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Evaluation of Neutrophil Lymphocytic Ratio, Platelet Lymphocytic Ratio and Red Cell Distribution Width in Patients with Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is a chronic autoimmune disease. Along with extra-articular symptoms and systemic consequences. It causes progressive symmetrical inflammation of the joints resulting in cartilage damage, bone erosion and articular disability.

Aim of Study: To study the Neutrophil lymphocytic ratio (NLR), platelet lymphocytic ratio (PLR) and red cell distribution width (RDW) levels in patients with RA, and the correlation between them and disease activity using DAS-28ESR.

Patients and Methods: Sixty-five RA patients were compared to 65 age and sex matched healthy volunteers as a control group. Disease activity was determined using DAS-28ESR. NLR and PLR were calculated and recorded with RDW level for all participants. They were analyzed and correlated to disease activity.

Results: NLR was statistically significantly higher in all RA patients and active RA patients compared to control group. PLR and RDW displayed statistically significantly higher values in all RA patients, active and inactive RA patients in comparison to control group. NLR had significant positive correlation with DAS-28ESR, VAS(0-100), TJC, and PtGA. PLR and RDW did not show any significant correlation with any of the clinical and laboratory parameters in RA patients, except for RDW which showed significant positive correlation with RF. NLR, PLR and RDW with AUC 0.677, 0.681 and 0.935 respectively could differentiate between RA patients and healthy controls. NLR, PLR and RDW with AUC 0.723, 0.702 and 0.933 respectively could differentiate between active RA patients and control individuals. PLR and RDW with AUC 0.649 and 0.939 respectively could separate inactive RA patients from healthy individuals.

Conclusions: NLR, PLR and RDW are present at higher levels in RA patients compared to normal population. NLR correlates with RA disease activity measured by DAS-28ESR. NLR correlates with RA disease activity.

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Key Words: *Rheumatoid arthritis – Disease activity – DAS-28 – DAS-28 ESR – Neutrophils – Lymphocytes – Red cell distribution width.*

Introduction

RHEUMATOID arthritis (RA) is a chronic autoimmune disease. Along with extra-articular symptoms and systemic consequences. It causes progressive symmetrical inflammation of the joints resulting in cartilage damage, bone erosion and articular disability **[1].**

Evaluation of the disease activity is crucial while monitoring RA patients **[2].** The European League Against Rheumatism (EULAR) suggests using the Disease Activity Score-28 (DAS-28) to assess disease activity in clinical studies **[3].** Erythrocyte sedimentation rate (ESR) and C-reactive protein

List of Abbreviations:

ACR : American College of Rheumatology.
Anti-CCP : Anticyclic citrullinated peptide antibodies.
AUC : Area under the curve.
CBC : Complete blood count.
CRP : C-reactive protein.
DAS-28 : Disease Activity Score-28joint count.
DMARDs : Disease-modifying antirheumatic drugs.
ESR : Erythrocyte sedimentation rate.
EULAR : European League Against Rheumatism.
IBD : Inflammatory bowel disease.
NLR : Neutrophil lymphocytic ratio.
PLR : Platelet lymphocytic ratio.
PtGA : Patient global assessment.
RA : Rheumatoid arthritis.
RDW: : Red cell distribution width.
RF: : Rheumatoid factor.
ROC curve: Receiver operating characteristic curve.
SLE : Systemic lupus erythematosus.
TJC : Tender joint count.
VAS : Visual analogue scale.

(CRP) constitute essential components of the DAS-28 score and are used to measure the inflammatory markers in RA patients' disease activity [4]. The non-specificity of these inflammatory measures and the fact that they have a flooring effect at reduced disease activity are the primary disadvantages in the laboratory measurement of inflammation [5]. Even at the lowest or nearly normal levels of ESR and CRP, synovial inflammation has been found to be visible using ultrasound and magnetic resonance imaging. However, these techniques are money and time consuming. So the demand for available, affordable and effective markers for disease activity and inflammatory process evolved [6].

Neutrophils, lymphocytes, and platelets all contribute to the regulation of inflammation. Neutrophil lymphocytic ratio (NLR) and platelet lymphocytic ratio (PLR) can be regarded as inflammatory indicators since systemic inflammation is linked to changes in circulating blood cell number and composition. Therefore, Neutrophil lymphocytic ratio (NLR), platelet lymphocytic ratio (PLR) can be considered inflammatory markers [7]. Recent studies have reported the numbers and ratios of complete blood count (CBC) subgroups in rheumatic diseases [8,9]. NLR, PLRand red cell distribution width (RDW) are inexpensive accessible laboratory indicators of systemic inflammation. It was recommended to do additional studies to determine whether NLR and PLR can be used as biomarkers to track RA activity in clinical practice or not [10]. Some studies displayed correlation between NLR and PLR with DAS-28 [9,11,12] but others did not [13,14]. Moreover, all studies issued RDW in RA were retrospective and only one of them mentioned DAS-28 correlation with RDW [15-19].

This controversy and deficiency in literature open the door for further studies. Up to our knowledge, there is no clinical study that evaluated the three markers NLR, PLR and RDW concurrently with RA activity in relation to DAS-28.

The aim of the study is to estimate the NLR, PLR and RDW levels in patients with RA and to find if there is a correlation between them and disease activity using DAS-28 ESR or not.

Patients and Methods

From April 2021 till August 2022, sixty five RA patients diagnosed according to the 2010 American College of Rheumatology/European League Against Rheumatism (ACR/EULAR) [20] were consecutively recruited from the our outpatient clinic of Rheumatology and Rehabilitation Department. They were compared to 65 age and sex matched healthy volunteers as a control group. Subjects with chronic diseases, acute or chronic infection, autoimmune diseases, malignancy, and pregnancy were excluded. Informed consent was obtained from the patients and controls. The study was performed with the approvalof the institutional review board (IRB).

RA patients were evaluated through full medical history, clinical examination and laboratory investigations that include serum rheumatoid factor (RF), anticyclic citrullinated peptide antibodies (anti-CCP), ESR, CRP and CBC. Disease activity wasdetermined using DAS 28-ESR. Different disease activity states were classified according to DAS-28 cut-offs: Remission (<2.6), low (2.6 to ≤3.2), moderate (3.2 to ≤ 5.1), and high (>5.1) [21]. On the same day of enrollment and examination 5ml of venous blood samples were collected for estimation of CRP and ESR and CBC. CRP was determined by using the immunonephelometric (Dade Behring N Latex High Sensitivity CRP mono assay) on a Behring Nephelometer II analyzer level, ESR was determined using citrated blood sample 1:4 dilution to be done by Westergren's method. Also, venous blood samples were collected from control group for evaluation of CBC. NLR and PLR were calculated and recorded with RDW level for all participants.

Statistical analysis:

The data were analyzed using the statistical package for the social sciences software version 21.0 (SPSS 21.0). Normal distribution of data was examined using the Kolmogorov–Smirnov test. Mean and standard deviation while median and range were recorded for parametric and non-parametric continuous variables respectively. Categorical variables were represented as number and percentage. Results were compared between the groups using student *t*-test and Mann-Whitney tests for parametric and non-parametric continuous data respectively. Chi-square test was used for comparison of categorical data. Correlation between variables was analyzed using Spearman's correlation test. $p<0.05$ was regarded as statistically significant.

Results

The study included 65 RA patients and 65 healthy controls. Mean ± SD of age in years was 46.7±14.4 and 44.1±13.7 ($p=0.3$) for RA patients and healthy controls respectively. There was no difference between both groups regarding sex differentiation ($p=0.8$). As in RA group 8 (12.3%) patients were males and 57 (87.7%) were females, and in control group 9 (13.8%) were males and 56 (86.2%) were females.

According to DAS-28 ESR, 25 (38.5%) patients were in remission while 8 (12.3%) and 32 (49.2%) patients were having moderate and high disease activity respectively. Table (1) demonstrates the clinical, laboratory characteristics and type of treatment in RA patients.

Table (1): The clinical, laboratory characteristics and type of treatment in RA patients.

Disease duration (years) median (range)	8 (0.4-35)
VAS (0-100) median (range)	60 (0-100)
TJC median (range)	5 (0-24)
SJC median (range)	3 (0-18)
PtGA median (range)	5 (0-9)
DAS-28 ESR median (range)	4.95 (1.89-7.4)
DAS-28 ESR for active RA patients (median ± SD)	5.9±1.1
DAS-28 ESR for inactive RA patients (median ± SD)	2.3±0.2
Morning stiffness duration (min.) median (range)	30 (0-75)
Patients with extra-articular manifestations No. (%)	8 (12.3%)
ESR (mm/hour) median (range)	22.85 (2.92-192)
CRP (mg/L) median (range)	27 (8-100)
RF:	
+ve No (%)	53 (81.5%)
–ve No (%)	12 (18.5%)
Anti-CCP:	
+ve No (%)	62 (95.4%)
–ve No (%)	3 (4.6%)
Patients on DMARDs No. (%)	50 (76.9%)
Patients on biological treatment (Anti TNF-α) No. (%)	15 (23.1%)

RA: Rheumatoid arthritis.
VAS: Visual analogue scale.
TJC: Tender joint count.
SJC: Swollen joint count.
PtGA: Patient global assessment.
DAS-28: Disease activity score using 28 joint counts.
ESR: Erythrocyte sedimentation rate.
CRP: C-reactive protein.
RF: Rheumatoid factor.
Anti-CCP: Anticyclic citrullinated peptide antibodies.
DMARDs: Disease-modifying antirheumatic drugs.
TNF-α: Tumor necrosisfactor alpha.

Table (2) displayed the level of different CBC components and hematological indices in RA patients, active RA patients, inactive RA patients compared to healthy controls. NLR was statistically significantly higher in RA patients and active RA patients compared to control group (p=0.001 and p<0.001 respectively). Moreover, PLR displayed statistically significant higher values in all RA patients, active and inactive RA patients in comparison to control group. (p<0.001, =0.001 and =0.03 respectively). In addition, RDW showed statistically significant higher values in all RA patients, active and inactive RA patients in comparison to control group. (p<0.001, <0.001 and <0.001 respectively).

There were no statistically significant differences between RA patients receiving disease-modifying antirheumatic drugs (DMARDs) and those receiving biological therapy regarding NLR, PLR and RDW (p=0.7, 0.8, 0.2 respectively).

NLR had significant positive correlation with DAS-28 ESR, visual analogue scale (VAS) (0-100), tender joint count (TJC), and patient global assessment (PtGA) (r=0.257, p=0.04), (r=0.267, p=0.033), (r=0.359, p=0.004) and (r=0.289, p=0.021) respectively. While it did not show any significant correlation with other parameters. PLR and RDW did not show any significant correlation with any of the clinical and laboratory parameters in RA patients, except for RDW which showed significant positive correlation with RF (r=0.436, p=0.006) (Table 3). Furthermore, no significant correlations were found between the three hematological parameters and all clinical and laboratory parameters in active RA patients.

Receiver operating characteristic curve (ROC curve) analysis was used to evaluate the potential role of the NLR, PLR and RDW as diagnostic biomarkers for RA patients. NLR, PLR and RDW with AUC 0.677, 0.681 and 0.935 respectively could differentiate between RA patients and healthy controls with cut off values 1.96, 126.5, 12.85 respectively (Fig. 1A). Moreover, NLR, PLR and RDW with AUC 0.723, 0.702 and 0.933 respectively could differentiate between active RA patients and control individuals with cut off values 1.98, 129.1, 12.85 (Fig. 1B). Only PLR and RDW with AUC 0.649 and 0.939 respectively could separate inactive RA patients from healthy individuals with cut off value 126.5 and 12.95 respectively (Fig. 1C). While NLR, PLR and RDW could not separate active RA patients from those in remission (Fig. 1D).

Table (2): The level of different CBC components NLR, PLR and RDW in RA patients, active RA patients, inactive RA patients compared control group.

	RA group			Control group (No.= 65)	p_1	p_2	p_3	p_4
	All patients (No.=65)	Active (No.=40)	Inactive (No.=25)					
RBCs ($\times10^3/\mu L$) mean ± SD	4.6±1.3	4.7±1.6	4.4±0.5	4.8±0.4	0.1*	0.6*	<0.001*	0.3*
Hb (gm/dl) mean ± SD	11.35±1.6	11.36±1.1	11.34±1.9	12.9±0.9	<0.001*	<0.001*	<0.001*	1*
WBCs ($\times10^3/\mu L$) mean ± SD median (range)	5.98 (3.2-15.6)	7.16±2.9	6.4±0.2	6.6 (4.9-10.9)	0.01#	0.1#	0.006#	0.009*
Neutrophils ($\times10^3/\mu L$) mean ± SD median (range)	3.3 (1.6-10.8)	4.49±2.3	3.8±1.6	4 (2.5-7.6)	0.02#	0.3#	0.004#	0.004*
Lymphocytes ($\times10^3/\mu L$) mean ± SD	1.7±0.55	1.67±0.6	1.7±0.48	2.48±0.6	<0.001*	<0.001*	<0.001*	0.3*
Platelets ($\times10^3/\mu L$) mean ± SD	243±75	243.7±71	242±82	279±51	0.002*	0.008*	0.04*	0.7*
NLR mean ± SD median (range)	2.25 (1.1-9.9)	2.4 (1.27-9.9)	2.24±0.8	1.7 (0.89-3.05)	0.001#	<0.001#	0.1#	0.1#
PLR mean ± SD median (range)	142.8 (62.1-473.1)	152.1 (62.1-473.1)	146.8±53.2	118.5±29.4	<0.001#	0.001#	0.03#	0.5#
RDW mean ± SD	14.1±1.4	13.9±1.3	14.2±1.5	12±0.7	<0.001*	<0.001*	<0.001*	0.08*

RA : Rheumatoid arthritis.
RBCs : Red blood cells.
Hb : Hemoglobin.
WBCs: White blood cells.
NLR : Neutrophil lymphocytic ratio.
PLR : Platelet lymphocytic ratio.
RDW: Red cell distribution width.
*Student *t*-test.
#Mann-Whitney.
p-value <0.05 is statistically significant.
*p*1 Comparison between all RA patients and control.
*p*2 Comparison between active RA patients and control.
*p*3 Comparison between inactive RA patients and control groups.
*p*4 Comparison between active and inactive RA patients.

Table (3): Correlation of NLR, PLR and RDW with different parameters in RA patients.

	NLR		PLR		RDW	
	r	*p*	*r*	*p*	*r*	*p*
Age	0.083	0.347	0.174	0.05	0.018	0.843
Sex	0.870	0.494	0.051	0.688	0.169	0.179
Disease duration	-0.225	0.073	-0.186	0.142	-0.060	0.637
Morning stiffness duration	-0.237	0.059	0.102	0.424	-0.035	0.785
VAS	0.267	0.033*	0.175	0.168	-0.08	0.527
TJC	0.359	0.004*	0.126	0.322	-0.045	.721
SJC	0.222	0.077	0.030	0.815	-0.117	0.354
PtGA	0.289	0.021*	0.181	0.152	-0.103	0.146
RF	0.051	0.761	-0.09	0.589	0.436	0.006*
Anti-CCP	-0.097	0.456	0.012	0.930	0.167	0.195
ESR	-0.08	0.532	0.154	0.224	-0.138	0.274
CRP	-0.071	0.652	-.0171	0.273	0.09	0.560
DAS-28ESR	0.257	0.04*	0.146	0.250	-0.121	0.338

NLR : Neutrophil lymphocytic ratio.
PLR : Platelet lymphocytic ratio.
RDW: Red cell distribution width.
VAS : Visual analogue scale.
TJC : Tender joint count.
SJC : Swollen joint count.
PtGA: Patient global assessment.
RF : Rheumatoid factor.
Anti-CCP: Anticyclic citrullinated peptide antibodies.
ESR : Erythrocyte sedimentation rate.
CRP : C-reactive protein.
DAS-28 : Disease activity score using 28 joint counts.
r: Spearman's correlation coefficient.
p-value <0.05 is statistically significant.

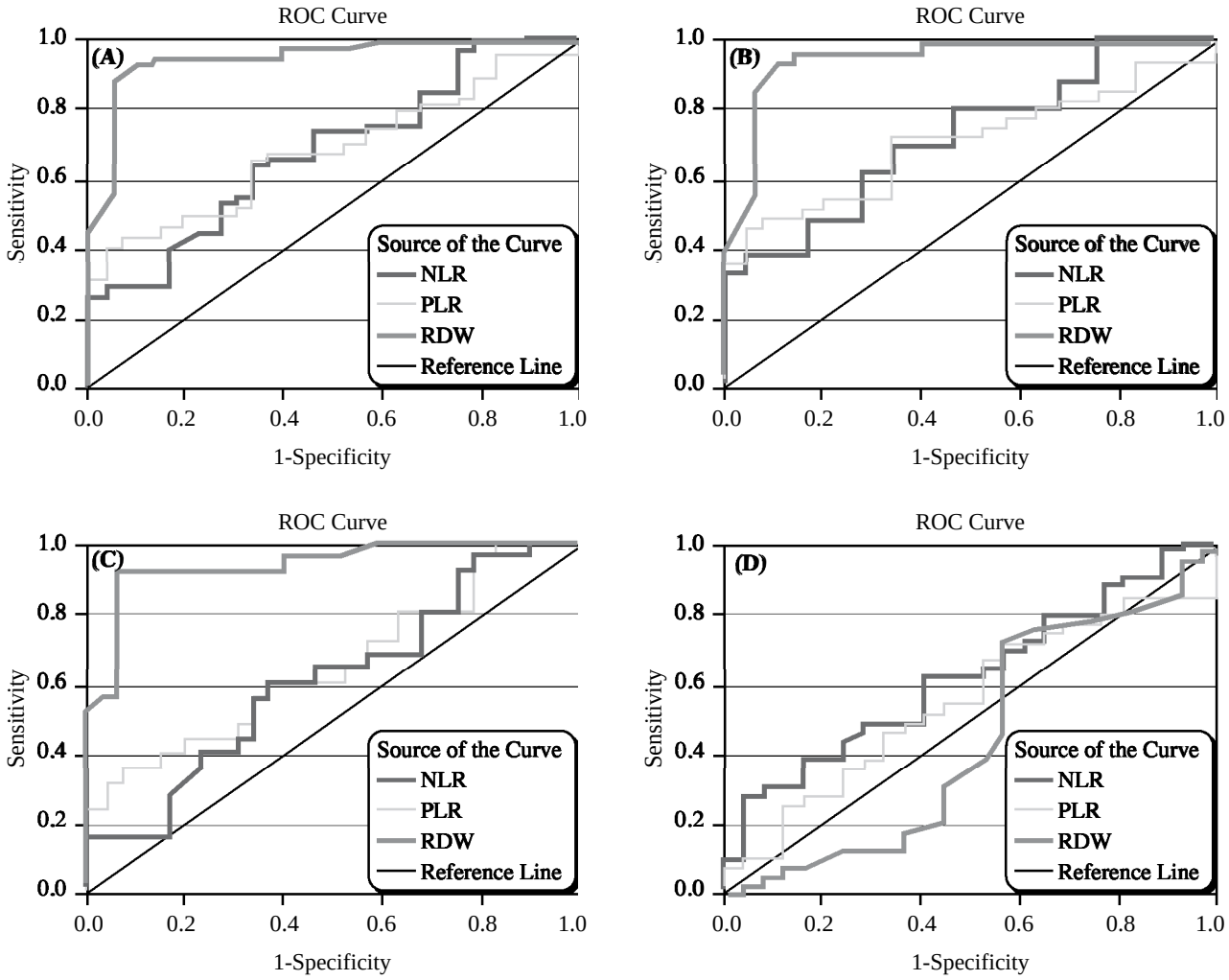

Fig. (1): ROC curve through NLR, PLR and RDW for differentiating between RA patients and control group (1A), between active RA patients and control group (1B), between inactive RA patients and control group (1C), between active and inactive RA patients (1D).

Discussion

Determining the disease activity in RA patients has become in importance since evaluating the disease activity makes it easier to make clinical decisions and personalize treatment *[22]*. Multiple studies have shown the limitations of ESR, CRP and DAS-28 in follow-up of disease activity *[23,24]*. Remission criteria could be attained in low ESR states, although patients might still have considerable amounts of swollen joints *[25,26]*. The development of measurable biomarkers that might aid in the quick and accurate diagnosis of RA is still necessary *[20,27]*. Even the ideal indicator of disease activity does not exist *[28]*.

NLR and PLR could be considered inflammatory markers owing to changes caused by the inflammation in neutrophils, platelets, and lymphocytes *[7]*. Lee et al. and Erre et al., in their meta-analysis advocated more research to clarify the clinical significance of NLR and PLR as biomarkers for tracking RA disease activity in routine practice *[10,29]*. Recent meta-analysis concluded that NLR and PLR could differentiate between RA patients with and without activity but recommended further studies to detect their utility in clinical practice *[30]*. Moreover, inflammatory conditions such inflammatory bowel disease (IBD), systemic lupus erythematosus (SLE), or Behçet's illness have been associated with increased RDW *[31-33]*. Erre et al. *[29]* in his meta-analysis stated that there are low numbers of studies evaluating NLR and PLR as markers for RA disease activity by DAS-28. In our study, we aimed to evaluate NLR, PLR and RDW in RA patients and their role as hematological markers for RA disease activity.

In our study, NLR and PLR were statistically significantly higher in all RA patients and active RA

patients compared to control group. This result is compatible with what stated in the literature [8,9, 11-14,34,35].

We did not find any statistically significant difference between inactive RA patients and control subjects regarding NLR. In agreement with our results Abd-Elazeem et al. [11] found the same results, while Zhang et al. and Koca et al. [14,15] found statistically significant difference between both groups. They included in their study a higher number of inactive RA patients (59 and 73 patients respectively) than ours. Moreover, they included more elderly patients [14,15]. On the other hand, we found a statistically significant difference between both groups (inactive RA group and control group) regarding PLR which is well matched with other studies [8,11,14,34].

Uslu et al. [36] found statistically significant difference in NLR and PLR between active and inactive RA patients. This does not match our results and could be attributed to the small number of inactive patients in our study (25 patients) compared to those in their study (64 patients).

RDW was found to be statistically significantly higher in RA patients, active RA and inactive RA patients compared to control group in this study. While there was no difference between active and inactive RA patients. These results are in accordance with other studies [15-19]. On the other hand, Koca et al., [15] concluded that there was statistically significant difference between active and inactive RA patients regarding RDW. In their study they contributed 73 inactive RA patients compared to 25 inactive RA patients that were included in our study.

Up to our knowledge, this is the first study that evaluated NLR, PLR and RDW as hematological biomarkers together in one study and its correlation to RA disease activity. In our study, NLR had significant positive correlation with DAS28-ESR, VAS (0-100), TJC and PtGA while PLR and RDW did not show any correlation with disease activity parameters or inflammatory markers.

Some studies as Gokman et al. and Zhang et al. [13,14] concluded no correlation of NLR and PLR with disease activity. Other studies as Mercan et al. [9], Abd-Elazeem et al. [11], Fawzy et al. [12] and Tecer et al., [16] stated that there are positive correlations of NLR, PLR and RDW with disease activity. These variations in results among studies could be attributed to the heterogenicity in included RA patients. Some studies included only newly diagnosed patients [12,34]. Other studies did not categorize the patients into active and in remission [8,9,13]. Moreover, the disease activity measured by DAS-28 in different studies was not similar.

In our study, NLR, PLR and RDW differentiate between all RA patients and active RA compared to healthy individuals. Jin et al., stated that NLR only could differentiate between all RA patients and healthy individuals with cut off value 2.13 [35]. Zhang et al. [14] documented that NLR and PLR could differentiate between active RA patients vs. control group, without mentioning to the cutoff value. Moreover, Peng et al. [34] found PLR could differentiate between active RA vs control group while NLR failed to differentiate.

In our study, NLR, PLR and RDW could not separate active RA patients from those in remission. In contrast to Chandrashekara et al. [6] who observed that NLR could differentiate between them. This could be explained by the small number of inactive RA patients (25 patients) in our study compared to 177 inactive RA patients in his study.

In this study, RDW showed diagnostic value in differentiating between RA patients and active and inactive RA patients versus control group with tremendous AUC, sensitivity, and specificity. This issue has not been studied widely in literature.

Our study has some limitations. Of these limitations, the relatively small number of included RA patients and the small number of inactive RA patients. Samples of this study have been taken during corona pandemic; most patients visited clinics were active patients. Chronic anemia is observed over time, but we did not consider vitamin B12, folic acid values which may affect the RDW values.

Conclusions:

NLR, PLR and RDW are present at higher levels in RA patients compared to normal population that indicates their role in the pathogenesis of RA. NLR correlates with RA disease activity. Therefore, it is valuable in evaluation and follow-up of the disease activity. NLR, PLR and RDW have suitable sensitivity and specificity for discrimination between RA patients and control group and between active RA patients versus control group. Moreover, RDW and PLR could discriminate between inactive RA patients and control group.

Ethics approval:

The study was validated and approved by the ethical committee of the Faculty of Medicine, Mansoura University (No: MS.20.09.1249). Written consents according to the Helsinki Declaration were taken from all participants prior to participa-

tion in the study that was approved by the ethical committee of the Faculty of Medicine, Mansoura University.

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Authors' contributions:

MN: Wrote the paper, followed-up the patients and analyzed the data, performed the research and statistical analysis. SMF and SH: Contribute to the research design, help in performing the research. SAB: Design the research study, performed the research, revised the paper and adjust the statistics.

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تقييم توزيع مدى تباين كرات الدم الحمراء ونسبة العدلات إلى الليمفاويات والصفائح إلى الليمفاويات فى مرضى الرثيان المفصلى

يعتبر الرثيان المفصلى مرض مناعى مزمن ويتسبب فى تدمير تدريجى للمفاصل وفقدان وظيفتها. يعد عدم خصوصية الدلالات الإلتهابية أحد القيود الرئيسية فى القياس المعملى للإلتهاب. أفادت دراسات التحليل التجميعى أن نسبة العدلات إلى الليمفويات ونسبة الصفائح الدموية إلى الخلايا الليمفاوية قد يكونان مؤشرات محتملة لتحديد مدى الإلتهاب وتقييم نشاط مرض الرثيان المفصلى، بالإضافة إلى ذلك، وجدت دراسات أخرى علاقة بين نشاط المرض وتوزيع مدى تباين كرات الدم الحمراء.

الهدف من هذه الدراسة هو قياس نسبة العدلات إلى الخلايا الليمفاوية، نسبة الصفائح الدموية إلى الخلايا الليمفاوية وتوزيع مدى تباين كرات الدم الحمراء فى مرضى الرثيان المفصلى كما تهدف إلى دراسة علاقتها بمؤشر نشاط المرض. ودراسة العوامل التى تؤثر على هذه العلامات.

أجريت هذه الدراسة في مستشفى جامعة المنصورة على ٦٥ مريض من مرضى إلتهاب الرثيان المفصلي المترددين على عيادة الامراض الروماتيزمية والقسم الداخلى للروماتيزم والتأهيل وتم مقارنتهم ب ٦٥ شخصاً من الأصحاء الذين لهم تقريبا نفس العمر والجنس. تضمن البحث تناول التاريخ المرضى للحالات وقت بداية الشكوى وتاريخ التشخيص والفحص السريرى الشامل وقياس نشاط المرض عن طريق مؤشر DAS–28 ESR والإختبارات المعملية. وصورة دم كاملة. كما تم حساب توزيع مدى تباين كرات الدم الحمراء، نسبة العدلات إلى الخلايا الليمفاوية ونسبة الصفائح الدموية إلى الخلايا الليمفاوية.

تتواجد نسبة العدلات إلى الخلايا الليمفاوية ونسبة الصفائح الدموية إلى الخلايا الليمفاوية ومستوى توزيع مدى تباين كرات الدم الحمراء بمعدل أعلى فى مرضى الرثيان المفصلى مقارنة بالأشخاص الطبيعيين. يرتبط نسبة العدلات إلى الخلايا الليمفاوية بنشاط مرض الرثيان المفصلى، وبالتالى فهو ذو قيمة فى تقييم نشاط المرض.