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Identification of inflammatory biomarkers in rheumatoid arthritis patients; a case-control study

Nasrin Bazgir[1], Elham Shafiei[2*]

[1]Department of Internal Medicine, School of Medicine, Shahid Mostafa Khomaeini Hospital, Ilam University of Medical Sciences, Ilam, Iran. [2]Non-Communicable Diseases Research Center, Ilam University of Medical Sciences, Ilam, Iran.

Rheumatoid arthritis is a chronic inflammatory autoimmune disease characterized by synovial involvement, inflammation, and joint destruction that, if not properly controlled, can damage cartilage, bone, ligaments, and tendons and, in some cases, lead to disability. The aim of this study was to identify inflammatory biomarkers in rheumatoid arthritis patients.

This case-control study was performed on 50 rheumatoid arthritis patients who referred to the Rheumatology Clinic of Shahid Mostafa Khomeini Hospital in Ilam and their healthy counterparts. All patients were examined by a rheumatologist for disease activity based on DAS28 (Disease Activity Score Calculator for Rheumatoid Arthritis) criteria.

The results of this study showed that the mean lymphocyte count in the case group was lower than the control group, and there was a statistically significant relationship between lymphocyte level in the two groups. The mean neutrophil count was higher in the case group than in the control group, and this relationship was significant. The mean neutrophil/lymphocyte ratio was higher in patients with rheumatoid arthritis than in controls and in women more than men. Stepwise logistic regression also showed that age, sex, DAS28, VitD (Vitamin D), RF (rheumatoid factor), and NLR (neutrophil-to-lymphocyte ratio) significantly predict the incidence of rheumatoid arthritis (*p value* < 0.05). Therefore, NLR can be used as a prognostic factor.

Keywords: Inflammatory biomarker, Neutrophil-to-lymphocyte ratio, Rheumatoid arthritis

Introduction_____

Rheumatoid arthritis (RA) is a chronic, multisystem disease of unknown etiology and the most common cause of inflammatory arthritis [1]. The disease can sometimes go off spontaneously, and it can often lead to severe and debilitating complications if not treated in a timely manner [2, 3].

The prevalence of the disease is estimated at 1% worldwide higher in women than in men [4]. In Iran, the prevalence of RA was reported as 0.19% in a population study [5]. The development of autoimmune diseases such as rheumatoid arthritis depends on the interaction of genetic and environmental factors. 50% of the causes of rheumatoid arthritis are genetically dependent [6]. One study found an increase in the incidence of the disease in women [7].

The basis for treatment of RA is the use of DMARDs (disease-modifying antirheumatic drugs), which depends on the drug dosage or how many drugs are active. Disease activity leads to the continuation of joint destruction (5). Therefore, knowing the methods of determining disease activity is important and can be useful in clinical evaluations. One of the most widely used clinical criteria is the DAS28 criterion, which determines the extent of disease activity based on clinical features and sometimes testing [8-10].

White blood cells alone cannot be used as a symptom. Both white blood cell inflammatory markers and CRP can be helpful in diagnosis, and when together, their positive predictive value increases measuredly. The researchers believe the neutrophil-to-lymphocyte ratio (Neut/Lym) is a more sensitive indicator than total leukocytes [11]. Therefore, the aim of this study was to identify inflammatory biomarkers in rheumatoid arthritis patients.

Materials and Methods_____

This case-control study was performed on 50 patients with rheumatoid arthritis whose diagnosis was based on clinical symptoms, physical examination, and laboratory

*Corresponding Author: Elham Shafiei., Non-Communicable Diseases Research Center, Ilam University of Medical Sciences, Ilam, Iran, E-mail: Shafiei-e@medilam.ac.ir , Telefax: +98-918-843-2410.

tests and confirmed by a specialist according to the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria (ACR/EULAR) [12].

Inclusion criteria comprised a diagnosis of RA according to ACR/EULAR criteria (for patient group) and over 16 years of age; exclusion criteria included heart, kidney, or liver disease, MCTD, overlap syndrome, pregnancy, hematologic malignancy, and infection within three weeks. Consent to participate was obtained from patients who met the inclusion criteria.

From each patient, a 2-cc blood sample was taken, and the neutrophil and lymphocyte cell counts were assessed in the laboratory. All patients were examined by rheumatologists for disease activity, and the DAS28 formula was used to evaluate the severity of disease activity. The control group consisting of healthy companions of the patients with no history of rheumatoid arthritis also submitted to blood sampling (2 cc) after the study objectives were explained to them and their informed consent was obtained. All data was transferred to the relevant questionnaires.

DAS28 values are divided into 4 categories: remission (< 2.6), mild (2.6-3.2), moderate (3.2-5.1), and severe (> 5.1). This formula is an international formula for evaluating disease activity in which t equals the number of painful joints, sw equals the number of swollen joints, and

pain scored based on the VAS. In this criterion, the patient scores the highest pain as 100 and the lowest pain intensity a score of zero.

Patients were treated with prednisolone and DMARD drugs.

Study variables for each patient were checked using a checklist which included patient age and gender, neutrophil level, lymphocyte level, and neutrophil-to-lymphocyte ratio.

Statistical Analysis

Data analysis was performed using Stata 12 software. Descriptive statistics (frequency, mean, standard deviation) and inferential statistics (independent t-test and logistic regression) were used. Significance level was considered for tests (p $value$ < 0.05).

Results

The mean age of RA patients was 53.54 ±14.1 years and of the controls was 36.82 ± 9.92 years. Patients in the case group were reported 8 months. The mean NLR in the case and control groups was 1.88 ± 0.774 and 1.49 ± 0.42, respectively, and there was a statistically significant relationship regarding mean NLR between the two study groups (Table 1).

Mean DAS28 in the case group was 2.97 ± 0.85 (mild) and in control group was 1.39 ±0.633 (remission).

Table 1. Frequency of demographic characteristics (age, duration of disease, and NLR) in two groups

Variable	Case group (*Mean ± SD*)	(Control group *Mean ± SD*)
Neutrophil (cmm)	58.26±1.28	54.36±1.136
Lymphocyte (cmm)	33.68±1.15	37.74±0.907
NLR (Ratio)	1.88±0.74	1.49±0.42
DAS28 score	2.97±0.85	1.39±0.63
VIT D (ng/ml)	43.4±25.26	32.69±18.69
PLT (×10^9/l)	235.87±72.85	252.36±52.95
ESR (mm/h)	34.2±57.57	11.54±11.09
Hb (g/dl)	11.46±1.39	14.26±1.46
TG (mg/Dl)	145.74±56.67	1166.64±62.86
Anti-CCP	19.95±54.7	4.49±1.29
Chol (mg/dl)	200.38±51.39	179.2±35.11

Stepwise logistic regression results showed that age, sex, DAS28, VitD, RF, and NLR can significantly predict the incidence of rheumatoid arthritis (p $value$ < 0.05).

Multivariate logistic regression results showed that DAS28, NLR, and age are good predictors of rheumatoid arthritis.

According to Table 2, the variable that has the most significant effect on the chance of developing rheumatoid

arthritis is DAS28 with (OR = 190), which indicates that with 1-unit change in the incidence of RA, the size is over 190. Thereafter, the NLR variable had the most significant effect on the incidence of RA (OR = 12.63), which indicates that patients have a 12-fold increased chance of developing this disease.

Table 2. Results of Logistic Regression Analysis Related to Predictors for Rheumatoid Arthritis

RA	Odds ratio	SE	z	sig	95% (Conf. Interval)
Sex(female)	1.78	3.36	0.31	0.76	(0.04-72.59)
DAS28	189.315	406.54	2.44	0.015	(2.81-12737.88)
Vitamin D	1.04	0.03	1.62	0.10	(0.99-1.11)
RF	10.45	17.62	1.39	0.16	(0.38-284.5)
Anti-CCP	1.09	0.07	1.32	0.18	(0.95-1.26)
NLR	12.63	16.65	1.92	0.05	(0.95-167.429)
Age	1.21	0.09	2.60	0.009	(1.05-1.41)

Discussion

Rheumatoid arthritis is one of the most common autoimmune diseases affecting about 1% of white people, with women being affected 2-3 times more than men.

The results of this study showed that the neutrophil-to-lymphocyte ratio in the patient and control group was 1.88 ± 0.774 and 1.49 ± 0.42, respectively, which was statistically significant. The new inflammatory marker is useful for the follow-up of RA, and changes in the neutrophil-to-lymphocyte ratio indicate the efficacy of biological agents but do not predict response to them.

Mean TG (*p value* < 0.01), anti CCP (*p value* < 0.049), ESR (*p value* < 0.007), and Chol (*p value* < 0.01) were higher in the patient group than the control group.

Mean Hb was lower in the patient group than in the control group. As the severity of the disease increased, platelet count also increased. The regression model, however, showed that factors such as DAS28, NLR, and age were influenced by the severity of the disease, and these indicators were a better predictor than other blood ones. The results of this study are in line with the results of the study.

Most studies have suggested that the anti-CCP, CRP, and ESR indicators can aid in determining the diagnosis and prognosis of RA [13, 14]. Little is known about the role of platelets in this disease, but some studies have shown that the number of platelets and secretory substances in platelets increase [7, 15].

Ivana et al. [16] reported that hemoglobin and platelet counts were lower in RA patients than in the control group. Other studies [17, 18] have also shown that hemoglobin levels are lower in patients with rheumatoid arthritis, with results similar to the current research.

According to the results of this study, hemoglobin and lymphocyte count decreased in the case group more than in the control group. Therefore, this blood indicator can be used as a prognostic factor.

Conclusion

The current results showed that NLR was higher in women than in men and was positively correlated with CRP and ESR. Moreover, it was more reliable than CRP and ESR for assessing disease activity.

It is suggested that future studies assessing hematocrit, MCV and MPV, platelet, and monocyte blood parameters be performed in RA patients.

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Availability of data and materials

The datasets used and/or analyzed in the current study are attainable from the corresponding author on reasonable request.

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Conflict of interest statement

None.

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