RESEARCH ARTICLE

NOVEL HEMATOLOGICAL MARKERS NEUTROPHIL TO HEMOGLOBIN LYMPHOCYTES RATIO AND PLATELETS TO LYMPHOCYTES RATIO AND ITS ASSOCIATION WITH THE DISEASE ACTIVITY IN RHEUMATOID ARTHRITIS

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ABSTRACT

Objective: *This study was done to find out the role of two new markers Neutrophil to Hemoglobin and Lymphocytes ratio (NHL) and the Platelet Lymphocytes ratio (PLR) in ascertaining the activity of disease in rheumatoid arthritis.*

Design: *A retrospective cross sectional study.*

Place and Duration: *The study was carried out on 290 patients in the Rheumatology outpatient department of Federal Government Polyclinic Hospital, Islamabad. The data under study was taken from Dec 2020 till June 2021.*

Methodology: *DAS28-ESR was calculated using the DAS28-ESR calculator and NHL & PLR were calculated.*

Results: *There were 238(82.1%) female and 52 (17.9%) male participants. The mean age of patients was 45.2± 12 years. Out of the 290 participants, 22.8% had inactive disease (<3.2 DAS28 score) while 77.2% had active disease (>3.2 DAS 28ESR). The spearman's rho analysis found a significant positive correlation between the disease activity score and the NHL and PLR ($p<0.001$). The receiver operating curves (ROC) for the NHL was 0.736 (95% Confidence Interval (CI):666–0.807, $p < 0.001$) , 0.672(95% CI:0.597-0.747, $p < 0.001$) for PLR and 0.756(95% CI: 0.687-0.824, $p < 0.001$) for ESR respectively.*

Conclusion: *Both NHL ratio and PLR can be used as a cheap and easily measurable tool for disease severity assessment in rheumatoid arthritis patients.*

Keywords: *Rheumatoid Arthritis (RA), Neutrophil to Hemoglobin and Lymphocyte Ratio (NHL), Platelet to Lymphocyte Ratio (PLR), Disease Activity score ESR (DAS28-ESR)*

INTRODUCTION

Rheumatoid arthritis is a common yet one of the most disabling disease amongst the autoimmune inflammatory diseases. Being an inflammatory disease it is bound to cause long term morbidity to the patients if not treated promptly and properly.[1] The degree of severity of inflammation is commonly assessed by the markers like sedimentation rate of the erythrocytes (ESR) and C-reactive protein (CRP) in day to day routine, but they alone are not sufficient to label the person as having high disease activity. Clinical assessment of painful, tender and swollen joints alongside the inflammatory markers is used to assess Disease activity score-28 (DAS28 CRP/ESR) and is used commonly as a severity index to monitor the disease and modify treatment accordingly.[2]

There is inclination towards identifying newer and cheaper biomarkers for the measurement of the disease activity. A lot of research is being done on utilizing simple blood complete picture and the different hematological indices for assessment of disease severity. The most commonly used ones are neutrophils to lymphocyte ratio (NLR)[3] red cell distribution width (RDW)[4] and the mean platelet volumes (MPV)[4] in

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different studies done throughout the world. The chronic inflammatory process in rheumatoid arthritis leads to changes in the neutrophils, lymphocytes and platelet counts as well as anemia of chronic disease. So, utilizing these simple entities can help in assessing the disease severity.

Platelets are pro inflammatory and they interact with leukocytes to bring about systemic as well as joint inflammation.[5] Platelet to lymphocyte ratio is obtained by dividing the platelet count by the lymphocyte count and it has been used as an inflammatory marker in rheumatoid arthritis as well as in other diseases like ankylosing spondylitis,[6] Systemic lupus erythematosus,[7] other inflammatory disorders like Crohn's disease, ulcerative colitis[8] and acute disorders like myocardial infarction.[9]

Neutrophils are the most important part of the inflammatory process going on in the body due to any systemic illness including rheumatoid arthritis. They release proteases, products of oxidative stress and prostaglandins thus leading to inflammatory process and ultimately damage the joints. There is relative neutrophilia and lymphocytopenia and thus a disturbed neutrophil to lymphocyte ratio. This was previously studied as a marker of severity of disease evidenced by degree of inflammation.[10] Also anemia because of chronic inflammation is commonly seen in rheumatoid arthritis so recently a new marker neutrophil to hemoglobin and lymphocyte score/ratio (NHL) has been introduced for assessing inflammation in rheumatoid arthritis and there has been only one study so far in Korea using NHL as a marker for rheumatoid arthritis disease actvitiy.[11] So the rationale behind conducting our study was to evaluate the role of NHL and PLR in rheumatoid arthritis and also find its association with disease activity in Pakistani cohort of RA patients. NHL ratio hasn't been used in any study previously in Pakistan.

MATERIALS AND METHODS

This study with a retrospective cross sectional design was carried out at IN/OUT patient department of Rheumatology in Federal Government Polyclinic Hospital Islamabad. The sample size was calculated using WHO formula for sample size in health sciences with a confidence interval of 95% and margin of error at 5%. The overall prevalence of rheumatoid arthritis in the world is 0.5-1% while in Pakistan in previous study in Karachi showed a prevalence of 0.14%.[12] A study done in India using NLR for severity of inflammation in rheumatoid arthritis was taken as the reference study for sample size estimation.[13] A total of 290 patients were randomly selected.

Inclusion criteria: Patients meeting the ACR /EULAR criteria for RA.

Exclusion criteria: Chronic renal failure, diabetics, anemia of chronic diseases, hematological malignancies, nutritional deficiency anemias, idiopathic thrombocytopenia, systemic lupus erythematosus and other disorders of the connective tissues were excluded from the study.

Disease activity 28 ESR score was calculated by counting the number of tender and swollen joints out of 28 joints (i.e small and large joints of the upper limbs excluding the distal interphalangeal joints and knee joints in the lower limbs), the patients global health on scale of 0 to 100 mm and adding the ESR in to the formula i.e

DAS28(4)=0.56*sqrt(t28)+0.28*sqrt(sw28)+0.70*Ln (ESR)+0.014*GH . [14]

The different disease activity categories are as follows.

DAS28-ESR SCORE/ DAS28 SCORE.[15]

DAS28 SCORE	ACTIVITY STATUS OF DISEASE
<2.6	Remission
2.6-3.2	Mild
3.2-5.1	Moderate
>5.1	Severe

Neutrophil to hemoglobin and lymphocyte ratio is a novel biomarker and it is calculated by dividing the neutrophil percentage by the hemoglobin of the patient and the percentage of lymphocytes thus making the unit of measurements g/dl.[11]

Platelets to lymphocyte ratio is calculated by dividing the number of platelets by the number of lymphocytes in the blood.

Previously a study was conducted in our department on association of RDW and NLR as markers of disease severity for RA done from December 2020 till June 2021 after getting approval from Ethical Review Board of our hospital. This study was done on the same data set and so no new approval was sought. Ethical approval was issued in the 76th meeting of ethical committee No. FGPC.1/12/2020. Data were entered on to the SPSS 23 version and then the analysis was done. NHL and PLR were calculated according to the formula described. DAS28-ESR score was calculated using the

DAS28-ESR calculator. Descriptive analysis was done and frequencies, percentages and where necessary standard deviations and mean values were calculated. The association between the DAS28-ESR score of the participants and the value of NHL and PLR was found out using the chi square tests. Spearman correlation analysis was done to find the significance and strength of relationship between abnormally distributed data among groups. The significant *p*-value was <0.05 in all the tests done. Among other important tests Mann Whitney U test was performed for both the variables in association with the DAS28-ESR activity. Receiver operator curves were also generated for the tested variables to define the sensitivity and specificity for NHL ratio and the PLR in reference to ESR of the patients.

RESULTS

There were total of 290 patients in the study. Among 290 patients, 238(82.1%) belonged to the female gender while male was 52 (17.9%). The mean age of patients was 45.2 ± 12 years. Out of the 290 participants, 8.9% were in remission of disease, 10% with low, 55.9% with moderate while 25.2% had high activity of disease based on the DAS28-ESR score.

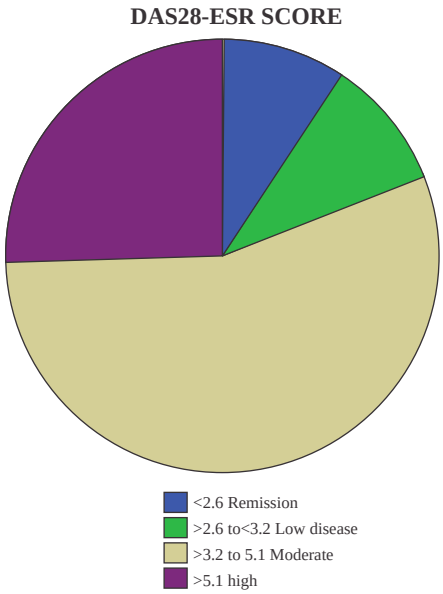

Fig 1: Percentage of the patients in different disease categories based on DAS28-ESR score

Baseline demographics of the study sample is shown in table I while table II shows the differences in the parameters of both the active and the inactive rheumatoid arthritis based on the DAS28 score with 3.2 being taken as a cut off to divide into active and inactive disease.

Table III shows the Spearman's rho correlation analysis, which suggests that there is significant positive correlation between the neutrophil to hemoglobin and lymphocytes ratio as well as the platelets to lymphocytes ratio ($p<0.001$).

Table I: Baseline Demographics of the study population

Variables	Minimum	Maximum	Mean ± Standard Deviation
Age (years)	16	75	45.02 ± 12.5
DAS28-ESR	1.20	8.34	4.29895 ± 1.26
ESR (mm/hr.)	5	110	33.75 ± 20.6
WBC (4000-11000/mm^3)	3700	16510	8630.48 ± 2342.45
Hemoglobin(12-14 g/dl)	9.50	16.60	12.41 ± 1.38
Platelets (150,000-400,000/mm^3)	120000	651000	310620.83 ± 99183.17
Neutrophils (40-70%)	40.00	87.0	66.84 ± 9.03
Lymphocytes (20-40%)	1.7	48.00	25.96 ± 8.10
Neutrophil to hemoglobin and lymphocytes ratio (NHL)	0.06	0.75	0.24 ± 0.11
Platelet to lymphocyte ratio (PLR)	23.29	698.63	157.67 ± 78.65

Table II: Comparison of characteristics in patients with inactive and active RA.

	INACTIVE RA (LOW AND REMISSION)	ACTIVE RA (MODERATE TO HIGH DAS28)	*p*-VALUE
Age,years+	43.29 ± 10.91	45.53 ± 12.90	
ESR (mm/h)	22.35 ± 12.50	37.62 ± 20.96	**<0.001**
WBC COUNT (x10^9/L)	8214.39 ± 2094.31	8753.08 ± 2401.29	0.122
Neutrophil %	61.69 ± 10.15	68.41 ± 8.01	**<0.001**
Lymphocyte% DAS28-ESR	31.12 ± 8.04	24.50 ± 7.35	**<0.001**
Platelet counts (x10^9/L)	292335.45 ± 90113.5	316008.48 ± 101260.7	0.153
Neutrophil to hemoglobin and lymphocytes ratio (NHL)	0.18 ± 0.10	0.26 ± 0.11	**<0.001**[a]
Platelet to lymphocyte ratio (PLR)	128.98 ± 55.05	166.7 ± 82.29	**<0.001**[a]
N (%)	66(22.76%)	224(77.24%)	

[a] Mann Whitney U test.

Table III: Spearman's rho correlation between DAS28-ESR and the Neutrophil to hemoglobin lymphocyte ratio (NHL), Platelets to lymphocytes ratio and ESR.

Factors		Spearman's Rho Correlation Co-efficient (r) and *p*-value
Neutrophil to hemoglobin lymphocyte ratio(NHL)	Spearman's rho Correlation Co-efficient	0.343
	p-value	<0.001
Platelets to lymphocyte ratio	Spearman's rho Correlation Co-efficient	0.250
	p-value	<0.001
ESR	Spearman's rho correlation coefficient	0.252
	p-value	<0.001

The receiver operator curves were also generated for NHL and PLR ratio to look at the sensitivity and specificity of these tests and identify the area under curves for each variable. Figure 2 and Table IV shows the ROC curves for these variables.

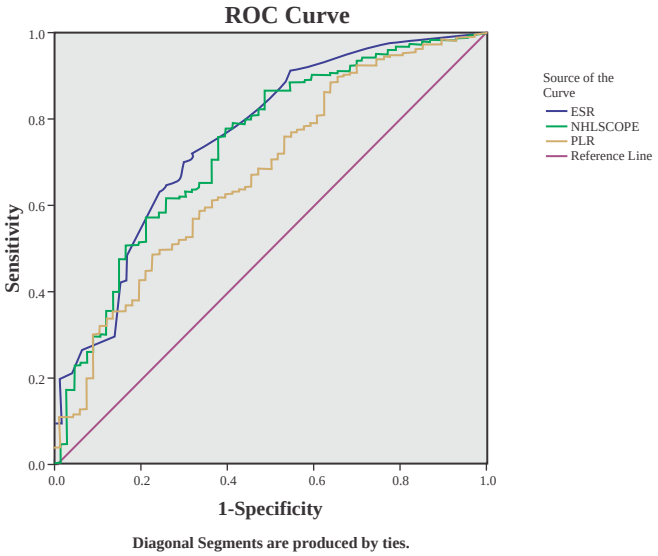

Fig 2: Showing ROC curves for the variables NHL, PLR and ESR.

Table IV: Showing Receiver operating curve characteristics for NHL , PLR and ESR in active and inactive RA. P<0.05 is significant.

Factors	Spearman's Rho Correlation Co-efficient (r)	*p*-value
Neutrophil to hemoglobin lymphocyte ratio (NHL)	0.343	<0.001
Platelets to lymphocytes ratio (PLR)	0.250	<0.001
ESR	0.252	<0.001

Table V: Difference in NHL And PLR Based On Patients Disease Activity using the Mann Whitney U test.

Variables	Outcome Disease activity	Median	IQR	Mean Rank	Test Statistics	*p*-Value
NHL	Inactive RA=66 Active RA=224	0.21	0.13	92.6 161.09	3900.5	<0.001
PLR	Inactive RA=66 Active RA=224	138.14	78.66	106.97 156.85	4849	<0.001

DISCUSSION

Rheumatoid arthritis is the commonest joint disease of inflammatory origin which involves the joints symmetrically i.e small and large joints of the body with a particular predilection for the small joints of the hand except the distal interphalangeal joint.[16] Joint inflammation is assessed by different parameters i.e DAS28-ESR, Simple Disease Activity Index(SDAI), Clinical Disease Activity Index (CDAI) scores and inflammatory markers like ESR and CRP have been extensively studied as predictor of inflammation.[17] As CRP and ESR are affected by certain confounding factors like anemia, hypergammaglobulinemia, gender of the patients, levels of fibrinogen and the changes in the viscosity of the blood so there is need to identify new relatively stable and cheaper biomarkers which could serve the function for assessing the disease activity in rheumatoid arthritis.[4]

The new markers are derived from the cells that are involved both in the coagulation and inflammatory cascade i.e platelets, lymphocytes, neutrophils and the effect on hemoglobin as a result of the chronic inflammatory process. These new biomarkers are Platelet lymphocyte ratio[4] and the neutrophil to hemoglobin and lymphocytes ratio. Although previously Neutrophil to lymphocyte ratio had been studied as a marker alongside platelet lymphocyte ratio but we in our study wanted to utilize this new marker.

Literature has shown that platelet lymphocyte ratio is higher in those having rheumatoid arthritis than the non-rheumatoid arthritis patients as shown by studies done by Erre et al in 2019 [18] and by Tarun khan et al in India 2022.[19] In our study, we found that there is a positive correlation between the disease activity in rheumatoid arthritis and the value of platelet lymphocyte ratio as evidenced by significant *p*-value on the spearman's rho correlation analysis. Those having active rheumatoid arthritis (moderate to high disease activity) had a higher value of PLR i.e 166.7 ± 82.29 vs inactive disease i.e (remission to low disease activity) 128.98 ± 55.05 with a *p*-value of <0.001. Findings similar to our study were

found in study done by Yingying Zhang et al in 2016 which showed a mean PLR of 142.6 for the active RA compared to 138.8 for inactive RA.[20] Similar findings were also observed in several other studies done by Rehab et al in which the group with active RA had a higher PLR while those with inactive RA had lower PLR.[21,22]

The area under the curve by ROC for the Platelet to lymphocyte ratio in our study was 0.672 which is almost similar to JY Choe et al's study (0.581)[11] and study done by Tarun Khan et al (0.651).[20] The ROC curve values though not very high but still did suggest the role in assessing the disease activity.

The other biomarker that was studied in our study with particular emphasis was neutrophil to hemoglobin and lymphocyte ratio/ score, a novel marker only previously been used once in study done by JY Choe et al.[11] in 2022. The findings in our study were comparable to that in the reference study showing mean values of 0.18 ± 0.10 for the inactive group while 0.26 ±0.11 for those with active rheumatoid arthritis with a *p*-value of <0.001 on the Mann Whitney u test while that in the reference study were 0.188 (0.112 - 0.238) for the low disease activity vs 0.192 (0.127 - 0.276) for those with moderate to high disease activity on Kruskal-Wallis test. Similarly the area under curves and ROC values for the NHL was 0.736 which were roughly similar to the parent study which showed a value of 0.705 for NHL score.[11] The findings thus suggest that NHL score/ ratio can be used as a novel biomarker which is cheap and has a relatively greater area under the curve compared to the PLR and almost similar to that found for the ESR group in our study population which had an area under curve of 0.756, which is the most common tool for assessing the inflammation in the joints in rheumatoid arthritis patients alongside the clinical assessment and an integral part of the DAS28-ESR score.

Thus using these two new biomarkers alongside assessment of the patients clinically, we can actually identify patients having high disease activity by only looking at the complete blood picture report of the patients and getting an idea about the degree of inflammation going on in the joints.

The study had a few limitations like the study was done retrospectively. Secondly the effect of steroids leading to slight rise in the neutrophil counts was not removed which could have contributed a bit higher values in those who were having high disease activity. The effect of different DMARDS patients were taking were not separately evaluated. The response to treatment after a follow up with repeat assessment was not done.

More studies are needed on the role of neutrophil to hemoglobin and lymphocyte ratio in future. It can also be used in other autoimmune disorders as well as a marker of disease activity. Prospective studies can be done to actually see the impact of various DMARDs on the NHL and PLR values and the change in disease activity with treatment.

CONCLUSION

Several hematological indices can be used as marker of inflammation in rheumatoid arthritis. In this study we used two new biomarkers i.e NHL score/ ratio and PLR as the markers of severity of disease activity in RA. And it showed that Neutrophil to hemoglobin and lymphocytes ratio and the platelets to lymphocytes ratio can be used as potential cheap but reliable markers of inflammation and thus reflect the disease activity in rheumatoid arthritis patients

Ethical Statement: This study was undertaken after taking approval from the institution based ethical review board in the 76th meeting of the ethical committee. All the participants gave a written consent for inclusion in the study.

Conflicts of interest: None.

Funding disclosure: None.

Disclaimers: None.

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