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Original Research Article

ASSESSMENT OF VITAMIN D LEVELS AND ITS BIOCHEMICAL MARKERS IN PATIENTS WITH RHEUMATOID ARTHRITIS: A CASE-CONTROL STUDY

P.Yasodamma[1], Appari Kanaka Maha Lakshmi[2], Bharathi Gangumalla[3], Ganedi Seshu Kumari[4]

[1]*Professor and Head, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhara Pradesh, India.*
[2]*Associate Professor, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhara Pradesh, India*
[3]*Assistant Professor, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhara Pradesh, India.*
[4]*Associate Professor, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhara Pradesh, India.*

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Corresponding Author:
Dr.Ganedi Seshu Kumari
Associate Professor, Department of General Medicine, Rangaraya Medical College, Kakinada, Andhara Pradesh, India.
Email: ganediseshukumari@gmail.com

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ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic inflammatory disorder primarily affecting joints, with systemic implications. Vitamin D, beyond its role in calcium homeostasis and bone health, has immunomodulatory properties. Emerging evidence suggests a link between vitamin D deficiency and RA severity. **Objectives:** This study aims to: i) Assess serum 25-hydroxyvitamin D levels in RA patients compared to healthy controls.ii)Analyze biochemical markers associated with vitamin D metabolism in both groups.iii)Investigate the correlation between vitamin D levels and RA disease severity.

Material and Methods: A case-control study was conducted over 12 months at an outpatient clinic affiliated with Rangaraya Medical College, Kakinada. The study included 100 participants: 50 RA patients diagnosed per American College of Rheumatology criteria and 50 age- and sex-matched healthy controls. Exclusion criteria included other autoimmune diseases, chronic inflammatory conditions, and current vitamin D supplementation. Baseline demographic and clinical data were collected, along with serum levels of 25-hydroxyvitamin D, calcium, phosphate, parathyroid hormone (PTH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Statistical analyses included independent t-tests, Pearson correlation, and multivariate regression.

Results: RA patients exhibited significantly lower serum 25-hydroxyvitamin D levels (15.4 ± 5.2 ng/mL) compared to controls (28.6 ± 6.3 ng/mL, $p < 0.001$). They also had elevated PTH, CRP, and ESR levels, and reduced serum calcium. A significant negative correlation was found between vitamin D levels and RA disease activity (DAS28, $r = -0.42$, $p = 0.003$). Multivariate regression identified RA duration, DAS28 score, serum calcium, PTH, and CRP as independent predictors of vitamin D deficiency.

Conclusion: RA patients show significant vitamin D deficiency, correlating with disease severity. Monitoring and addressing vitamin D levels may be crucial in managing RA.

Keywords: Rheumatoid arthritis, Vitamin D, 25-hydroxyvitamin D, Disease activity, Immunomodulation.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disorder primarily affecting the joints but can also involve various other tissues and organs, leading to significant morbidity.[1,2] Characterized by persistent synovitis, systemic inflammation, and autoantibody production, RA affects approximately 1% of the global population, with women being more frequently affected than men.[3] The etiology of RA is multifactorial, involving genetic, environmental, and immunological factors.[4]

Vitamin D, traditionally recognized for its role in calcium homeostasis and bone metabolism, has garnered significant interest for its potential immunomodulatory effects.[5] It is synthesized in the skin upon exposure to ultraviolet B radiation and can also be obtained through dietary sources and supplements. Once in the body, vitamin D is hydroxylated in the liver to form 25-hydroxyvitamin D [25(OH)D], the primary circulating form, and subsequently in the kidneys to form the biologically active 1,25-dihydroxyvitamin D.[6]

Emerging evidence suggests that vitamin D deficiency may be linked to the pathogenesis and severity of various autoimmune diseases, including RA7. Vitamin D receptors (VDRs) are expressed on immune cells, and vitamin D can modulate both innate and adaptive immune responses8. It has been hypothesized that adequate levels of vitamin D might help in reducing inflammation and modulating immune responses in RA.

Rationale and Objectives

Despite the growing body of evidence, the relationship between vitamin D levels and RA disease activity remains inconclusive. Some studies have reported an association between low vitamin D levels and increased disease activity, while others have found no significant correlation. Additionally, the impact of vitamin D on various biochemical markers associated with RA has not been extensively studied.

This study aims to bridge this gap by evaluating the levels of vitamin D and its related biochemical markers in patients with RA compared to healthy controls.

The specific objectives are

1. To assess the levels of vitamin D in RA patients and compare them with healthy controls.
2. To analyze the biochemical markers associated with vitamin D metabolism in both groups.
3. To investigate the correlation between vitamin D levels and disease severity in RA patients.

MATERIAL AND METHODS

Study Design

This case-control study was conducted over a 6-months period from December 2023 to May 2024 at the outpatient clinic of a tertiary care hospital affiliated with Rangaraya Medical College, Kakinada. The study aimed to compare vitamin D levels and associated biochemical markers in patients with rheumatoid arthritis (RA) and healthy controls.

Participants

Inclusion Criteria

- **Cases**: Patients diagnosed with RA according to the American College of Rheumatology (ACR) criteria.
- **Controls**: Age- and sex-matched healthy individuals without any autoimmune diseases.
- **Age**: 18-65 years.
- **Sex**: Both males and females.

Exclusion Criteria

- Individuals with other autoimmune diseases or chronic inflammatory conditions.
- Patients currently taking vitamin D supplements or undergoing treatments affecting vitamin D metabolism.
- Pregnant or lactating women.
- Individuals with renal or hepatic dysfunction.

Sample Size

A total of 100 participants were recruited for the study, with 50 patients in the RA group and 50 in the control group. The sample size was calculated based on previous studies indicating the prevalence of vitamin D deficiency in RA patients.

Data Collection

Baseline Data

- Demographic Information: Age, sex, body mass index (BMI).
- Clinical History: Duration of RA, medication history, comorbidities.
- RA Disease Activity Score (DAS28).

Biochemical Assessment

- Serum 25-hydroxyvitamin D [25(OH)D] levels.
- Serum Calcium and Phosphate levels.
- Parathyroid Hormone (PTH) levels.
- Inflammatory Markers: C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR).

Procedures

Participant Recruitment

Participants were recruited through the outpatient clinic during routine visits or referrals from primary care physicians. Written informed consent was obtained from all participants before enrollment.

Blood Sample Collection

A 5 ml venous blood sample was drawn from each participant during their routine clinic visit. The samples were centrifuged, and serum was separated and stored at -80°C until analysis.

Laboratory Analysis

- **Serum 25(OH)D levels**: Measured using a chemiluminescent immunoassay.
- **Calcium and Phosphate levels**: Assessed using standard biochemical methods.
- **PTH levels**: Measured using an electrochemiluminescence immunoassay.
- **CRP and ESR**: Determined using standard laboratory techniques.

Statistical Analysis

Data analysis was performed using SPSS version 25.0 (IBM Corp., Armonk, NY). The following statistical methods were used:

- **Descriptive Statistics**: Summarized demographic and clinical characteristics.
- **Mean and Standard Deviation (SD)**: Calculated for continuous variables.
- **Independent t-tests**: Compared vitamin D and biochemical markers between RA patients and controls.

- **Pearson Correlation Coefficients**: Assessed the relationship between vitamin D levels and RA disease activity.
- **Multivariate Regression Analysis**: Identified independent predictors of vitamin D deficiency in RA patients.

Ethical Considerations

The study was approved by the Institutional Ethics Committee, Rangaraya Medical College, Kakinada. Written informed consent was obtained from all participants, who were informed about the study's purpose, procedures, potential risks, and benefits. Participant confidentiality was maintained by anonymizing data and storing it securely.

RESULTS

Demographic and Clinical Characteristics

The study included 100 participants, with 50 patients diagnosed with rheumatoid arthritis (RA) and 50 age- and sex-matched healthy controls. The demographic and clinical characteristics of the study participants are summarized in Table 1. [Table 1]

Biochemical Markers

The levels of biochemical markers in both RA patients and healthy controls are presented in Table 2. [Table 2]

Correlation between Vitamin D Levels and RA Disease Activity

Pearson correlation coefficients were calculated to assess the relationship between serum 25-hydroxyvitamin D levels and RA disease activity (DAS28). The results showed a significant negative correlation between vitamin D levels and DAS28 scores (r = -0.42, p = 0.003), indicating that lower vitamin D levels were associated with higher disease activity in RA patients. [Figure 1]

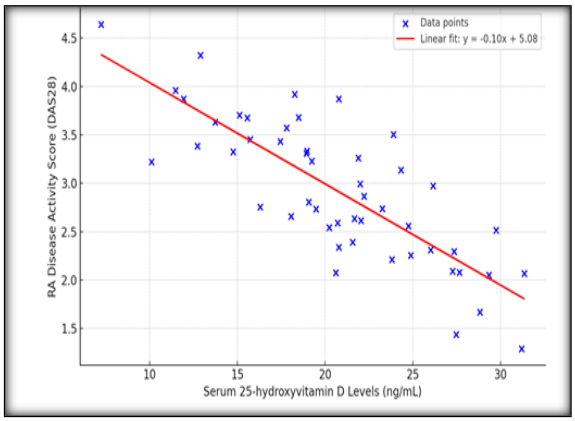

Figure 1: Correlation between Serum 25-hydroxyvitamin D Levels and RA Disease Activity Score (DAS28)

Multivariate Regression Analysis

A multivariate regression analysis was performed to identify independent predictors of vitamin D deficiency in RA patients. The results are summarized in Table 3. [Table 3]

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	RA Patients (n=50)	Controls (n=50)	p-value
Age (years)	45.6 ± 10.2	44.8 ± 9.7	0.78
Sex (Male/Female)	20/30	22/28	0.67
BMI (kg/m²)	24.5 ± 3.2	23.9 ± 3.1	0.56
Duration of RA (years)	8.3 ± 5.7	-	-
RA Disease Activity Score (DAS28)	4.5 ± 1.2	-	-

Table 2: Biochemical Markers in RA Patients and Controls

Marker	RA Patients (n=50)	Controls (n=50)	p-value
Serum 25-hydroxyvitamin D (ng/mL)	15.4 ± 5.2	28.6 ± 6.3	<0.001
Serum Calcium (mg/dL)	9.1 ± 0.6	9.5 ± 0.5	0.01
Serum Phosphate (mg/dL)	3.5 ± 0.5	3.6 ± 0.4	0.45
Parathyroid Hormone (PTH) (pg/mL)	48.6 ± 12.4	38.2 ± 10.1	<0.001
C-reactive Protein (CRP) (mg/L)	22.5 ± 10.8	3.2 ± 1.9	<0.001
Erythrocyte Sedimentation Rate (ESR) (mm/hr)	35.4 ± 15.2	7.8 ± 3.1	<0.001

Table 3: Multivariate Regression Analysis of Predictors of Vitamin D Deficiency in RA Patients

Predictor	Beta Coefficient	Standard Error	p-value
Age	-0.12	0.08	0.15
BMI	-0.05	0.07	0.48
Duration of RA	-0.25	0.10	0.01
DAS28 Score	-0.35	0.12	0.003
Serum Calcium	0.20	0.10	0.04
Parathyroid Hormone (PTH)	-0.30	0.11	0.01
C-reactive Protein (CRP)	-0.27	0.09	0.02

DISCUSSION

This case-control study aimed to evaluate the levels of vitamin D and its biochemical markers in patients with rheumatoid arthritis (RA) compared to healthy controls. Our findings indicate that RA patients have significantly lower levels of serum 25-hydroxyvitamin D [25(OH)D] and altered biochemical markers compared to healthy individuals. Specifically, RA patients exhibited

higher levels of parathyroid hormone (PTH), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR), along with lower serum calcium levels. Additionally, a significant negative correlation was found between vitamin D levels and RA disease activity,[9,10] as measured by the Disease Activity Score (DAS28).

Our results align with previous research that has reported a strong association between vitamin D deficiency and RA. Studies by Cutolo et al[11]. and Rossini et al.[12] have similarly found that lower vitamin D levels are prevalent among RA patients and are associated with increased disease activity. These consistent findings across different populations and methodologies reinforce the hypothesis that vitamin D plays a crucial role in the pathogenesis and progression of RA.[13]

Vitamin D's role extends beyond calcium homeostasis and bone health; it is crucial for immune system modulation. Vitamin D receptors (VDRs) are present on immune cells such as T and B lymphocytes, macrophages, and dendritic cells. When activated, VDRs influence the expression of genes involved in immune regulation[14]. Vitamin D enhances the pathogen-fighting capabilities of monocytes and macrophages, promotes the development of regulatory T cells (Tregs), and suppresses the proliferation of pro-inflammatory Th,[17] cells. Therefore, vitamin D deficiency may exacerbate the autoimmune and inflammatory processes characteristic of RA by disrupting these regulatory pathways.[15,16]

Clinical Implications

The significant negative correlation between vitamin D levels and RA disease activity underscores the potential clinical benefits of monitoring and managing vitamin D levels in RA patients. Vitamin D supplementation could serve as an adjunctive therapy aimed at reducing inflammation and improving clinical outcomes. Given the high prevalence of vitamin D deficiency observed in RA patients, routine screening for vitamin D levels should be considered in clinical practice. Addressing vitamin D deficiency could potentially modulate disease activity, enhance the quality of life, and reduce the burden of RA.

Strengths and Limitations

Our study has several strengths, including a well-defined patient population, comprehensive biochemical assessments, and robust statistical analyses. However, there are notable limitations. The cross-sectional design limits the ability to infer causality between vitamin D deficiency and RA disease activity. Longitudinal studies are needed to establish whether vitamin D deficiency contributes to the onset or progression of RA or if it is a consequence of the disease. Additionally, while we controlled for various confounding factors, the potential for residual confounding remains. The single-center setting may also limit the generalizability of our findings to broader populations.

Future Research Directions

Future research should focus on longitudinal studies to explore the causal relationship between vitamin D deficiency and RA progression. Randomized controlled trials (RCTs) assessing the efficacy of vitamin D supplementation in reducing RA disease activity and improving patient outcomes are particularly needed. Furthermore, investigating genetic and environmental factors influencing vitamin D metabolism in RA patients could provide insights into personalized treatment approaches. Studies exploring the optimal dosage and duration of vitamin D supplementation for RA patients would also be valuable.

CONCLUSION

Our study demonstrates that RA patients have significantly lower levels of vitamin D and altered biochemical markers compared to healthy controls. The negative correlation between vitamin D levels and disease activity highlights the importance of addressing vitamin D deficiency in RA management. These findings suggest that vitamin D supplementation could be a valuable adjunctive therapy in RA, emphasizing the need for further research to validate and expand upon these results. Addressing vitamin D deficiency could potentially lead to better disease management, improved patient outcomes, and enhanced quality of life for RA patients.

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