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Assessment of Vitamin D_3 level in patients of rheumatoid arthritis and its relationship with disease activity using DAS 28-CRP in tertiary care center of Bihar, India

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ABSTRACT

Background: Rheumatoid arthritis (RA) is most common type of inflammatory arthritis. It is chronic autoimmune disease that affects joints as well as extra-articular structures. The prevalence of this condition could be due to both genetic and non-genetic factors (e.g., environmental, viral, and hormonal). **Aims and Objectives:** The aim of the study was to determine and correlate 25(OH) D level with disease severity in patients of RA. **Materials and Methods:** The prospective observational study consists of 70 patients of RA and 70 healthy controls. Assessed level of 25(OH) D, C-reactive protein (CRP), anti-cyclic citrullinated peptide (CCP), erythrocyte sedimentation rate (ESR), and Disease Activity Score (DAS) 28-CRP score were compared in both cases and controls. Correlation of Vitamin D level with parameters such as CRP, Anti-CCP, and ESR was performed. Correlation between Vitamin D level deficiency and disease activity among the RA patients was assessed. **Results:** The mean age of patients in the RA group was 37.77 ± 13.31 years. The mean CRP in cases was 40.57 ± 25.94 mg/L and in controls was 3.26 ± 1.95 mg/L with significant intergroup difference ($P<0.001$). The mean anti-CCP in cases was 62.18 ± 27.21 U/mL and in controls was 4.99 ± 1.52 U/mL with significant intergroup difference ($P<0.001$). The mean 25(OH) D level in cases was 12.65 ± 5.88 and in controls was 32.18 ± 10.22. The 25(OH) D was significantly decreased in cases than controls ($P<0.001$). A significant inverse relationship between serum 25(OH) D levels and DAS28 was observed. Receiver operating characteristic (ROC) curves results showed that 25(OH) D < 11.7 ng/mL indicates severe disease activity (Area under ROC curve = 84%, Sensitivity 77.78%, and specificity 83.33%,). 25(OH) D between 11.7 ng/mL and 19.83 ng/mL indicates moderate disease activity. 25(OH) D>19.83 ng/mL indicates low disease activity value below 4.13 is alarming situation. **Conclusion:** The study reveals high prevalence of Vitamin D deficiency and insufficiency in RA patients. There is also significant inverse correlation exist between serum Vitamin D levels and RA disease activity. Vitamin D level is good disease activity predictor in patients of RA.

Key words: Rheumatoid arthritis; Vitamin D_3; C-reactive protein, DAS28; ROC curve

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INTRODUCTION

Rheumatoid arthritis (RA) is most common type of inflammatory arthritis. It is chronic autoimmune disease that affects joints as well as extra-articular structures. The small joints of the hands and feet are most typically affected by RA, which manifests itself as pain, stiffness, and loss of function in symmetrical pattern.[1] RA affects 1% of the world's adults.[2] RA occurs more commonly in females than in males, with 2:1–3:1 ratio.[3] The studies of RA from

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some of the Latin American and Caucasians show an even greater predominance of disease in females compared to males, with ratios of 6:1 in Latin Americans compared to 3:1 in Caucasians.[4] The incidence of RA increases between 25 and 55 years of age.[5] It can lead to significant morbidity and mortality. Early diagnosis and treatment are helpful in decreasing the burden of this disease, but the pathogenesis and etiology of RA remains unclear.

The prevalence of this condition could be due to both genetic and non-genetic factors (e.g. environmental, viral, and hormonal).[6] First-degree relatives of RA patients have a higher risk of developing the disease, especially if the individuals have severe disease or are seropositive for rheumatoid factor (RF).[7] Identical twins have greater disease concordance rates than non-identical twins, indicating genetic predisposition.[8,9] Non-inherited variables are also important in RA because it is a polygenic and genetically diverse disease.[10] Some genes are responsible for severity of illness than for its occurrence. On the short arm of chromosome 6, there is a substantial genetic major histocompatibility complex area that has been related to RA.[11] Infectious agents such as Epstein-barr virus, Parvovirus B19, Mycobacterium tuberculosis, *Escherichia coli*, and Proteus mirabilis have all been suggested as probable RA triggers; however, the evidence is inconsistent.[12,13] Hormonal variables may also play a role in the disease's etiology, as evidenced by female preponderance, high incidence during the premenopausal or postpartum period, and the preventive effect of oral contraceptive pills, probably due to their progesterone content.[14] Diet and stress have also been suggested as potential factors in illness manifestation.[15]

Vitamin D3 (1,25-(OH)2 Vitamin D, active form of Vitamin D) has been shown to act as a key player in the onset and pathogenesis of RA. Vitamin D_3 has been implicated in preventing the onset and RA pathogenesis. The prevalence of RA has been found to decrease in individuals with its high intake, including both dietary and supplemental forms.[16,17] It is a secosteroid hormone that can be gained by food or synthesized in skin and its significance in calcium control and bone mineralization has long been recognized. It has lately been found to have a variety of extra skeletal effects.[18] The prominent among these are proposed role of Vitamin D3 in the pathophysiology of autoimmune disease, such as autoimmune thyroid disease,[19] multiple sclerosis (MS), inflammatory bowel disease (IBD), and RA.[20] The storage form of Vitamin D is 25-hydroxy Vitamin D {25(OH)D}. Thus the study was to determine and correlate 25(OH)D level with disease severity in patients of RA.

Aims and objectives

The aim of the study was to determine and correlate 25(OH) D level with disease severity in patients of RA.

MATERIALS AND METHODS

This prospective observational study was conducted among out-patients attending in the Department of General medicine, Rheumatology and Orthopaedics of IGIMS, Patna after institutional ethical approval (IRB number: 46/Academic). The study was designed with the purpose to assess the 25(OH)D level in RA patients and to find its relationship with RA disease activity. The study was carried out between February 2018 and January 2019 (12 months). The study consists of 70 patients of RA of age group between 18–60 years and 70 matched healthy controls. The control group was mostly recruited from patients' relatives who lived with them to minimize the impact of diverse lifestyles on 25(OH)D status.

All newly diagnosed patients fulfilling American College of rheumatology criteria (2010).[21] for RA with age group of 18–60 years were included. People younger than 18 years and older than 60 years, malabsorption diseases (celiac disease, inflammatory IBD, etc.), GFR <40 mL/min/1.73 m^2, pregnancy, Lactation, osteoporosis, and patient on Vitamin D supplementation are excluded from the study.

Fasting blood samples (overnight) were collected from all the participants in the morning under full aseptic precautions. Newly diagnosed RA cases were confirmed by RF and anti- cyclic citrullinated peptide (CCP) along with clinical examinations. A 28-joint count disease activity score (DAS) index was used to determine disease activity (Figure 1). DAS 28-C-reactive protein (CRP) score include a 28 TJC, 28 SJC, CRP and its interpretation shown in Table 1.[22]

DAS28-CRP formula = $[0.56\sqrt{TJC28} + 0.28\sqrt{SJC28} + 0.36 \log_{nat} (CRP+1] \times 1.10 + 1.15$ (https://www.das-score.nl)

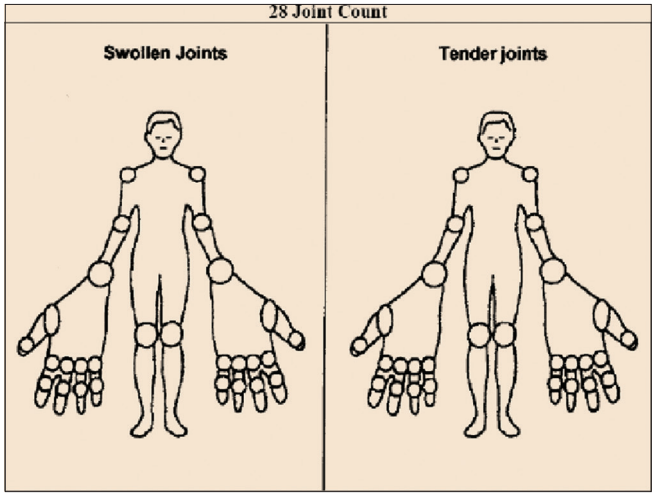

Figure 1: Showing 28 joints

The major storage form of Vitamin D is 25(OH) D and is present in blood up to 1000 fold higher concentration compared to the active 1,25-$(OH)_2$ Vitamin D. 25(OH)D has half-life of 2–3 weeks and 4 h for 1,25-$(OH)_2$ Vitamin D. Therefore 25(OH)D is the analyte of choice for determination of vitamin D status. 25(OH) D assay was estimated by chemiluminence immunoassay method with Chemimmunoassay Analyzer Access 2 (Beckman Coulter) in department of biochemistry. 25(OH) D levels of 30ng/mL (75 nmoL/L) are considered optimal, whereas serum levels of 21–29 ng/mL (50–75 nmol/L) are considered insufficient, and serum levels of 20 ng/mL (50 nmol/L) are considered deficient. CRP level was measured by quantitative turbidimetric method known as Turbilatex Method. Normal value adults up to 6 mg/L. RF measured by particle enhanced turbidimetric immunoassay known as Turbilatex method. The normal range of RF is from 0–20 u/mL. RF above 20 u/mL is considered RA positive. Anti-CCP is an immunoenzymatic test (ELISA). The normal level of Anti-CCP is <20 u/mL. Erythrocyte sedimentation rate (ESR) measured by fully automated analyzer. Reference value: Men: 0–15 mm in 1st Hr, Women: 0–20 mm in 1st Hr.

Assessed level of 25(OH) D, CRP, Anti-CCP, ESR, and DAS 28-CRP score was compared in both cases and controls and its correlation with parameters such as CRP, Anti-CCP, and ESR were performed. 25(OH)D level was reviewed according to disease activity in patients suffering from RA. Then correlation between Vitamin D level deficiency and disease activity among the RA patients was assessed. The receiver operating characteristic (ROC) curves is used to determine the Vitamin D level thresholds that can be used to identify distinct disease activity.

Statistical analysis

The data were entered into an MS Excel spread sheet, and statistical analysis was performed using SPSS 16.0 software (Statistic Package for Social Sciences). The variables were summarized using frequency distributions and Mean±SD. The quantitative variables were compared between groups using ANOVA and unpaired *t*-test. Qualitative variables are expressed as frequencies and percentages and assessed using Chi-square/Fisher's exact test. Results were interpreted at level of 95% confidence. ROC curve is made to identify the threshold values of Vitamin D to predict Disease activity. For such thresholds, Sensitivity and Specificity are calculated. $P < 0.05$ is considered statistically significant.

Table 1: The interpretation of disease activity score

DAS28-CRP	Interpretation
<2.6	Disease remission
2.6–≤3.2	Low disease activity
>3.2–5.1	Moderate disease activity
More than 5.1	Severe disease activity

CRP: C-reactive protein

RESULTS

Among the 70 patients in the RA group, 32 (45.71%) were male and 38 (54.29%) were female. Among the 70 participants in the control group, 33 (47.14%) were male and 37 (52.86%) were female. There is no significant difference between gender wise variation in cases and controls (Table 2).

The cases and controls were classified into five different age groups. The mean age of patients in the RA group was 37.77±13.31 years, whereas the mean age of participants in the control group was 36.93±12.73 years. Most common age group affected was 41–50 years (27.14% cases) followed by 51–60 years (22.86% cases). There was no statistically significant difference ($P = 0.351$) between age group of cases and controls.

The mean CRP in cases was 40.57±25.94 mg/L and in controls was 3.26±1.95 mg/L with significant intergroup difference ($P < 0.001$). The mean ESR in cases was 50.81±16.96 mm/h and in controls was 9.67±4.43 mm/h with significant intergroup difference ($P < 0.001$). The mean anti-CCP in cases was 62.18±27.21 U/mL and in controls was 4.99±1.52 U/ml with significant intergroup difference ($P < 0.001$). Hence, elevation of CRP, ESR, and anti-CCP was highly significant in cases.

The mean 25(OH) D level in cases was 12.65±5.88 and in controls was 32.18±10.22. The 25(OH) D was significantly decreased in cases than controls ($P < 0.001$). Whereas the mean DAS Score in cases was 4.98±0.98 and in controls was 2.19±0.46 suggested that there is significant increase in DAS score in cases than controls ($P < 0.001$).

There is significant difference between disease activity and 25(OH) D level. Low disease activity has mean 25(OH) D levels as 22.45±3.07 ng/mL, moderate disease activity has 15.44±4.83 ng/mL while severe disease activity has 9.24±4.28 ng/mL.

Table 2: Gender-wise distribution of cases and controls

Gender	Cases		Controls		P-value
	n	%	n	%	
Male	32	45.71	33	47.14	0.433
Female	38	54.29	37	52.86	
Total	70	100	70	100	

Since significant correlation existed between Vitamin D levels and Disease activity, we tried to find out the threshold values of 25(OH) D level that can be used to classify various disease activities. 25(OH)D < 11.7 ng/mL indicates severe disease activity. 25(OH) D between 11.7 ng/mL and 19.83 ng/mL indicates moderate disease activity. 25(OH) D >19.83ng/mL indicates low disease activity. Value below 4.13 is alarming situation (Table 3 and Figure 2).

Since significant correlation existed between Vitamin D levels and Disease activity, we tried to find out the threshold values of Vitamin D level that can be used to classify various disease activities. Vitamin D < 11.7 ng/mL indicates severe disease activity. Vitamin D between 11.7 ng/mL and 19.83 ng/mL indicates moderate disease activity. Vitamin D >19.83 ng/mL indicates low disease activity. Value below 4.13 is alarming situation (Table 3 and Figure 2).

The ROC curves for determining optimal 25(OH)D cutoff points in predicting low and high disease activity, respectively, are shown in Figures 3 and 4. The area under the curve (AUC) was 0.892 (95% CI=0.777–1.000, $P = 0.012$), and the sensitivity and specificity were 83.33% and 100.00%, respectively, and >19.83 ng/mL was the optimal 25(OH) D cutoff value for predicting low RA disease activity as shown in Figures 3 and 4 shows that in predicting high disease activity, the AUC was 0.840 (95% CI=0.743–0.937, $P = 0.000$), sensitivity was 77.78%, specificity was 83.33%, and the optimal 25(OH) D cutoff point was <11.7ng/mL.

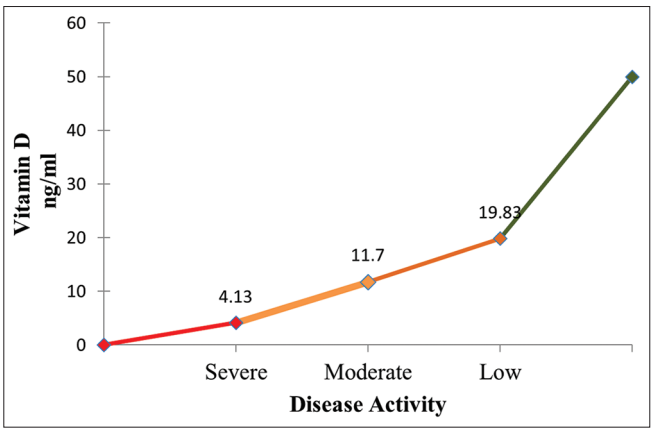

Figure 2: Showing threshold values of Vitamin D level that can be used to classify various disease activities

DISCUSSION

RA is the most common chronic polyarthritis. In this study, it was observed that Vitamin D play important role in etiology of RA. There was significant correlation between Vitamin D deficiency and RA. Vitamin D deficiency or insufficiency was more common in patients of RA than in healthy control. RA was most commonly seen in middle age females. In the study mean age of cases was 37.77±13.31 years, whereas the mean age of controls were 36.93±12.73 years. Most common affected age group was 41–50 years (27.14% cases) followed by 51–60 years (22.86% cases). There was no statistical difference between age group of cases and controls. Among the 70 patients in the RA group, females (54.29%) were more than males (45.71%). Similar findings were observed by Meena *et al*.., that the mean age of cases were 44.92±13.06 years, and the majority (86%) were female, whereas the mean age in the control group was 44.02±11.65 years and 84% were females.[23] Raised inflammatory markers such as ESR, CRP, and anti-CCP are one of the major criteria for diagnosis and assessment of RA. The mean ESR in cases and controls were 55.24±15.61 mm/h and 9.67±4.43 mm/h, respectively, with significant intergroup difference ($P < 0.001$). The mean CRP in cases was 40.57±25.94 mg/L and in controls 3.26±1.95 mg/L with significant intergroup difference ($P < 0.001$). The mean anti-CCP in cases was 62.18±27.21 U/mL and in controls was 4.99±1.52 U/mL, this difference is also highly significant. Similar finding was observed by Alexandru *et al*.., in cases as ESR level was 74.11±18.47 mm/h and in controls ESR was 8.45±2.99 mm/h. The mean CRP in RA patients was 60.34±27.8 mg/L and in healthy controls was 2.88±0.98 mg/L. The difference is highly significant ($P < 0.00001$).[24]

RA is an inflammatory disease characterized by flares and remissions, flares being characterized by pain. Clinical diagnosis of RA includes tender and swollen joints. This study suggested that the mean value of tender joints and swollen joints in cases 13.89±6.8 and 1.94±1.73 respectively whereas in controls 1.06±0.95 and 0.39±0.69, respectively (according to DAS28-CRP score). The intergroup difference of tender joints and swollen joints in cases and controls was highly significant.

Table 3: Identifying optimal Vitamin D cutoff point in predicting RA disease activity

Disease activity	25(OH) D	AUROC	P-value	Sensitivity (%)	Specificity (%)
Low	>19.83	0.892	0.012	83.33	100.00
Severe	<11.7	0.840	<0.001	77.78	83.33

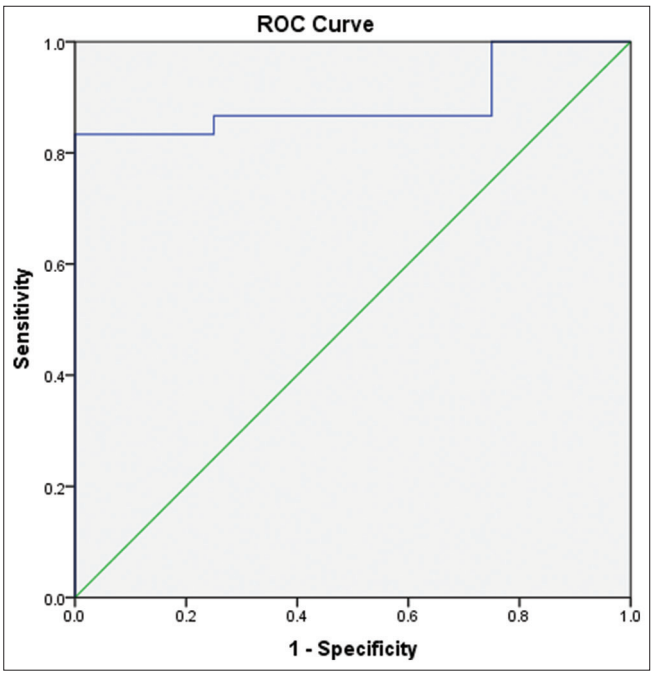

Figure 3: ROC curve for identifying optimal Vitamin D cutoff points in predicting low disease activity

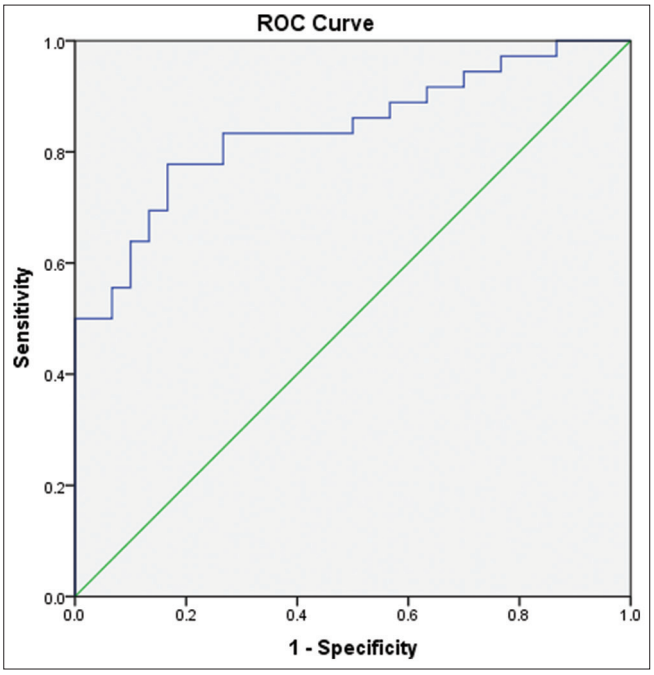

Figure 4: ROC curve for identifying optimal Vitamin D cutoff points in predicting high disease activity

The serum 25(OH) D levels in the RA group were substantially lower (mean value of 12.65±5.88 ng/mL) than in the control group (mean value of 32.18±10.22 ng/mL) in the current study. In a prospective comparison research found that serum 25(OH) D levels were considerably lower in the RA group (mean value of 21.05±10.02 ng/mL) than in the control group (mean value of 32.87±14.16 ng/mL).[23] Cen *et al*.., in their study, also suggested that the mean serum 25(OH) D level was significantly lower in RA patients (35.99±12.59 nmol/L) as compared to the normal participants (54.35±8.20 nmol/L).[25]

The DAS28-CRP is a measure of disease activity in RA. DAS stands for 'DAS' and the number 28 refers to the 28 joints that are examined in this assessment. The mean DAS Score in cases was 4.98±0.98 and in controls was 2.19±0.46 suggested that there is significant increase in DAS score in cases than controls ($P < 0.001$). In the study, there was a significant inverse correlation between serum 25(OH) D levels and RA disease activity. The mean serum 25(OH) D levels were 22.45±3.07 ng/mL,15.44±4.83 ng/mL, and 9.24±4.28 ng/mL, in low disease activity, moderate disease activity, and high disease activity groups, respectively. No cases found in remission.

In the study done by Meenaet *et al*.., found that the mean serum 25(OH) D levels in the remission, low disease activity, moderate disease activity, and high disease activity groups were 35.28±9.0 ng/mL, 33.80±4.1 ng/mL, 22.47±6.18 ng/mL, and 14.21±6.97 ng/mL, respectively. These variations were statistically significant (P 0.05).[23] Another study done by Attar SM with 100 RA patients and 100 controls, not on Vitamin D3 supplements, noticed that patients with high disease activity had the lowest 25(OH) D levels (18.25±8.3 nmol/L) compared to patients with moderate (35.13±15.2 nmol/L), and low (38.05±7.3 nmol/L) disease activity. Serum 25(OH) D was negatively correlated with DAS28, which was statistically significant. Significantly lower 25(OH) D values were found in patients who were poorly responding to treatment, and were not in a state of disease remission.[26] Braun-Moscovici (2011) studied on 85 RA patients but did not find correlation with Vitamin D levels.[27] Hajjaj-Hassouni (2017) studied with the aim to evaluate Vitamin D status in 1413 RA patients in 15 countries and analyzed that inverse relationship between RA patients and low levels of Vitamin D.[28] Since significant correlation existed between Vitamin D_3 levels and disease activity, we tried to find out the threshold values of Vitamin D_3 level that can be used to classify various disease activities. Figure 2 shows the best cut off points of Vitamin D_3 for high and low disease activity. The sensitivity (true positive) values in predicting low and high disease activity were 83.33% and 77.78%, respectively, according to ROC curves (Figures 3 and 4), which are good results. Furthermore, the AUC for both curves was greater than 0.7, showing that they were accurate. A test with an area larger than 0.9 has high accuracy, whereas 0.7–0.9 suggests moderate accuracy, 0.5–0.7, poor accuracy, and 0.5, a chance result, according to one interpretation of the area under the ROC curve. As a result, 25(OH) D levels are a reliable predictor of RA disease activity. In the study, the optimal 25(OH) D level <11.7 ng/mL can be a strong predictor of high

disease activity, between 11.7 ng/mL and 19.83 ng/mL for moderate disease activity and greater than 19.83 is for low disease activity. DAS28-CRP and Vitamin D3 status could thus be used to classify the disease activity of RA patients.

Azzeh and Kensara (2015) proposed similar findings in their study. They also found the best 25(OH) D cutoff values for predicting disease activity of RA. The AUC for high disease activity was 0.716 (95% CI=0.613–0.819, *P* value 0.001), and sensitivity and specificity were 82.6% and 75.4%, respectively, in his ROC curve. 25(OH) D level of 12.3 ng/mL (30.7 nmol/L) was found to be optimal cutoff point (J) for predicting high RA disease activity. On the other hand, AUC of 0.728 (95% CI=0.622–0.834, *P* value 0.001), sensitivity of 78.4%, specificity of 70.9%, and 25(OH) D cutoff point (J) of 17.9 ng/mL (44.7 nmol/L) found optimal in predicting low disease activity.[29]

However, there are some drawbacks. The study was done at a single institute, with a small sample size. To corroborate the findings of our study, more multi-centered prospective research studies with bigger sample numbers and greater diversity are needed. Furthermore, research should be carried out for therapeutic doses of Vitamin D3 supplementation to investigate improved therapy options.

Limitations of the study

This was a small population study and is single centered. Climatic variations and nutritional status may be confounding factors.

CONCLUSION

Vitamin D_3 deficiency and insufficiency are prevalent in RA patients and a strong inverse relationship exist between them. The optimal 25(OH) D cutoff criteria for sensitivity and specificity are <11.7% for severe disease activity, 11.7–19.83 for moderate disease activity, and >19.83 for low disease activity, respectively. A proper measurement of Vitamin D_3 levels in a broad population of RA patients is required to ensure that the recommended Vitamin D_3 intake are met.

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