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CORRELATION OF SERUM 25 HYDROXY VITAMIN D LEVELS AND DISEASE ACTIVITY IN A NEWLY DIAGNOSED RHEUMATOID ARTHRITIS

General Medicine

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ABSTRACT

Background: 25 hydroxy vitamin D levels and DAS 28 ESR score were calculated in newly diagnosed Rheumatoid arthritis patients and analyze the correlation between them. Hypovitaminosis is seen in those patients. **Aim of the study:** To assess the correlation between 25 hydroxy vitamin D levels and disease activity using DAS28 ESR in newly diagnosed Rheumatoid arthritis. **Study Design:** This is a prospective observational study conducted at the Department of General medicine, Sri Ramachandra Medical College and Research Institute, Chennai from February 2019 to October 2020. In this study, newly diagnosed Rheumatoid arthritis patients admitted to the ward and from rheumatology and General Medicine OPDs, who fit into the inclusion criteria were taken and in those patients, 25 hydroxy vitamin D levels were analyzed and correlated with the DAS 28 ESR score calculated from the tender, swollen joints, ESR and PGA and to see what relation exists between them and whether it is significant or not in patients without treatment. **Results:** A total of 43 patients were analyzed and the mean age of the population is 49.79 ± 10.45, maximum are females constituting 86%, with a mean duration of symptoms of 7.19 ±7.46 months. When mean vitamin D levels and DAS 28 ESR were analyzed 23.43 ± 13.33 and 5.52 ± 1.26 respectively. Using the pearson correlation, there exists an indirect correlation between 25 hydroxy vitamin D levels and DAS 28 ESR (r=-0.164; p=0.029). **Conclusion:** Hypovitaminosis is seen in patients with newly diagnosed Rheumatoid Arthritis and require an even larger sample size to establish a significant relationship between 25 hydroxy vitamin D and DAS 28 ESR. It may even depend on environmental, genetic, and regional factors.

KEYWORDS

DMARD naïve Rheumatoid arthritis, DAS 28 ESR, 25 hydroxy vitamin D levels, Disease activity

INTRODUCTION

Rheumatoid arthritis is a chronic inflammatory disorder of unknown etiology characterized by symmetric polyarthritis. Nearly 0.5-1% of the adult population were affected by Rheumatoid arthritis.[1] Prevalance of Rheumatoid arthritis in Africa and Asia is found to be 0.2-0.4% in contrast to native American Yakima, Pima, and Chippewa tribes of NorthAmerica which is 7%.[2]Synovium is the first to be affected followed by cartilage and bone. Other organs affected in Rheumatoid arthritis other than the musculoskeletal system are the skin, eye, lungs, heart, blood vessels, kidney, bone marrow, and central and peripheral nervous system seen in 40% of the patients with Rheumatoid arthritis over a lifetime of a disease .[3,4] Etiology of RA involves interactions between genetic and environmental factors .[5]

Vitamin D is a fat soluable vitamin formed from 7-dehydro cholesterol in the skin by ultraviolet radiation and it is also present in foods like fish liver. Vitamin D acts through single vitamin D receptor(VDR) present in nucleated cells. It acts through intracellular receptors in target tissues and regulate gene expression.[6]Vitamin D carry out calcium and phosphorous absorption from bones and gut. It has a role in pancreas, prostrate and immune system.[7]A systematic review analysed 219 published studies and concluded that vitamin D have a role in preventing autoimmunity.[8]

Vitamin D have effects on immunity like inhibition of T cell proliferation, prevents the release of Th1 cytokines like IL-2,INF r, TNF-alfa and modulates indirectly cytokine induced MMP and PGE2 production.[9]Around 50-90% of the Indians including all age groups and both sexes are deficient in vitamin D.[10] As vitamin D has effects on immunity, so vitamin D supplementation may be beneficial these patients. Here we conducted a cross-sectional periodic study to measure 25 hydroxy vitamin D levels in rheumatoid arthritis patients and to assess its correlation with disease activity.

MATERIALS AND METHODS:

Study setting:
This study is conducted in the department of general medicine at sri Ramachandra university, porur, Chennai-600116.

Study population:
Patient with rheumatoid arthritis as defined by the study methodology who consent to share their clinical, laboratory and treatment information as required by the study were included.

Study design : Prospective observational study

Sample size: Period dependent sample size from February 2019 to October 2020

Sampling method:
All the eligible subjects were recruited in to the study consecutively by convenient sampling till the end of study period.

Study duration:
The data collection for the study was done from February 2019 to October 2020

Inclusion criteria:
- Age of 18 to 64 years who are DMARD naïve, newly diagnosed rheumatoid arthritis according to ACR EULAR criteria(2010)

Exclusion criteria:
- Patients with renal and liver diseases.
- Patients with malignancy, inadequate exposure to sunlight for more than 6 months, diseases like sarcoidosis.
- DMARD intake.
- History of intake of drugs which effect vitamin D metabolism were excluded from the study.
- Pregnancy and patients with disorders of parathyroid.History of intake of vitamin D supplements.

Methodology And Data Collection:
In patients with evidence of Rheumatoid arthritis, after obtaining informed consent, personal history, chief complaints, Joints involved, history of co-morbid illness and drug intake were elicited. General and systemic examination findings were noted. Through Health assessement quessionnaire, disability index was calculated and PGA (1-100) measured using visual analog scale.

Laboratory data was obtained from our hospital database. Results of patients complete blood count, RFT, Serum electrolytes, LFT, ESR, CRP, serum calcium, phosphorous, serum 25 hydroxy vitamin, RF,ACPA and PTH were tabulated. ESR calculated by westerngrens method.25 hydroxy vitamin D levels and PTH were analysed by Chemiluminescence immuno assay(CLIA);CRP by ELISA;RF by nephelometry; ACPA by Electro chemiluminescence(ECL) and serum calcium by end point method.

25(OH) VITAMIN D LEVELS(ng/ml)	
<20	DEFICIENT
20-30	INSUFFICIENT
>30	NORMAL

Radiological data comprising of radiographs of hands and feet and degree of joint damage is assessed using vander Heijde modified sharp scoring system.

SVDH SCORE

HANDS			
EROSION SCORE	Depends on the surface area of the joint	JSN	JOINT SPACE NARROWING
1	If exist discretely	1	Focal or doubtful
2	Larger	2	>50% original joint space
3	Extend over the middle of bone	3	<50% original joint space,subluxation
5	Complete collapse	4	Ankylosis,complete luxation
FEET			
EROSION SCORE	0-10		

HAQ -DI: (HEALTH ASSESSEMENT QUESTIONNAIRE-DISABILITIY INDEX):

The complete HAQ is a comprehensive instrument designed to assess patients disability, discomfort, medication side effects, costs and mortality. HAQ-DI is used in clinical trials and clinical practise. Through 20 questions it evaluates the patient ability to perform activities of daily living. These questions are organised in to eight categories: Dressing, rising, eating, walking, hygiene, reach, grip and usual activities and each question is scored on a four level scale (0-3). 0= no difficulty;1= some difficulty;2=much difficulty and 3= inability to do. The final HAQ index ranges from 0-3,which is the mean of scores of all the eight categories and score of <0.3 is normal. Higher HAQ scores indicate increased disability.

Caluclation Of Significant Scores:

Disease activity is calculated using DAS 28 scoring system. DAS 28 score is calculated using either CRP or ESR as both are not same. In my study I calculated DAS28 using ESR.DAS 28 used to decide on dosing of DMARD.

DAS 28 SCORE =0.56xSquare root of tender joint count + 0.28 x square root of swollen joint count + 0.70 * ln [ESR] + 1.14 x (patients global assessment on a scale of 1-100,measured using visual analog scale.

DISEASE ACTIVITY SCORE	
>5.1	HIGH DISEASE ACTIVITY
>3.2-5.1	MODERATE DISEASE ACTIVITY
2.6-3.2	LOW DISEASE ACTIVITY
<2.6	REMISSION

Disease activity is calculated using the above formula in patients with rheumatoid arthritis obeying inclusion criteria and the values are correlated with the 25 hydroxy vitamin D levels.

Statistical Analysis And Interpretation:

Data was entered into Microsoft excel (windows 7) and analysis were done using the IBM SPSS version 21. Descriptive analysis was carried out by mean and standard deviation for quantitative variables, frequency and proportion for Categorial variables. Data was also represented using appropriate diagrams like bar and pie diagrams.

Inferential statistics:

Quantitiative outcome:

The association between vitamin D and lab parametres were assessed by comparing the mean values. were used to assess statistical significance. The association between vitamin D and TLC, RF, CRP, Anti CCP and duration of symptoms (in months) were assessed by comparing the median and interquartile range (IQR). Krushkal wallis test was used to assess the statistical significance. The association between disease activity and lab parametres were assessed by comparing the mean values. Independent sample t-Test was used to assess the statistical significance. The association between TLC, RF, anti CCP, CRP and duration of symptoms were assessed by comparing the median and interquartile range (IQR). Mann-whitney U test was used to assess statistical significance. Correlation between vitamin D and lab parametres were assessed by using pearson correlation coefficient and the data was represented in scatter diagrams.

Categorial outcome:

The association between categorial variables with vitamin D and disease activity were assessed by cross tabulation and comparision of percentages. Chi -Square test was used to test the statistical significance.

P value <0.05 was considered statistically significant.

OBSERVATION AND RESULTS:

A total of 43 patients who were newly diagnosed Rheumatoid arthritis,25 hydroxy vitamin D levels were analysed over a period of 20 months and correlated with various parametres mainly DAS 28 ESR.

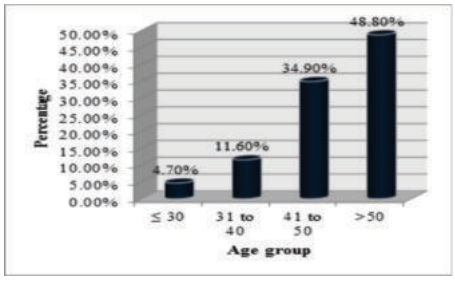

Fig 1: Bar chart of age distribution in study population (N=43)

The mean age of the study population(N=43) is 49.79 ± 10.45(95% C.I) and the median age is 50 years with a minimum age of 23 years and the maximum age of 64 years.Bar diagram(Fig.1) shows 48.8% belongs to more than 50 years of age,34.9% to 41-50 years of age,11.6% to 31-40 years and 4.7% to less than 30 years in the study population.

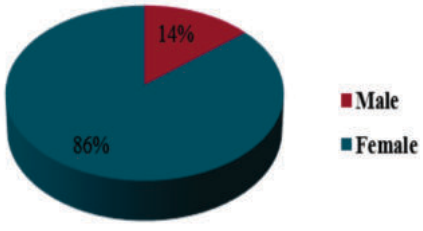

Fig. 2: Bar chart of gender distribution in study population (N=43) Out of 43 patients with rheumatoid arthritis 86% were females and 14% were males.

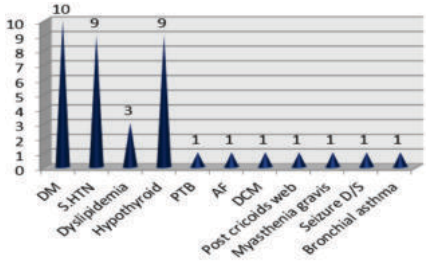

Fig 3: Bar chart of co morbidities distribution in study population (N=43)

Of the entire study population,11(25.6%) had diabetes,9(20.9%) had hypothyroid,9(20.9%) had systemic hypertension and 3(7%) patients with dyslipidemia.PTB,Atrial fibrillation,Post cricoid web,Myasthenia gravis,Seizure disorder,Dilated cardiomyopathy and Bronchial asthma each constitute 2.3%(1).

Table 1: Descriptive analysis of Laboratory parameter in study population (N=43)

Laboratory Parameters	Mean ± SD
DURATION OF SYMPTOMS (in months)	7.19 ±7.46
Tender joints	11.84 ± 7.53
Swollen joints	8.47 ± 7.6
HB(g/dl)	10.92 ± 1.73

TLC	8000 (6900, 9800)
TLC	8595.35 ± 8000
Platelets	3.30 ± 1.11
S.Creatinine(mg/dl)	0.6 ± 0.19
ALP	98.33 ± 27.95
S.calcium	8.76 ± 0.49
RF(IU/ml)	115.58 ± 152.84
ANTI CCP	96.50 ± 78.95
ESR(mm/hr)	54.79 ± 32.41
CRP	3.92 ± 7.09
PTH	27.01 ± 8.32
Vitamin D(ng/ml)	23.43 ± 13.33
SVDH Score	38.65 ± 26.28
DAS 28 ESR	5.52 ± 1.26
PGA(0-100mm)	24.56 ± 14.93
HAQ(0-3)	1.30 ± 0.94

When clinical and laboratory parametres were analysed in the study population mean of tender and swollen joints was 11.84 ± 7.53 and 8.47 ± 7.6 respectively. While mean haemoglobin was 10.92 ± 1.73,TC was 8000(6900,9800),platelets was 3.30 ± 1.11,S.creatinine was 0.6 ± 0.19,Alkaline phosphatase was 98.33 ± 27.95 and s.calcium was 8.76 ± 0.49.Mean of rheumatoid factor and anti-CCP was 115.58 ± 152.84 and 96.50 ± 78.95 respectively.When clinical parametres were analysed ,mean PGA was 24.56 ± 14.93,HAQ 1.30± 0.94 and the duration of onset of symptoms was 7.19 ± 7.46 months.Mean of acute phase reactants ESR and CRP was 54.79 ± 32.41 and 3.92 ± 7.09 respectively.Mean of SvdH score was 38.65 ± 26.28 ,25 hydroxy vitamin D was 23.43 ± 13.33,DAS 28 was 5.52 ± 1.26 and PTH was 27.01 ± 8.32.

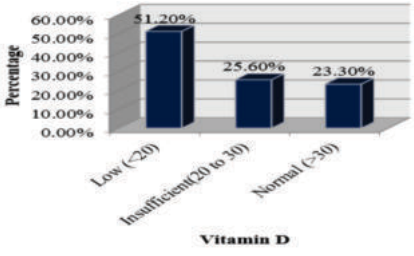

Fig 4: Bar chart of vitamin D distribution in study population (N=43)

When 25 hydroxy vitamin D levels were analysed in the study population(N=43), 51. 2%(22) of the patients have low(<20 ng/ml) vitamin D levels, 25. 6%(11) have insufficient(20-30ng/ml), while 23. 3%(10) have normal(>30ng/ml) vitamin D levels.

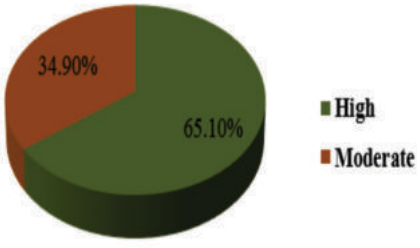

Fig 5: Bar chart of disease activity distribution in study population (N=43)

In study population, majority of the patients with RA have high disease activity with 65. 1%(28) while the patients with moderate disease activity constitute 34. 9%(15) and no patients with low disease activity.

Table 2: Comparison of mean laboratory parameter between disease activity (N=43)

Parameter	Disease Activity		P value
	High	Moderate	
Duration of symptoms	8. 38 ± 8. 39	4. 98 ± 4. 78	0. 098^
Tender joints	14. 75 ± 7. 25	6. 40 ± 4. 49	<0. 001
Swollen joints	11. 79 ± 7. 37	2. 27 ± 2. 37	<0. 001
HB	10. 90 ± 1. 41	10. 97 ± 2. 27	0. 906
TLC	8100 ± 2405. 09	9520 ± 4149. 22	0. 237^
Platelets	3. 43 ± 1. 18	3. 07 ± 0. 98	0. 290
S. CR	0. 55 ± 0. 17	0. 69 ± 0. 20	0. 034
ALP	101. 04 ± 31. 82	93. 27 ± 18. 69	0. 320
S. Calcium	8. 68 ± 0. 48	8. 91 ± 0. 48	0. 157
RF	150. 61 ± 177. 20	50. 19 ± 49. 90	0. 082^
ANTI CCP	87. 38 ± 76. 41	113. 51 ± 83. 44	0. 323^
ESR	67. 50 ± 31. 19	31. 07 ± 18. 70	<0. 001
CRP	4. 85 ± 8. 11	2. 18 ± 4. 36	0. 168^
Vitamin D	20. 03 ± 9. 92	29. 76 ± 16. 66	0. 05
PTH	26. 41 ± 8. 38	28. 12 ± 8. 38	0. 528
SVDH Score	46. 71 ± 27. 07	23. 60 ± 16. 89	0. 001
PGA	30. 18 ± 14. 50	14. 07 ± 9. 07	<0. 001
HAQ	1. 71 ± 0. 81	0. 53 ± 0. 64	<0. 001

^ indicates Mann whitney U test is used to calculate the statistical significance

Mean tender and swollen joints, ESR, SvdH score, PGA, CRP, RF, duration of symptoms and HAQ are more in patients with High disease activity, while vitamin D levels and ACPA are high in patients with moderate disease activity. There is a statistically significant relation between tender joints (p <0. 001), swollen joints (p<0. 001), ESR (P<0. 001), PGA (P<0.001), HAQ(P<0. 001), SVDH (p=0. 001) and Vitamin D(p=0. 05) using independent sample t-test. Mann whitney U test is used to calculate the significance of CRP, RF, ACPA, TLC and duration of symptoms.

Table 3: Comparison of disease activity between Vitamin D (N=43)

Disease activity	Vitamin D			Total	P value
	Low (<20)	Insufficient (20 to 30)	Normal (>30)		
High	19 (86. 4%)	3 (27. 3%)	6 (60%)	28 (65. 1%)	0. 003
Moderate	3 (13. 6%)	8 (72. 7%)	4 (40%)	15 (34. 9%)	
Total	22 (100%)	11 (100%)	10 (100%)	43 (100%)	

Out of 43 patients, majority of the patients with low vitamin D levels (86. 4%)have High disease activity, while most of the them with vitamin D levels between 20-30ng/ml (72. 7%) are with moderate disease activity. Using one way ANOVA when disease activity compared across the vitamin D leves, there is a statistically significant relation between them(p=0. 003).

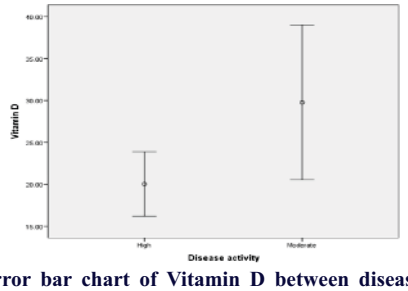

Fig 6: Error bar chart of Vitamin D between disease activity (N=43)

Fig.6 shows the error bar chart of vitamin D and disease activity;there is a less overlap in the standard error deviations between high disease activity and moderate disease activity, so there is probably no statistically significant difference between them and further statistical tests should be done draw a conclusion.

Table 4: Pearson correlation between Laboratory parameter and Vitamin D in study population (N=43)

Laboratory Parameters	Correlation	P value
DURATION OF SYMPTOMS	0. 132	0.400
Tender joints	-0. 087	0.577
Swollen joints	-0. 031	0.841
HB	-0. 212	0.172
TLC	-0. 293	0.057
PLATELETS	-0. 259	0.094
S. CR	0. 016	0.917
ALP	-0. 023	0.885
S. calcium	0. 026	0.868
RF	0. 058	0.714
ANTI CCP	0. 210	0.177

ESR	-0. 050	0.752
CRP	-0. 283	0.066
PTH	-0. 368	0.015
SVDH Score	-0. 157	0.313
DAS 28	-0. 164	0.029
PGA	-0. 335	0. 028
HAQ(0-3)	-0. 512	<0. 001

Correlation between 25 hydroxy vitamin D levels and other parametres were assessed using pearsoncorrelation;there is a statistically significant, negative correlation exists between the DAS 28 (r=-0. 164;p=0. 029), PGA(r= -0. 335;p=0. 028), HAQ(r=-0. 512;p=<0. 001) and PTH(r=-0. 368;p=0. 015). correlation between the parametres and vitamin D is diagrammatically represented below with scatter plots at 95%C. I.

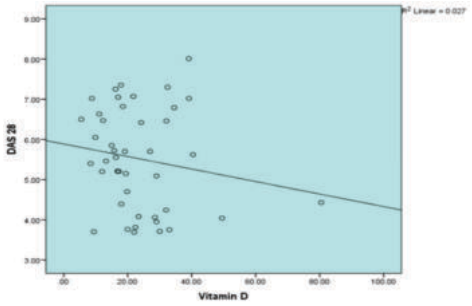

Fig 7: Scatter plot diagram of Vitamin D and DAS 28 (N=43)

Fig.7 shows the scatter plot between the DAS 28 and vitamin D levels and there exists a negative correlation between vitamin D levels and DAS 28**(r = - 0. 164;95% C. I)**

DISCUSSION:

In B cells antibody secretion and autoantibody production are inhibited by vitamin D. Few studies stated that vitamin D deficiency is associated with an increased risk of autoimmune diseases like type 1 diabetes mellitus, Rheumatoid arthritis, SLE, and Multiple sclerosis. [11] In our study newly diagnosed RA patients were taken and in those 25 hydroxy vitamin D levels and DAS 28 ESR were correlated.

Age And Rheumatoid Arthritis:

The mean age in our study population was 49.79±10.45 years, while the median age was 50 years. One study done by RamdossRamu et al. [12] in India showed mean age was 42.2± 12.3years and the median age was 40.5years. Another study done on Thai patients by rattapolpakchotanol et al. [13] showed the mean age was 58.85 ± 12.56 years and the median was 60 years. In our study, there was no statistically significant relation between Vitamin D levels and age; same with the disease activity in other study done in India.

Gender And Rheumatoid Arthritis:

In our study majority were females (86%) and in that most of them were with low vitamin D levels and high disease activity, though there was no statistically significant relation between vitamin D levels(p=0.16) and sex; the same with disease activity (p=0. 07). In a COMORA study done in 1413 rheumatoid arthritis patients by NajjaHajjaj-Hassouni et al. [14] showed the majority of the study population were females and most of them were with insufficient vitamin D levels than low vitamin D levels as in our study, but there was no statistically significant relation between vitamin D levels and female sex.

In a longitudinal retrospective study done by Di Franco et al., [15] in 37 RA patients showed a majority of them were females with insufficient vitamin D levels than deficiency and no statistically significant relationship between vitamin D levels and sex. This indicates vitamin D levels and disease activity were independent of gender and they may depend on the other parameters. Further studies have to be done to know whether hormones like testosterone and estrogen have any influence on disease activity and vitamin D levels.

Comorbidities:

In our study majority of the study, the population was with diabetes (23. 3%) followed by systemic hypertension (20. 9%) and hypothyroid (20. 9%). Dyslipidemia constitutes about 7% and all the other comorbidities like PTB, AF, DCM, Post cricoid web, Myasthenia Gravis, Seizure disorder, and bronchial asthma each constitute 2.3%. No statistically significant relationship between vitamin D levels and comorbidities, while there was a statistically significant relation between disease activity and dyslipidemia(p=0.014). In a COMORA study done in 1413 known rheumatoid arthritis patients by NajjaHajjaj-Hassouni et al., [14] majorities of patients with hypertension (35%) followed by ulcer and stomach diseases(11.8%); Diabetes constitute 9.4% and none with hypothyroid. 54.1% of the patients with dyslipidemia were in the vitamin d insufficiency group, while in our study it was almost the same in 3 groups. In this study, there was a statistically significant relation between lung diseases and vitamin D levels, which is not in our case.

Duration Of Symptoms With Vitamin D Levels And Disease Activity:

In our study Mean duration of symptoms was 7.19 ±7. 46 months. When compared with the vitamin D levels, the mean duration of symptoms was more in patients with normal vitamin D levels than in low and insufficient vitamin D levels, and no statistically significant correlation between vitamin D levels and duration of symptoms (r =0.132, P=0.400). The mean duration of symptoms was more in patients with high disease activity compared with moderate disease activity, but there was no statistically significant relation between disease activity and duration of symptoms(p=0.098). In a study done by RamdossRamu et al. [12] in 40 patients of newly diagnosed rheumatoid arthritis, the mean duration of symptoms were 15 months and did not show any significant correlation with disease activity and duration of symptoms like in our study. In our study mean duration of symptoms was more in patients with disease activity but was not statistically significant, so we require a large sample size to prove any significant relation between disease activity and duration of symptoms and the same with vitamin D levels as well.

Tender And Swollen Joints With Vitamin D And Disease Activity:

In our study mean of tender joints was 11.84 ± 7.53 and when compared between vitamin D levels mean was more in patients with normal vitamin D levels than low and insufficient vitamin D individuals and exists a negative correlation between vitamin D levels and tender joints(r=- 0.087), but it is not statistically significant (p=0.577). The mean of tender joints was more in patients with high disease activity compared to moderate disease activity and there was a statistically significant relation between disease activity and tender joints(p=<0.001). The mean of swollen joints was 8.47 ± 7.6 and it was comparatively more in patients with normal vitamin D levels compared to the low and insufficient vitamin D levels and not significantly correlated with the vitamin D levels (r=-0.031, p=0. 841). The mean of swollen joints was more in patients with high disease activity than moderate disease activity and there existed a statistically significant relation between them (p <0.001).

A study done by Zahra Zakeri et al. [16] in 66 RA patients showed a significant correlation between vitamin D with tender and swollen joints, but in our study, there was no significant correlation between vitamin D levels and tender and swollen joints. A longitudinal retrospective study done in 37 treatment naïve RA patients by Manuela Di Franco et al.[15] showed no statistically significant relationship between vitamin D levels and tender and swollen joints, it was statistically significant after 12 months of treatment(p <0. 05).

In our study, there was a significant relation between tender, swollen joints, and disease activity, and the means of both were more in patients with high disease activity and these parameters were actually used in the calculation of DAS 28.

Crp With Vitamin D And Disease Activity:

In our study mean CRP levels were 3.92 ±7.09 and mean CRP levels were more or less the same in low, insufficient, and normal vitamin D level groups, while more in patients with high disease activity than with low disease activity. There was no statistically significant relation with both vitamin D and disease activity and there exists a negative correlation between vitamin D levels and CRP(r -0.283;p 0.066). A study done by Zahra Zakeri et al., [16] done in 66 patients showed the same results as our study. A study done by Turhanoglu et al., [17] in 65 patients with known RA showed a significant negative correlation between vitamin D levels and CRP (r -0. 276; p 0.026).

This indicates that in patients with newly diagnosed rheumatoid arthritis vitamin D levels did not correlate significantly with CRP, but

that's not true with known RA patients on DMARDS and steroids.

Esr With Vitamin D And Disease Activity:
In our study, mean ESR levels were 54.79 ± 32. 41. Mean ESR levels were more in patients with normal vitamin D levels. ESR was negatively, but not significantly correlated with the vitamin D levels (r -0. 050;p 0. 752). ESR levels were higher in patients with high disease activity and there was a significant relation between disease activity and ESR(p <0. 001).

A study done by Zahra Zakeri et al., [16] showed mean ESR levels of 29. 9 ± 23. 7 and have a positive correlation, but not significantly correlated with vitamin D levels.

A study done by Manuela Di Franco et al. [15] showed mean ESR levels were more in patients with vitamin D levels >20ng/dl and there was no significant relation between vitamin D levels and ESR like in our study and the same relation even after the 12 months of treatment.

A study done by Burhan FatihKocyigit et al., [18] showed no significant correlation between vitamin D levels and ESR.

Svdh Score With Vitamin D And Disease Activity:
In our study mean SVDH score was 38. 65 ± 26. 28 and it was negatively correlated with vitamin D levels and this correlation is statistically not significant (r -0.157;p 0. 313). SVDH score is almost the same in patients with vitamin D deficiency and normal levels and there was a statistically significant relation between vitamin D levels and SVDH score using one-way ANOVA(P 0. 042). SVDH scores are higher in patients with high disease activity and a statistically significant relationship exists between them (p 0.001) using an independent t-test. This indicates radiological damage is more in patients with high disease activity.

In a study done by RamdossRamu et al., [12] showed no significant correlation between vitamin D levels and SVdH score like in our study. This study did not show a significant correlation with DAS 28 but after univariate regression analysis, SvdH is one of the significant predictors of DAS28(P 0. 03).

A study done by Burhan FatihKocyigit et al., [18] showed no significant correlation between vitamin D levels and SvdH scores like in our study, and DAS 28 significantly and negatively correlated with BMD of the lumbar spine, femur neck total, and femur neck.

This indicates there was no significant correlation between vitamin D levels and SvdH score in DMARD naïve and known RA patients, but a larger sample size may be required for correlation.

RF/ACPA With Vitamin D And Disease Activity:
In our study mean RF levels were 115. 58 ± 152. 84 and RF levels were more in patients with normal vitamin D levels and there was no statistically significant correlation between vitamin D and RF(r 0.058;p 0.714). RF levels were more in patients with high disease activity than in moderate disease activity, but there was no significant relation between DAS 28 and RF levels(p 0. 082). RF positivity is 67.4% in our study.

In our study mean ACPA levels were 96.50 ± 78.95 and ACPA levels were more in patients with vitamin D insufficiency, but there was no statistically significant correlation between vitamin D and ACPA(r 0.210;p 0.177). In contrast to RF, ACPA was more in patients with moderate disease activity and there was no statistically significant correlation between DAS 28 and ACPA (p 0. 323).

A study done by RamdossRamu et al. [12] showed no significant correlation of RF positivity with both DAS 28 and vitamin D levels like in our study. RF positivity is 60%.

A study done by Manuela Di Franco et al., [15] showed no significant relationship between vitamin D levels and ACPA at baseline and after 12 months of treatment, but the same does not apply for RF, because in this study significant relation exists between vitamin D levels and RF(p 0. 002) after 12 months of treatment and not at baseline. Our study is not a follow-up study, so not able to establish the relationship between vitamin D levels and RF.

A study done by F. E. Abourazzak et al. [19] done with 167 RA patients did not show any significant relation between vitamin D levels and ACPA/RF positivity like in our study.

HAQ With Vitamin D And Disease Activity:
In our study mean HAQ was 1.30 ± 0. 94 and HAQ scores were more in patients with vitamin D deficiency and high disease activity. HAQ was significantly and negatively correlated with Vitamin D levels(r -0.512;p<0. 001).There also exists a significant relation between DAS 28 and HAQ (P <0. 001).

A study done by A Turhanoglu et al.[17] showed there was a significant negative correlation between 25 OH vitamin D levels and HAQ like in our study, but there was no significant relation between DAS 28 and HAQ though maximum HAQ scores were in patients with HDA like in our study.

In a study done in India by RamdossRamu et al.,[12]showed a significant correlation between DAS 28 and HAQ (p<0. 001)by univariate and multivariate regression analysis like in our study. The mean HAQ in this study was 1.36 ± 0. 7.

PGA With Vitamin D And Disease Activity:
In our study mean PGA was 24. 56 ±14. 93 and it was high in patients with low vitamin D levels; significantly and negatively correlated with the vitamin D levels(r -0.335;p=0.028). PGA was high in patients with HDA and there was a significant relation between DAS 28 and PGA(P <0.001). This indicates patients with High disease activity and low vitamin D levels will have high PGA. PGA is used in the calculation of DAS 28.

A study done by Zahra Zakeri et al.[16] showed a negative correlation between 25 OH vitamin D levels and PGA, which was statistically significant(r -0. 709, p <0. 001) like in our study.

In a study done by Manuel Di Franco et al., [15] did not show any significant correlation between 25 OH vitamin D levels and PGA at baseline, but after 12 months of treatment, the relation was statistically significant between them(p<0. 001).

Disease Activity And Vitamin D:
In our study majority of the patients were with HDA (65.1%) and 86. 4% of the patients with HDA had 25 OH vitamin D deficiency. Mean DAS 28 was 5.52 ± 1.26, indicating high disease activity in DMARD naïve patients. Mean 25 OH Vitamin D levels were 23.43 ± 13.33 ng/ml. DAS 28 was negatively correlated with the 25 OH vitamin D levels (r -0.164) and there was a significant correlation between them (p=0. 029). Vitamin D levels are more in patients with MDA than HDA and there was a significant relation between 25 OH vitamin D and DAS 28 using an independent sample t-test (p=0.05). This indicates there was an inverse association between the DAS 28 and 25 OH Vitamin D levels.

In a study done by RamdossRamu et al.,[12]showed a significant correlation between DAS 28 and 25 OH vitamin D levels like in our study. Mean DAS 28 was 5.73 ± 1.47 and 25 OH vitamin D levels were 30± 24.5 ng/ml.

In a study done by Haque et al. [20] done in 62 patients showed a significant correlation between 25 OH vitamin D levels and DAS 28 with active RA (DAS 28>2. 6) and no significant correlation in patients in remission. A study done by Manuela Di Franco et al. [15] did not show any significant relation between 25 OH Vitamin D and DAS 28 at baseline, but statistically significant after 12 months of treatment.

In "the COMORA study"[14] done in 1413 patients with RA across 15 countries showed a mean DAS 28 of 3.5± 1.4. This study showed a negative correlation between 25 OH vitamin D levels and DAS 28 and it was statistically significant (**β -0.05;p 0.03). 3**

Serum 25 (OH) vitamin D is a single measurement that changes with time and it is influenced by drugs and other systemic disorders. DAS 28 scores can be correlated with 25(OH) Vitamin D levels at that point in time. SvdH scores are not correlated with 25 (OH) vitamin D levels, because radiological progression may or may not be constant. Cause and effect cannot be derived from this study in view of the small sample size.

Limitations Of The Study:

- Major limitation is the small sample size and further cases cannot be collected in view of the COVID-19 pandemic.
- In developing countries like India, vitamin D deficiency is common in the general population, so the status of vitamin D is not known, Though in our study we correlated it with disease activity.
- Though there is an inverse relationship between DAS 28 and 25(OH) vitamin D levels, the cause and effect can not be obtained from this study.
- Follow up study would have been done, to see the association between DAS 28 and 25(OH) vitamin D levels in patients with RA before and after treatment and with the other parameters after the treatment.
- Long term study with a larger sample size is required to establish the association between DAS 28 and 25(OH) vitamin D levels.

CONCLUSION

- In our study Age, sex, Duration of symptoms, RF, ACPA, and CRP were not significantly correlated with 25 Hydroxy Vitamin D levels and Disease activity(DAS 28), while HAQ, SvdH score, and PGA have a statistically significant associations with both 25 Hydroxy vitamin D levels and DAS 28.
- Above association suggests that radiological damage is more in patients with high disease activity through radiological damage occurs over time.
- In our study DAS 28 was significantly and negatively correlated with 25 Hydroxy vitamin D levels and most of our study population have vitamin D levels below the normal range and high disease activity. This indicates that hypovitaminosis is common in rheumatoid arthritis.
- HAQ scores were high in patients with low vitamin D levels and high disease activity in our study population, this indicates that rheumatoid arthritis affects the quality of life.
- Large long-term study is required to establish the cause-and-effect relationship between vitamin D and DAS 28 because still exists a controversy between the association between vitamin D levels and DAS 28.
- Correlation even depends on the environmental and genetic causes with respect to region and population.

DAS 28 - Disease activity score 28
IL - Interleukin
INF- r - Interferon gamma
TNF - Tumour necrosis factor
MMP - Matrix metalloproteinase
PG- Prostaglandin
DMARD- Disease modifying anti-rheumatic drugs
ESR - Erythrocyte sedimentation rate
CRP- C- reactive protein
RF- Rheumatoid factor
ACPA - Anti citrullinated protein antibody
HAQ - Health assessment quessionnaire
DI- Disability index
PGA - Patient global assessment
VAS - Visual analog scale
SvdH - Sharp Vander Heijde score
HDA - High disease activity
MDA - Moderate disease activity
LDA - Low disease activity
ACR - American college of Rheumatology
EULAR- European League against Rheumatism
HB - Haemoglobin
TLC - Total leucocyte count
RFT - Renal function test
S.CR- serum creatinine
LFT – Liver function test
PTH- Parathyroid hormone
DM- Diabetes mellitus
S.HTN - Systemic Hypertension
PTB - Pulmonary tuberculosis
DCM -Dilated cardiomyopathy
AF - Atrial fibrillation
VITAMIN D- 25 hydroxy vitamin D
ANOVA-Analysis of variance
C.I- Confidence interval
SD- Standard deviation

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