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Relationship Between Vitamin D and Neutrophil-Lymphocyte Ratio with Rheumatoid Arthritis Disease Activity

Putri Handayani[1], Andi Raga Ginting[2], Deske Muhadi Rangkuti[2]

[1]Department of Internal Medicine, [2]Division of Rheumatology, Department Internal Medicine, Faculty of Medicine, University of North Sumatera, Medan, Indonesia

Corresponding Author: Putri Handayani

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ABSTRACT

Introduction: Rheumatoid arthritis is a chronic inflammatory disease characterized by peripheral and symmetrical polyarthritis. Vitamin D deficiency has been linked to the course of several autoimmune diseases, and there is a link. In the process of autoimmune disease, the value of the neutrophil lymphocyte ratio is also known to be one of the parameters to assess the degree of activity. This study was conducted to assess the relationship between neutrophil lymphocyte ratio and rheumatoid arthritis disease activity.

Method: This research is a cross-sectional study with a retrospective approach, conducted from January to March 2023. This research was conducted on rheumatoid arthritis sufferers at Haji Adam Malik Hospital, Medan. Subsequent research subjects underwent anamnesis and physical examination to assess the DAS28 score. Blood sampling for examination of the ratio of neutrophil lymphocytes and vitamin D was carried out at Haji Adam Malik Hospital. The data collected was then analyzed using SPSS version 26.

Results: There were 67 research subjects with an age range of 43-47 years. The research subjects consisted of 20 men and 47 women. MTX treatment history in the remission group was 56.7%, in the low disease activity group was 80% and in the moderate disease activity group was 81%. Leflunomide treatment history in remission group 43.3%, low disease activity group 20% and moderate disease activity group 19%. Correlation test of vitamin D levels and rheumatoid arthritis disease activity showed a p-value of 0.380. Correlation test of neutrophil lymphocyte ratio and rheumatoid arthritis disease activity showed a p-value of 0.862. Correlation test of vitamin D levels and neutrophil lymphocyte ratio with DAS28 using the Spearman correlation showed p-values of 0.133.

Conclusion: There was no significant relationship between vitamin D levels and neutrophil lymphocyte ratio with rheumatoid arthritis disease activity (p-value 0.133).

Keywords: Vitamin D, Lymphocyte Neutrophil Ratio, DAS28, Rheumatoid Arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by peripheral and symmetrical polyarthritis [1]. Based on data from the Global Burden of Disease Study in 2017, there was an increase in the prevalence of RA by 7.4% between 1990–2017 [2]. RA has a significant impact on daily activities and quality of life of patients [3]. Factors such as pain, decreased muscle strength and aerobic capacity, limited joint motion, fatigue, and decreased physical activity also result in decreased physical function and quality of life in patients [4].

Until now, it is known that there are several tools to measure RA disease activity scores, namely Disease Activity Index including on 28-joint count (DAS28), Simplified Disease Activity Index (SDAI), European League

Against Rheumatism Response Criteria (EULARC), Modified Health Assessment Questionnaire. (M-HAQ) and Clinical Disease Activity Index (CDAI). The DAS-28 score is a score that assesses RA disease activity as measured by joint tenderness, amount of joint swelling using a count of 28 joints, erythrocyte sedimentation rate or C reactive protein levels, and the patient's general condition as measured by VAS [5]. There is a clear correlation between the mean DAS28 values and the amount of radiological damage that occurs over a given time period. DAS28 and assessment of disease activity (high or low) have been validated [6].

The neutrophil to lymphocyte ratio (NLR) is the proportion of the absolute neutrophil count to the lymphocyte count, which is obtained from a routine complete blood count. It has been widely useful that the NLR is a powerful tool for evaluating inflammatory activity in chronic inflammatory disorders. Research in Turkey in 2015 where the neutrophil lymphocyte ratio can be used as an inflammatory biomarker because of the physiological response of circulating leukocytes in the human body due to an increase in neutrophils and a decrease in lymphocytes [7].

Efforts are being made to identify and understand the role of vitamin D beyond its function in calcium homeostasis, including in auto-immune diseases [8]. Vitamin D can inhibit monocytes and macrophages to release pro-inflammatory cytokines, also inhibits T lymphocytes in the production of pro-inflammatory cytokines. VDR expression is found in cartilage and pannus junctions in RA patients, and is associated with various cell types, including chondrocytes, macrophages, synoviocyte fibroblasts, B cells and endothelial cells [9].

Several previous studies have begun to show the potential relationship of vitamin D with RA disease activity. Larissa Lumi, in her research on experimental rat populations, showed that the use of vitamin D affected RA disease activity with TNF and IL markers. Another cohort study in Denmark also showed that severe vitamin D deficiency was associated with a high DAS28 score in RA patients [10]. However, until now no one has examined the relationship between neutrophil lymphocyte ratio and vitamin D with RA disease activity using the DAS-28 indicator, especially in populations in Indonesia.

MATERIALS & METHODS

This research is an analytical study with a cross-sectional design with a retrospective approach which carried out in January until March 2023, at Rheumatology Outpatient Clinic of H. Adam Malik General Hospital with the approval from Research Ethics Commission, Faculty of Medicine University of North Sumatera. Sampling by means of consecutive sampling.

Samples were rheumatoid arthritis patients who came to the Rheumatology Outpatient Clinic at H. Adam Malik General Hospital and met the inclusion criteria, namely men or women aged > 18 years, able to communicate, willing to participate and sign the consent forms, willingness to undergo NLR and vitamin D examinations and did not meet the exclusion criteria, namely patients with autoimmune diseases (systemic lupus erythematosus, Type 1 DM, autoimmune thyroid disease), severe chronic diseases (heart failure, chronic kidney disease and malignancy), and severe infectious diseases. Subsequent research subjects underwent physical examination to assess the DAS28 score. Blood sampling for examination of the ratio of neutrophil lymphocytes and vitamin D was carried out at H. Adam Malik General Hospital.

STATISTICAL ANALYSIS

Basic characteristics data of the study population are presented in graphs/tables of frequency distribution and analyzed descriptively. The Kolmogorov-Smirnov test is used to test data normality. To analyze the relationship between neutrophil lymphocyte ratio and DAS28 score,

Independent T-test was used if the data was normally distributed. If the data is not normally distributed, Mann Whitney test is performed. Then proceed with the Pearson correlation test if the data is normally distributed and the Spearman correlation test if the data is not normally distributed. The desired deviation (α) is 0.05. Statistically significant if p<0.05.

RESULT

In this research, demographic characteristics of 66 patients included in the analysis were described which 47 patients (71.2%) were female while 19 patients (28.8%) were male. The mean age of the patients was 43.93± 12.78 in the remission group, 42.13±14.49 in the low disease activity group, dan 47.57±11.84 in the moderate disease activity group. All data on the characteristics of the study subjects showed a significance value of > 0.05 which indicated that these characteristic variables were not a bias factor except for the duration of illness which indicated a significant difference in terms of disease severity.

Tablee 1. Demographic Characteristics of the Research Sample

Karakteristik	**Remission** n = 30	**LDA** n = 15	**MDA** n = 21	**P-value**
Age (years old), mean ± SD	43,93 ± 12,78	42,13 ± 14,49	47,57 ± 11,84	0,424
Gender, n (%)				0,490
Male	10 (33,3)	5 (33,3)	4 (19)	
Female	20 (66,7)	10 (66,7)	17 (71,2)	
DAS-28, median (min-max)	1,94 (1,02-2,52)	2,8 (2,62-4,34)	3,24 (1,18-3,62)	-
CRP, median (min-max)	0,7 (0,7 – 11,2)	1,11 (0,7 – 2,8)	0,7 (0,7 – 2,8)	0,182
Duration of illness (years), median (min-max)	5 (2 – 7)	3 (1 – 7)	2 (1 – 6)	<0,001
Drug history, n (%)				0,109
MTX	17 (56,7)	12 (80)	17 (81)	
Leflunomide	13 (43,3)	3 (20)	4 (19)	
* LDA: Low disease activity, MDA: Moderate disease activity				

Table 2 presents data from the correlation analysis of vitamin D levels on rheumatoid arthritis disease activity using the Spearman test. Statistically, the average of vitamin D level is 22.95. Vitamin D and DAS28 variables show linear data so that they meet the requirements for a correlation test. The correlation test used is Spearman's test.

Table 2 Correlation of Vitamin D Levels on Rheumatoid Arthritis Disease Activity

	Median	**Min - Max**	**R**	**P-value***
DAS28, n = 66	2,67	1,02 – 4,34	-0,110	0,380
Vitamin D, n = 66	22,95	5,1 – 36,2		

*Spearman's correlation test

Table 3 presents data from the results of the correlation analysis of NLR values on rheumatoid arthritis disease activity. The NLR and DAS28 variables show linear data so that they meet the requirements for a correlation test. The correlation test used is Spearman's test.

Table 3 Correlation of NLR Values on Rheumatoid Arthritis Disease Activity

	Median	**Min - Max**	**R**	**P-value***
DAS28, n = 66	2,67	1,02 – 4,34	-0,022	0,862
NLR, n = 66	2,99	0,7 – 11,1		

*Spearman's correlation test

Table 4 presents data from the analysis of the relationship between vitamin D levels and rheumatoid arthritis disease activity. Statistically, vitamin D levels were highest in the remission group and it was concluded that there was no relationship between vitamin D levels and rheumatoid arthritis disease activity.

Table 4 Relationship of Vitamin D Levels to Rheumatoid Arthritis Disease Activity

	Mean ± SD	**F**	**P-value***
Remission, n = 30	24,11 ± 6,01	1,132	0,329
LDA, n = 15	23 ± 8,67		
MDA, n = 21	21,2 ± 6,42		

*Analysis of Variance

Table 5 presents data from the analysis of the relationship between NLR values and rheumatoid arthritis disease activity. Statistically using the Kruskal Wallis test, the NLR value was highest in the remission group and it was concluded that there was no relationship between NLR levels and rheumatoid arthritis disease activity.

Table 5 Relationship of NLR Values to Rheumatoid Arthritis Disease Activity

	Median	Min - Max	Nilai p*
Remission, n = 30	3,09	1,13 – 11,1	0,438
LDA, n = 15	2,8	0,7 – 5,13	
MDA, n = 21	3,1	1,49 – 5,5	

*Kruskal Wallis test

DISCUSSION

From the results of the study, it was found that there were more females than males, namely 47 female subjects, 19 male subjects. This was explained, that there were 10 male subjects with degrees of remitting activity, 5 male subjects with low disease activity and 4 male subjects with moderate disease activity. In women, there were 20 subjects with degrees of remitting activity, 10 subjects with low disease activity and 17 subjects with moderate disease activity. In general, rheumatoid arthritis (RA) is more common in female than male, with a lifetime risk of RA of 3.6% in female compared to 1.7% in male. The risk of RA also increases with age, with a peak incidence between the ages of 65 and 80 years [4].

In this study, it was known that the mean age of the research subjects was 43.93 ± 12.78 for degree of remission, 42.13 ± 14.49 for low disease activity, and 47.57 ± 11.84 for moderate disease activity. Study by Pasha et al. (2017) found 119 patients at RSCM aged 54 (21-75) years. Several clinical indices can be used to evaluate RA disease activity, including the Disease Activity Score (DAS28) [11]. DAS28 is a measure of ongoing disease activity and usually shows a normal distribution in the population with RA. DAS28 integrates physical examination measures (number of tender and swollen joints), acute phase reactants (erythrocyte sedimentation rate (ESR) or C-reactive protein (CRP), and the patient's own global health assessment; the combination of these variables into one score may be more comprehensive shows disease severity rather than individual variables [12]. This is consistent with the results of this study, namely the average DAS28 at the degree of remission was 1.94, at the degree of low disease activity was 1.11, and at the degree of moderate disease activity was 0.7.

This study was dominated by female patients, namely 47 subjects. Study by Pasha et al. (2017) in 119 patients at RSCM, the majority of patients were female, namely 107 people (89.9%). This study is also in line with the descriptive study by Pratama et al. (2017) in 97 RA patients at Dr. Hassan Sadikin. The prevalence of RA is found to be five times higher in women than men, which is related to the important role of estrogen in activating the inflammatory response in RA pathogenesis [11]. The study conducted by Medeiros et al. (2015) in 111 RA patients, the majority of the study results were female, namely 108 people, aged 55.6 years. Women are more likely to experience various types of recurrent pain and report higher scores. Pain threshold was found to be lower in women and that they experience more physical pain than men to the same noxious stimulus [13].

In this study, the average CRP was 0.7 in remission degree, 1.11 in low disease activity, 0.7 in moderate disease activity. Hopkins et al. (2014) found that the average CRP was 6 (<4-180) mg/dL, the DAS28 average was 4.8 (2.63-7.98). This is in accordance with the results of Junaidi's study (2016) on 46 patients using medical records, where in patients who were experiencing flares with a high DAS28 score, the increase in their CRP score also increased 100% [14].

The results of this study also obtained the average value of vitamin D from the degree of disease activity. In the degree of remission activity, the average was 24.11 ± 6.01; in the low disease activity degree, the average was 23 ± 8.67; in moderate disease

activity, the average was 21.2 ± 6.42. This is in line with a study conducted by Larissa Lumi, in her research on experimental rat populations showing that the use of vitamin D affected RA disease activity with TNF and IL markers. Another cohort study in Denmark also showed that severe vitamin D deficiency was associated with a high DAS28 score in RA patients [10].

The study subjects also obtained the average value of the neutrophil lymphocyte ratio, namely in the degree of remission activity the average was 3.09, in low disease activity the average was 2.8, and in moderate disease activity the average was 3.1. In this study, there was a significant positive correlation between the neutrophil lymphocyte ratio and the DAS28 score, which assesses the degree of rheumatoid arthritis disease activity. In Andi Nadya's research (2022), the results obtained were 86.2% become severe RA in the >2.96 group and 15.0% for moderate RA. The NLR value of >2.96 has 35.17 times the risk of serious disease activity (95% CI 7.70-178.01) [15].

The results of a similar study were found in a study conducted by Hanik R (2014), a cross-sectional study conducted on outpatients at Brawijaya Hospital, where 24 subjects met the diagnostic criteria according to ACR/EULAR 2010. In this study, vitamin D was measured in the blood and TNF ELISA method, the severity of clinical manifestations of rheumatoid arthritis was assessed with a DAS28 score. The results of this study stated that there was a significant correlation between vitamin D and patient age (p=0.005, r=-0.553), with length of illness (p=0.009, r=-0.522), with DAS28 (p=0.001, r =-0.615), number of painful joints (p=<0.001, r=-0.733), and swollen joints (p=0.045, r=-0.413), patient VAS (p=0.006, r=-0.541) and with TNF level (p=0.048, r=-0.408) and patient's functional status (p=0.039, r=-0.424). The results prove that blood levels of vitamin D have a relationship with the degree of activity of rheumatoid arthritis [16].

CONCLUSION

There was no significant relationship between vitamin D levels and neutrophil lymphocyte ratio with rheumatoid arthritis disease activity (p-value 0.133).

Declaration by Authors

Ethical Approval: Ethics approval and consent to participate. Permission for this study was obtained from the Ethics Committee of Universitas Sumatera Utara and Haji Adam Malik General Hospital.

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