MEASUREMENT OF SERUM VITAMIN D LEVEL AND ITS ASSOCIATION WITH RHEUMATOID ARTHRITIS, A PROSPECTIVE STUDY AT EASTERN REGION OF NEPAL

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ABSTRACT

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease characterized by synovitis. The etiology and pathogenesis of RA remain obscure and many factors may be associated with its pathogenesis. Recent findings support that low levels of serum Vitamin D is associated with an increased risk for the development of RA. Recently, the role of vitamin D deficiency in the pathogenesis of RA, as well as the relationship between vitamin D deficiency and the activity of RA has also been an area of interest for many researchers.

Objectives

The objectives of this study is to analyse the vitamin D levels in RA patients and investigate the association between the serum levels of vitamin D and disease activity of RA patients from Eastern region of Nepal.

Methodology

A prospective study was conducted at Birat Medical college teaching hospital, Tankisinwari from September 2022 to August 2023. A total of 150 patients, after fulfilling exclusion and inclusion criteria were enrolled with convenience sampling technique. A control group (n=97) of patients were also enrolled for comparison. After collection of all data, ANOVA test was used to compare continuous variables between control and vitamin D groups.

Result

Vitamin D deficiency was found in 35 patients (23.3%), Vitamin D insufficiency found in 94 patients (62.6%), and is normal in only 21 patients (14%). Also patients with RA had significantly lower vitamin D levels in comparison to patients with control activity. The difference between these two groups was statistically significant (P<0.05).

Conclusion

Our study finds a significant inverse relationship between blood vitamin D levels and RA activity. However, due to the small number of patients included in the current study, further research with a larger sample size is needed to gain a better understanding of the relationship between RA activity and vitamin D.

KEYWORDS

C reactive protein, ESR, Rheumatoid arthritis, Vitamin D

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease of unknown aetiology affecting approximately 1% of the world population.[1] It is a systemic autoimmune disease that frequently results in joint deformities and subsequent disabilities. Clinically, the main characteristic feature of established RA is persistent inflammatory synovitis, usually involving small peripheral joints in a symmetrical distribution causing cartilage damage, bone erosion and joint destruction.

A renewed interest in area of RA research has been the association of dietary and supplemental vitamin D with RA incidence & disease severity. Higher intake of vitamin D was inversely associated with risk of RA.[2] It may be mentioned that worldwide one billion people are estimated to have vitamin D deficiency or insufficiency.[3] Whereas the deficiency of vitamin D is very common (50-90%) in all age groups, more than ninety percent of individuals above 50 years of age have vitamin D deficiency.[4-7] 1,25-dihydroxyvitamin D3 (1,25 (OH)2D3) the active metabolite of vitamin D3, is regulator of bone and calcium metabolism. It also exerts immunomodulation via the nuclear vitamin D receptor (VDR) expressed in antigen-presenting cells (APC) and activated T/B cells.[8] Main target of vitamin D immunomodulation are the dendritic cells (DCs) as indicated by inhibition of DC differentiation and maturation. This ultimately leads to vitamin D induced inhibition of DC-dependent T-cell activation.[9] VDR agonists also inhibit the T-cell production of IL-17 which is a pro-inflammatory cytokine produced by T-cells in models of organ-specific autoimmunity in the brain, synovium, heart, and intestines.[9] The net effect of vitamin D is an enhancement of innate immunity with multifaceted regulation of adaptive immunity.[10] Rheumatoid arthritis (RA) is an immune-mediated disease, these immunomodulatory activities of vitamin D might be particularly efficient in RA patients and may support the therapeutic role of 1,25(OH)2D3 in such a disease. Deficiency of vitamin D is involved in the pathogenesis as well as disease activity of RA. The decrease in vitamin D is related to older age, female gender, and a higher degree of RA activity.[11,12] To the best of our knowledge no study has been conducted in our region to establish the relationship between RA and vitamin D; thus, we designed this study to find out the correlation between rheumatoid arthritis disease activity and serum vitamin D level.

METHODOLOGY

This is a cross-sectional study conducted in Rheumatoid arthritis patients who were attending our orthopaedic OPD, at at Birat Medical College in Biratnagar, Nepal, from August 2022 to April 2023. We enrolled 150 patients of Rheumatoid arthritis patients, along with 97 controlled group participants.

a) Inclusion criteria:

Diagnosed cases of rheumatoid arthritis according to 2010 ACR-EULAR[13] criteria with age > 18 years were included in the study after obtaining informed consent.

b) Exclusion criteria:

Obese patients (BMI >30kg/m^2), patients on glucocorticoids, and those with liver/kidney/thyroid diseases, malabsorption syndrome, and other auto-immune diseases were excluded from the study.

All participants were divided into two groups, the control group (η = 97) and the RA group (η = 150) group. The RA group includes 118 female and 32 male patients with RA diagnosed by the Department of orthopaedics, Birat medical college teaching hospital. The control group includes 67 females and 30 males, which has no significant differences in sex and age from the RA group.

Study tools:

1. Anti-CCP antibody was estimated by electro chemiluminescence immunoassay (ECLIA). All values >17 IU/ml are considered positive with > 50 IU/ml as highly positive.
2. Rheumatoid factor: IgM RF was determined by immunoturbidimetry/nephelometry. A value of <15.9IU/ml was considered normal.
3. C-reactive protein was estimated by the latex agglutination method and a value of 1-3 mg/dl was considered normal.
4. Erythrocyte sedimentation rate (ESR) was determined using the Westergren method and expressed in millimeters at the end of the first hour. A value of >20mm/1st hour in males and >30mm/1st hour in females was considered high.
5. Serum vitamin D was measured by using an ELISA reader at wavelength 450nm using an ELISA test kit of Immunodiagnostic Germany by the method of Hollis BW. A value of ≥ 50 nmol/ml is taken as normal, and<50 nmol/ml as reduced.
6. The DAS28 score was used to assess disease activity score using parameters of Tender joint count (TJC-28), swollen joint count (SJC-28), erythrocyte sedimentation rate (ESR), and BMI. Participants from the RA group were further divided into four subgroups according to DAS28 score and vitamin D level in RA patients.
   DAS28 score, < 2.6(Remission)
   DAS28 score ≥2.6 but ≤ 3.2(Mild disease activity)
   DAS28 score ≥ 3.2 but ≤5.1 (Moderate disease activity)
   DAS28 score ≥5.1 (Severe disease activity)

Accordingly, a correlation between serum vitamin D and disease activity of RA was analyzed in both control and RA groups.

Statistical analysis: The ANOVA test was used to compare continuous variables between the control and vitamin D groups. In addition, we employed correlation analysis to compare continuous variables when covariates were determined to be significant in univariate analysis at the p 0.05 level. The odds ratios (OR) with the 95% confidence interval (CI) were computed. Descriptive and inferential statistical analysis has been carried out in the present study. Results on continuous measurements are presented on

Mean, and SD, and results on categorical measurements are presented in frequency and percentage. A p-value of < 0.05 was considered statistically significant. Student t-test (two-tailed, independent), Chi-square/ Fisher Exact test has been used to find the significance of study parameters on a categorical scale between two or more groups.

Statistical software: The Statistical software namely SPSS 22.0 and R environment ver.3.2.2 was used for the analysis of the data and Microsoft Word and Excel have been used to generate graphs, tables, etc.

Approval of Research Ethics Board and Informed consent: The study was approved by an institutional review board of Birat medical college (IRC-PA-231).

RESULTS

We investigated a total of 247 cases and divided them into two groups, group RA with 150 patients with rheumatoid arthritis (RA) and a control group with 97 patients with similar multiple joint pain without RA. Most of the participants in both groups were found to be female (74.9%) and the average age of the study participants was 35 years to 55 years. The demography of the study population is as shown in Table 1. The average height and weight of the participants in this study were 141 to 170 cm and 51 to 70 kg, respectively.

Table 1: Demographic variables of rheumatoid arthritis patients and healthy controls

Variable	RA	Controls	P-Value
Age	49.67±6.37	47.01±6.87	0.002
Weight	57.96±5.74	60.74±5.53	0
Height	162.61±5.78	161.68±6.03	0.227
BMI	22.03±2.72	23.32±2.64	0

Between groups, there were significant differences in the number of tender and swollen joints. The tender joint count has a lower bound and an upper bound of 16.85±18.83, which is greater than the control group (4.77±5.91), and the swollen joint count has a lower bound and an upper bound of 13.63±14.66 which is likewise higher than the control group (1.73±2.64). Similarly, the duration of morning stiffness, visual activity scale, ESR, and RF of the patients with rheumatoid arthritis (RA) and control group participants was examined through the distribution of ANOVA and displayed in the table 2. The lower and the upper of the duration of morning stiffness (14.71±15.42), visual activity scale (3.728±4.085), ESR (38.96±42.15), and RF (7.77±9.86) of the patients with rheumatoid arthritis were found to have lower and upper bounds that were larger than those of the control group participants of morning stiffness (1.92±2.39), visual activity scale (2.130±2.798), ESR (14.00±16.33) and RF (4.60±5.59) respectively.

Patients with rheumatoid arthritis (RA) had significantly lower vitamin D levels in comparison to patients with control activity (Table. 2).

According to the serum vitamin D level, RA patients were further divided into a reduced group (<50 nmol/L) and a normal group (>50 nmol/L). The association between clinical data and disease activity in two groups was analyzed to explore the correlation between these parameters and serum vitamin D levels and is as shown in Table 3.

We found no significant differences in age, sex, height, weight, BMI, disease duration, swelling and tenderness joint count, duration of morning stiffness, visual analog scale (VAS), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF), and DAS28 score (> 0.05).

Table 2: Distribution of Serum vitamin D level in RA and control group

25 (OH) D Level	RA (n=150)	Controls (n=97)	P Value
Tested Level(nmol/l)	37.6±12.1	58±15.2	0
Deficiency(<25nmol/l)	35 (23.3%)	3 (3.1%)	0
Insufficiency(25-50nmol/l)	94 (62.6%)	24 (24.7%)	0
Normal(>50nmol/l)	21 (14%)	70 (72.2%)	0

Table 3: Comparison analysis of RA patients with vitamin D deficiency and normal levels

25 (OH) D Level	RA (n=150)	Controls (n=97)	P Value
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Table 3: Comparison analysis of RA patients with vitamin D deficiency and normal levels

Characteristics	Normal	Deficiency	P value
Age	48±5	50±6	0.372
Weight	60±6	58±6	0.904
Height	162±6	163±6	0.688
BMI	23±3.2	21.9±2.6	0.139
Disease duration	29±6	28±6	0.283
DAS28	5.49±.92	5.87±.82	0.273
Patient global VAS (cm)	3.6±1	4±1.1	0
Swollen joint count (0-28)	13±5	14±4	0
Tender joint count (0-28)	15±6	18±6	0
ESR (mm/H)	41±11	40±10	0
CRP (mg/L)	16.5±13.5	16.4±12.4	0
RF (IU/ML)	8±6	9±7	0.422

DISCUSSION

Vitamin D deficiency increases the risk of developing autoimmune diseases such as multiple sclerosis, inflammatory bowel disease, type I diabetes mellitus, and especially RA [14-16] where low vitamin D level is implicated as an independent risk factor in RA development.[2] Although the exact etiology is still unknown, many environmental and genetic factors play an important role. RA which is the most

common chronic systemic polyarthritis is mediated by T-helper1 cells (Th-1). Vitamin D has immunoregulatory activity and vitamin D receptors are present in a number of cells of the immune system, antigen-presenting cells, activated T lymphocytes, and activated B lymphocytes. Vitamin D leads to the induction of regulatory T cell and NK T cells and inhibits TH1 cell response so, vitamin D suppresses experimental autoimmunity.[17]

Monolagas et al[18] found that a significantly greater proportion of seropositive RA patients (76%) had lymphocytes possessing vitamin D receptors compared with healthy controls (18%). Cherniak[19] also studied the 84% prevalence of vitamin D insufficiency (serum level <30 ng/ml) in RA patients. We also found a similar result in our study, where vitamin D deficiency or insufficiency is inversely related to the severity of RA.

Regarding the disease activity, numerous studies have linked low vitamin D levels with increased disease activity in RA.[2,20-23] We also found that patients of RA having high disease activity (with increased inflammatory burden) had significantly lower vitamin D levels compared to patients of RA having low disease activity. Patel et al also found similar results which showed an inverse association between disease activity and vitamin D metabolite concentration in early polyarthritis patients.[20] Kerr GS et al also concluded that 25(OH) vitamin D deficiency, but not insufficiency, was independently associated with higher tender joint counts and highly sensitive CRP levels.[24] We also found a significant negative correlation between disease activity measured by DAS-28 score and serum vitamin D level. Our results are compatible with the study done by Turhanoglu et al which also showed a significant negative correlation between disease activity measured by DAS-28 score and vitamin D level.[25]

Sanjeev Patel et al showed there was an inverse relationship between Vitamin D levels and the tender joint count and DAS28 score.[22] Each 10-ng/ml increase in the level of Vitamin D was associated with a decrease in the DAS28 score of 0.3 and in the CRP level of 25%. In our study, the DAS 28 score shows a direct relationship with vitamin D deficiency with a p-value of < 0.001. Tamrakar BK et al, found that in RA patients serum 25-hydroxy vitamin D levels were negatively correlated to anti-CCP antibody levels (rs = 0.72, p <0.001), and ESR (rs = 3.95, p <0.005).[26] Similar result is also shown in our study that vitamin D deficiency level is inversely proportional to ESR value with a p-value of <0.001.

Patel et al reported vitamin D level was an independent predictor of greater disability in persons with active RA, even after controlling for age, race, and disease activity.[20] Vitamin D deficient patients had six times the odds of needing assistance with activities of daily living. In patients with moderate to high disease activity, vitamin D deficiency was associated with higher DAS scores, pain, and disability. In our study, both swollen joint count and tender joint count are more with a decrease in vitamin D level with a significant p-value of <0.001.

CONCLUSION

There was a significant association between vitamin D deficiency and disease activity as well as ESR, TJC, and SJC. Hence, we propose that Vitamin D deficiency is an independent risk factor for increasing the severity of the disease process in RA. Therefore, all RA patients must be given vitamin D supplementation in order to reduce the severity of the disease process.

LIMITATION OF STUDY

Due to the small number of patients included in the current study, further research with a larger sample size is needed to gain a better understanding of the relationship between RA activity and vitamin D. Also more study is needed before Vitamin D's antiproliferative, immunomodulatory, and anti-inflammatory characteristics may be used to treat a range of autoimmune rheumatic disorders.

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CONFLICT OF INTEREST

None

FINANCIAL DISCLOSURE

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