was reduced. The ESR subgroup analysis does not support this finding. This may be because the small sample size reduced the reliability of the ESR subgroups. Further research is needed to clarify this connection and explore its potential explanation (Bjorkman MP, 2009).

Our study also focused on the effect of vitamin D supplementation on the self-inflammatory response and hormonal immune response of rheumatoid arthritis patients. Therefore, we systematically evaluated the levels of CRP and PTH in rheumatoid arthritis patients and found no significant improvement after VD supplementation. Bjorkman et al, and Moghimi et al, reported results consistent with this finding. CRP concentration is related to bone turnover but not to vitamin D status. Serum vitamin D levels in patients with rheumatoid arthritis may not be related to PTH secretion or activity (Moghimi J, Sadeghi A, 2012).

This study has several limitations. First, the overall sample size of this study is limited. Some of the included studies have a small sample size, and the level of evidence defined by GRADE method is not high. Second, most studies included did not assess the effects of sun exposure and dietary intake, and did not indicate whether the patient's rheumatoid arthritis was in the early or late stage, which may have affected the results of the meta-analysis. We conducted a sensitivity analysis of the included RCTs and found that two studies may be the source of most of the heterogeneity.

In both studies, the study design was a single-center study with a small number of participants, which may have had an impact on the overall measurement results. In addition, language and publication biases limited our research. Finally, the evaluation included only randomized controlled trials.

In the future, more research diversity is needed, such as cooperation between multiple centers, more rigorous clinical reports and prospective research.

We summarize the research status of vitamin D supplementation in patients with rheumatoid arthritis and provide data to support future clinical treatment and trials of rheumatoid arthritis.

Although this study shows that supplemental vitamin D can effectively control the DAS28, TJC, and ESR levels of patients with rheumatoid arthritis, the current evidence, potential bias due to the low quality of the research methods and the observed clinical heterogeneity of the examined studies suggest that these findings should be carefully investigated.

### 4.1.2. Conclusion:

Compared with the control interventions, vitamin D supplementation seemed to be an effective intervention for patients with rheumatoid arthritis.

Different doses of vitamin D and the duration of intervention will produce different effects.

More RCTs with rigorous research designs are needed to determine the efficacy of vitamin D supplementation in the treatment and improvement of symptoms and inflammatory responses in patients with rheumatoid arthritis and to apply vitamin D supplementation in daily interventions for rheumatoid arthritis patients to improve the symptoms of rheumatoid arthritis and other related chronic diseases.

## References:


- Aranow, C. (2011). Vitamin D and the immune system. Journal of Investigative Medicine, 59(6), 881–886.
- Bahrami, L. S., Ranjbar, G., Norouzy, A., & Arabi, S. M. (2020). Vitamin D supplementation effects on the clinical outcomes of patients with coronary artery disease: a systematic review and meta-analysis. Scientific Reports, 10(1), 12923.
- Bartley, J. (2010) Vitamin D: emerging roles in infection and immunity. Expert Rev Anti Infect Ther 8: 1359–1369.
- Bellan M, Sainaghi PP, Pirisi M. Role of vitamin D in rheumatoid arthritis. Adv Exp Med Biol. (2017) 996:155–68. 10.1007/978-3-319-56017-5_13.
- Bjorkman MP, Sorva AJ, Tilvis RS. C-reactive protein and fibrinogen of bedridden older patients in a six-month vitamin D supplementation trial. J Nutr Health Aging. (2009) 13:435–9.
- Bouillon R. Vitamin D: from photosynthesis, metabolism, and action to clinical applications. In: Jameson JL, De Groot LJ, de Kretser DM, et al, eds. Endocrinology: Adult and Pediatric. 7th ed. Philadelphia, PA: Elsevier Saunders; 2016:chap 59.
- Buleu, F. N., Luca, C. T., Tudor, A., Badalica-Petrescu, M., Caraba, A., Pah, A., Georgescu, D., Christodorescu, R., & Dragan, S. (2019). Correlations between vascular stiffness indicators, OPG, and 25-OH vitamin D3 status in heart failure patients. Medicina, 55(6), 309.
- Chernecky CC, Berger BJ. Vitamin D (cholecalciferol) -- plasma or serum. In: Chernecky CC, Berger BJ, eds. Laboratory Tests and Diagnostic Procedures. 6th ed. St Louis, MO: Elsevier Saunders; 2013:1182-1183.
- DiBenedetti, D. B., Zhou, X., Reynolds, M., Ogale, S., & Best, J. H. (2015). Assessing methotrexate adherence in rheumatoid arthritis: a cross-sectional survey. Rheumatology and Therapy, 2, 73–84.

- Erythrocyte Sedimentation Rate (ESR). labtestsonline.org. Retrieved 2019-12-12.
- Handout on Health: Rheumatoid Arthritis. National Institute of Arthritis and Musculoskeletal and Skin Diseases. August 2014. Archived from the original on June 30, 2015. Retrieved July 2, 2015.
- Harris Jr, E. D. (1990). Rheumatoid arthritis: pathophysiology and implications for therapy. New England Journal of Medicine, 322(18), 1277–1289.
- Harris, E. D. (2000). Clinical features of rheumatoid arthritis. Kelley's Textbook of Rheumatology.
- Heiberg T, Kvien TK. Preferences for improved health examined in 1,024 patients with rheumatoid arthritis: pain has highest priority. Arthritis Rheum. (2002) 47:391–7.
- Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357:266-228.
- Holick, M. (2011) Vitamin D: evolutionary, physiological and health perspectives. Curr Drug Targets 12: 4–18.
- Holick, M. F., MacLaughlin, J. A., Clark, M. B., Holick, S. A., Potts Jr, J. T., Anderson, R. R., Blank, I. H., Parrish, J. A., & Elias, P. (1980). Photosynthesis of previtamin D3 in human skin and the physiologic consequences. Science, 210(4466), 203–205.
- Jankosky, C., Deussing, E., Gibson, R. and Haverkos, H. (2012) Viruses and vitamin D in the etiology of type 1 diabetes mellitus and multiple sclerosis. Virus Res 163: 424–430.
- Jeon, S.-M., & Shin, E.-A. (2018). Exploring vitamin D metabolism and function in cancer. Experimental & Molecular Medicine, 50(4), 1–14.
- Jones, B. J., & Twomey, P. J. (2008). Issues with vitamin D in routine clinical practice. In Rheumatology (Vol. 47, Issue 9, pp. 1267–1268). Oxford University Press.

- Kanfer EJ, Nicol BA (January 1997). "Haemoglobin concentration and erythrocyte sedimentation rate in primary care patients" (Scanned & PDF). Journal of the Royal Society of Medicine. 90 (1): 16–8. doi:10.1177/014107689709000106. PMC 1296109. PMID 9059375.
- Khajoei S, Hassaninevisi M, Kianmehr N, Seif F, Khoshmirsafa M, Shekarabi M, et al.. Serum levels of adiponectin and vitamin D correlate with activity of rheumatoid arthritis. Mol Biol Rep. (2019) 46:2505–12.
- Lips, P. (2004). Which circulating level of 25-hydroxyvitamin D is appropriate? The Journal of Steroid Biochemistry and Molecular Biology, 89, 611–614.
- Majithia V, Geraci SA (November 2007). "Rheumatoid arthritis: diagnosis and management". The American Journal of Medicine. 120 (11): 936–939.
- Mason, R., Sequeira, V. and Gordon-Thomson, C. (2011) Vitamin D: the light side of sunshine. Eur J Clin Nutr 65: 986–993.
- Moghimi J, Sadeghi A, Malek M, Ghorbani R. Relationship between disease activity and serum levels of vitamin D and parathyroid hormone in rheumatoid arthritis. Endocr Regul. (2012) 46:61–6.
- Pediatric Inflammatory Bowel Disease Collaborative Research Group , Mack DR, Langton C, Markowitz J, LeLeiko N, Griffiths A, et al. (June 2007). "Laboratory values for children with newly diagnosed inflammatory bowel disease". Pediatrics. 119 (6):1113–9. doi:10.1542/peds.2006-1865. PMID 17545378. S2CID 5558076. Lay summary in: Bauchner H (July 13, 2007). "Lab Screening in Children with Suspected Inflammatory Bowel Disease". NEJM Journal Watch.
- Poisbeau P, Aouad M, Gazzo G, Lacaud A, Kemmel V, Landel V, et al. Cholecalciferol (Vitamin D3) reduces rat neuropathic pain by modulating opioid signaling. Mol Neurobiol. (2019) 56:7208–21.
- Prevoo ML, van 't Hof MA, Kuper HH, van Leeuwen MA, van de Putte LB, van Riel PL. Modified disease activity scores that include twenty-eight-joint

counts. Development and validation in a prospective longitudinal study of patients with rheumatoid arthritis. Arthritis Rheum. (1995) 38:44–8.

- Saha, Amit K; Schmidt, Brendan R; Wilhelmy, Julie; Nguyen, Vy; Do, Justin; Suja, Vineeth C; Nemat-Gorgani, Mohsen; Ramasubramanian, Anand K; Davis, Ronald W (2018-11-21). "Erythrocyte Deformability As a Potential Biomarker for Chronic Fatigue Syndrome". Blood. 132 (Suppl 1): 4874. doi:10.1182/blood-2018-99-117260. ISSN 0006-4971. Retrieved 2019-06-19.
- Salaffi F, Di Carlo M, Farah S, Di Donato E, Carotti M. Prevalence of frailty and its associated factors in patients with rheumatoid arthritis: a cross-sectional analysis. Clin Rheumatol. (2019) 38:1823–30.
- Silman, A. J., & Hochberg, M. C. (2001). Epidemiology of the rheumatic diseases. (Issue Ed. 2). Oxford University Press.
- Singh JA, Wells GA, Christensen R, Tanjong Ghogomu E, Maxwell L, Macdonald JK, Filippini G, Skoetz N, Francis D, Lopes LC, Guyatt GH, Schmitt J, La Mantia L, Weberschock T, Roos JF, Siebert H, Hershan S, Lunn MP, Tugwell P, Buchbinder R (February 2011).
- Tabatabaeizadeh SA, Avan A, Bahrami A, Khodashenas E, Esmaeili H, Ferns GA, et al.. High dose supplementation of vitamin D affects measures of systemic inflammation: reductions in high sensitivity C-reactive protein level and neutrophil to lymphocyte ratio (NLR) distribution. J Cell Biochem. (2017) 118:4317–22.
- US Preventive Services Task Force; Krist AH, Davidson KW, et al. Screening for vitamin D deficiency in adults: US Preventive Services Task Force Recommendation Statement. JAMA. 2021;325(14):1436-1442. PMID: 33847711 pubmed.ncbi.nlm.nih.gov/33847711/.
- Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, et al. (GBD 2015 Mortality and Causes of Death Collaborators) (October 2016).
- Zamanpoor, M. (2019). The genetic pathogenesis, diagnosis and therapeutic insight of rheumatoid arthritis. Clinical Genetics, 95(5), 547–557.

## الهدف من هذه الدراسة

هو تلخيص العالقة بين فيتامين D وتطور ونتائج اإلصابة بمرض التهاب المفاصل الروماتيدي وتأثير فيتامين Dعلى واعراض وعالمات هذه المرض. الدراسات الوبائية أظهرت ان هنالك ارتباط ممكن بين التعرض القليل ألشعة الشمس وخصوصا االشعة فوق البنفسجية نوع B والنظام الغذائي الذي يحتوي على كمية قليلة من فيتامين D وفي مخاطر اإلصابة بمرض التهاب المفاصل الروماتيدي. وفي هذه الدراسة وجد ان هنالك عالقة بين تركيز فيتامين D في المصل واحتمالية إلصابة بالمرض حيث ان زيادة تركيز فيتامين D في المصل من خالل التعرض الكافي ألشعة الشمس واخذ مكمالت غذائية تحتوي على فيتامين D يمنع الى حد كبير خطر اإلصابة بمرض التهاب المفاصل الروماتيدي.