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A comprehensive look at inflammation in RLS: assessing NLR, MLR, PLR, SII, SIRI, and microR

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ABSTRACT

Aims: Restless legs syndrome (RLS) has been linked to systemic inflammation. The number of studies investigating inflammation in RLS patients is extremely limited. The purpose of this study is to examine the possible role of proinflammatory parameters in RLS, specifically neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), platelet-to-lymphocyte ratio (PLR), systemic immune-inflammation index (SII), systemic inflammatory response index (SIRI), and microR.

Methods: The study included 100 patients admitted to the neurology outpatient clinic diagnosed with RLS using the International Restless Legs Syndrome Study Group ((IRLSSG) scale and 100 healthy controls. Hemogram results were obtained from both RLS patients and healthy controls, while ferritin, folate, vitamin D and B12, and C-reactive protein (CRP) levels were obtained only from RLS patients.

Results: The median age of the patient group was 52.50 (43-60.75), while the median age of the healthy group was 51.00 (50-53). The patient group is 37% male, while the healthy group is 34% male. It doesn't vary by age or gender (p=0.658). The two groups showed significant differences in PLR (<0.001), MLR (0.035), microR (p=0.023), and SIRI (p=0.022). There was no statistically significant difference in NLR, SII, and macroR levels between the two groups.

Conclusion: In the current study, the inflammatory variables PLR, MLR, and microR were significantly lower, and SIRI was significantly higher from healthy control groups.

Keywords: Restless legs syndrome, NLR, PLR, MLR, SII, SIRI, microR

INTRODUCTION

Restless legs syndrome (RLS), also known as Willis-Ekbom illness, was first identified in 1945 by Dr Karl Ekbom. RLS is a sensory-motor neurological condition characterized by an impulse to move the legs, aberrant feelings in the legs, and dysaesthesia while at rest.[1] RLS affects 3-10% of the population. The etiology of RLS remained unknown until recently. Dopaminergic dysfunction is the most commonly accepted explanation for the etiology of RLS. RLS can be classified as idiopathic or secondary. Secondary RLS can have multiple reasons. The primary causes include iron deficiency, terminal renal failure, Parkinson's disease, polyneuropathy, pregnancy, and medications. Certain medications, including antiemetics, antipsychotics, antihistamines, antiepileptics, and antidepressants, can induce or aggravate RLS.[2]

Although the pathomechanism is clearly unknown, dopaminergic dysfunction, brain iron deficit, and inflammation are likely to be key contributions to the pathophysiology of idiopathic RLS. Neuroinflammation and oxidative stress have been linked to the development and progression of chronic neurodegenerative diseases.[2] RLS is related to systemic inflammation.[3] The number of studies examining inflammation and oxidative stress in RLS patients is extremely low. A study demonstrated high C-reactive protein (CRP) levels and enhanced inflammation in patients with RLS.[4] A recent study discovered a high neutrophil/lymphocyte ratio (NLR) in RLS patients compared to controls, highlighting the role of inflammation in illness pathogenesis.[5] However, to our knowledge, no study has been conducted in the literature to investigate the relationship between RLS and inflammatory parameters monocyte lymphocyte ratio (MLR), platelet lymphocyte ratio (PLR), systemic immune-inflammation index (SII), system inflammation response index (SIRI), and microR.

In this study, we investigated the potential role of inflammatory parameters NLR, MLR, PLR, SII, SIRI, and microR in RLS.

METHODS

The study was carried out with the permission of the Kastamonu University Clinical Researches Ethics Committee (Date:12.06.2023, Decision No:2023-KAEK-156). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. All study participants provided informed consent forms. This study was conducted in Kastamonu Training and Research Hospital between January 2024 and February 2024. RLS was diagnosed using the International Restless Legs Syndrome Study Group (IRLSSG) questionnaire, which includes four questions: (a) Do you have an urge to move your legs accompanied by uncomfortable or disagreeable feelings? (b) Do the uncomfortable or disagreeable feelings begin or worsen during inactive periods? (c) Do the uncomfortable or disagreeable feelings decrease with activity? (d) Are the uncomfortable or disagreeable feelings more prominent in the evening or at bedtime? Patients who replied 'yes' to every question were diagnosed with RLS. Exclusion criteria included polyneuropathy, lumbosacral radiculopathy, malignancy, acute infection, severe liver or renal failure, or being younger than 18 and older than 65.

Hemogram values, ferritin, folate, vitamin D and B12, and CRP levels were measured. According to IRLSSG the severity scale consisted of ten items, each graded from 0 to 4, for a total score of 0 to 40. An IRLS score of 1 to 10 correlates to mild RLS, 11 to 20 moderate, 21 to 30 severe, and 31 to 40 very severe RLS. Patients were divided into two groups based on their scores: mild-moderate-severe disease (0-30 points) and very severe disease (31-40 points). To strengthen the statistical power of the study, we defined two categories in the IRLSSG score subgroup (mild-moderate-severe as group 1 and very severe as group 2). The NLR, MLR, PLR, SII, and SIRI were computed as follows:

NLR=Neutrophil count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

MLR=Monocyte count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

PLR=Platelet count ($x10^9$/L) / Lymphocyte count ($x10^9$/L)

SII=Platelet count ($x10^9$/L) x NLR

SIRI=Neutrophil count ($x10^9$/L) x MLR.

Statistical Analysis

Data were analyzed with IBM SPSS V23. Compliance with normal distribution was examined using Shapiro-Wilk and Kolmogorov-Smirnov tests. The chi-square test was used to compare categorical variables according to groups. Independent two-sample t-test was used to compare normally distributed data according to binary groups, and Mann-Whitney U test was used to compare non-normally distributed data. The Pearson correlation coefficient was used to analyze relationships between normally distributed data, whereas Spearman's rho correlation coefficient was used to examine relationships between non-normally distributed data. The significance level was taken as $p<0.050$.

RESULTS

One hundred patients and 100 healthy controls were included in our study. Median age (p=0.133) and gender (p=0.658) do not differ according to the groups. The average time to onset of disease symptoms in the patient group was 6.79 years. 86 (86%) of the patient group is married (Table 1).

A comparison of hemogram parameters and immune response-related markers (NLR, MLR, LMR, PLR, SII,

Table 1. Demographic characteristics of each group

	Patients	Healthy Controls	Total	Test Statistic	p
Age	51.31±11.05	50.72±0.45	51.02±7.80	U=4402.000	0.133
	52.50 (21.00-73.00)	51.00 (50.00-51.00)	51.00 (21.00-73.00)		
Gender					
Male	37 (37)	34 (34)	71 (35.5)	x^2=0.197	0.658
Female	63 (63)	66 (66)	129 (64.5)		
Year	6.79±6.11	----	6.79±6.11	----	---
	5.00 (0.00-30.00)	----	5.00 (0.00-30.00)		
Marital Status					
Married	86 (86)	----	86 (86)	----	---
Single	14 (14)	----	14 (14)		

U: Mann-Whitney U test statistic, x^2: Chi-square test statistic, frequency (percentage), mean±s. deviation, median (minimum-maximum)

and SIRI) in the patient and control groups is given in Table 2. A statistically significant difference was found between the two groups in WBC (10^3/uL) (p=0), PLT (10^3/uL) (p=0.029), PCT(%) (p=0.038), NEUT (10^3/uL) (p=0), LYMPH (10^3/uL) (p=0), MONO (10^3/uL) (p=0.001), EO (10^3/uL) (p=0.007) , BASO (10^3/uL) (p=0.007), IG (10^3/uL) distributions (p=0.038), microR (%) (p=0.023), SIRI (p=0.022) No statistically significant difference was detected in the other data stated in the table. In Table 3, patients are divided into two groups according to the UHBSSG score: mild-moderate disease (0-30 points) and severe disease (31-40 points) and compared. In addition to the data given in Table 2, serum ferritin, folate, vitamin B12, vitamin D, albumin, and CRP levels were also examined in both groups. A statistically significant difference was found between the creatine medians according to the UHBSSG score groups in both groups (p=0.029). A statistically significant difference was found between HGB (g/dL) and HCT (%) averages according to UHBSSG score groups (p=0.01, p=0.023).

DISCUSSION

In this study, PLR, MLR, and microR were significantly lower, and SIRI was significantly higher from healthy control groups. We observed a statistically significant relationship in HGB and HCT between the groupings mild-moderate-severe and very severe according to the IRLSSG rating scale. We also found a significant association between CRP and the total score of the IRLSSG rating scale.

The most common explanations of RLS etiology are dopamine dysregulation and iron deficiency.[6] Although there is various research on the subject, the association between systemic inflammation and RLS has just recently been investigated. Many inflammatory and autoimmune diseases, such as chronic liver disease, Sjogren's syndrome, rheumatoid arthritis, sarcoidosis, systemic lupus erythematosus, inflammatory bowel disease, and multiple sclerosis, have been linked to an increased risk of developing RLS.[7,8] Additionally, patients with recurrent and severe RLS have consistently been observed to have these infectious-inflammatory conditions.[7,8] These findings improve the possibility that inflammatory factors play a role in the etiopathogenesis of RLS.

The association between NLR and several neurological disorders has been established in the literature. NLR has been demonstrated to be effective in predicting neurological disorders as well as prognosis and death following critical neurological diseases.[9,10] Two cross-sectional investigations found increased NLR in RLS than in controls.[4,11] However, Dowsett et al.[12] observed no connection between RLS and NLR in Danish blood donors after controlling for sex, age, alcohol use, smoking status, and BMI. Furthermore, Tak et al.[13] found no statistically significant link between NLR and RLS in their research of

Table 2. Comparison of parameters according to groups

	Patients		Healthy Controls			
	Means±S. Deviation	Median (min.-max.)	Mean±S. Deviation	Median (min.-max.)	Test Statistic	p
WBC(10^3/uL)	7.64±2.50	7.20 (4.67-24.91)	6.29±3.32	4.63 (2.78-12.13)	U=2419	<0.001
RBC(10^6/uL)	4.93±0.44	4.88 (4.05-6.64)	4.87±0.53	4.89 (2.99-6.42)	t=0.842	0.401
HGB(g/dL)	14.05±1.38	14.00 (9.60-17.40)	13.64±1.59	13.75 (9.10-18.60)	t=1.912	0.057
HCT(%)	42.67±3.72	42.80 (32.20-52.80)	41.65±4.29	41.85 (26.50-54.50)	t=1.727	0.086
MCV(fL)	86.62±4.33	87.00 (70.80-94.40)	85.68±4.90	85.45 (68.60-99.00)	U=3781.5	0.075
PLT(10^3/uL)	268.12±82.04	256.00 (116.00-748.00)	248.20±77.37	230.00 (37.00-499.00)	U=3632	0.029
NEUT#(10^3/uL)	4.362±1.934	3.920 (1.570-17.810)	3.579±2.209	2.520 (1.180-9.910)	U=2631	<0.001
LYMPH#(10^3/uL)	2.430±0.673	2.360 (1.200-4.950)	1.995±1.105	1.575 (0.690-6.990)	U=2486.5	<0.001
MONO#(10^3/uL)	0.592±0.233	0.550 (0.320-1.980)	0.518±0.232	0.430 (0.240-1.280)	U=3187	0.001
EO#(10^3/uL)	0.212±0.207	0.150 (0.030-1.370)	0.158±0.176	0.100 (0.000-1.240)	U=3431	0.007
BASO#(10^3/uL)	0.048±0.026	0.040 (0.010-0.140)	0.040±0.027	0.030 (0.000-0.150)	U=3447.5	0.007
MicroR(%)	2.902±3.697	1.900 (0.400-27.400)	3.502±4.194	2.500 (0.300-29.400)	U=3600	0.023
MacroR(%)	3.961±0.428	3.900 (2.900-5.600)	3.918±0.580	3.850 (3.000-7.800)	U=3975.5	0.205
NLR	1.889±0.751	1.750 (0.610-4.320)	1.901±1.055	1.680 (0.570-8.930)	U=4160	0.440
PLR	117.35±46.15	107.18 (55.14-387.56)	143.70±55.38	138.76 (30.76-305.41)	U=3035.5	<0.001
MLR	0.255±0.097	0.240 (0.120-0.600)	0.292±0.127	0.270 (0.080-0.890)	U=3658.5	0.035
SII	520.50±325.72	451.68 (97.66-2464.91)	473.15±306.87	413.63 (57.81-2446.25)	U=3891	0.136
SIRI	1.190±0.934	0.940 (0.270-7.120)	1.044±0.862	0.730 (0.260-4.600)	U=3588	0.022

t: Independent two sample t test statistic, U: Mann-Whitney U test statistic

Table 3. Comparison results according to UHBSSG score groups in the patient group

	Mild-Moderate-Severe		Very Severe			
	Mean±s. deviation	Median (min.-max.)	Mean±s. deviation	Median (min.-max.)	Test statistic	p
FERRITIN (ng/mL)	45.92±43.90	27.15 (3.70-157.40)	35.66±34.52	18.00 (2.00-109.00)	U=727	0.211
FOLATE (ng/mL)	9.364±4.529	8.000 (4.500-23.500)	9.646±4.345	9.000 (3.200-23.500)	U=801	0.553
VITAMIN B12 (pg/mL)	307.06±218.74	262.50 (0.51-1500.00)	246.32±120.46	235.00 (102.00-656.00)	U=687	0.109
VİTAMIN D (ng/dL)	20.06±17.36	15.00 (3.00-116.00)	17.63±9.47	15.00 (9.00-52.00)	U=705.5	0.813
CRP (mg/L)	5.287±8.582	2.325 (0.150-53.000)	5.799±5.710	3.230 (0.430-22.000)	U=611.5	0.160
WBC(10^3/uL)	7.649±2.835	7.080 (4.670-24.910)	7.632±1.756	7.680 (4.780-12.380)	U=814	0.464
RBC(10^6/uL)	4.986±0.451	4.915 (4.180-6.640)	4.831±0.402	4.730 (4.050-5.480)	t=1.599	0.113
HGB(g/dL)	14.33±1.34	14.40 (10.70-17.40)	13.54±1.32	13.50 (9.60-15.60)	t=2.641	0.010
HCT(%)	43.32±3.63	43.35 (35.60-52.80)	41.45±3.62	41.10 (32.20-47.70)	t=2.321	0.023
MCV(fL)	87.00±3.67	87.25 (75.70-94.40)	85.92±5.36	86.60 (70.80-94.20)	U=788.5	0.341
PLT(10^3/uL)	266.36±93.57	248.00 (116.00-748.00)	271.42±55.59	260.00 (173.00-406.00)	U=744	0.182
NEUT#(10^3/uL)	4.362±2.208	3.830 (1.570-17.810)	4.363±1.308	4.080 (2.620-8.090)	U=822	0.507
LYMPH#(10^3/uL)	2.443±0.684	2.405 (1.280-4.950)	2.405±0.663	2.350 (1.200-3.900)	t=0.25	0.803
MONO#(10^3/uL)	0.604±0.261	0.550 (0.320-1.980)	0.568±0.170	0.550 (0.340-0.980)	U=853.5	0.695
EO#(10^3/uL)	0.191±0.133	0.140 (0.040-0.630)	0.251±0.299	0.160 (0.030-1.370)	U=889	0.931
BASO#(10^3/uL)	0.049±0.027	0.040 (0.010-0.140)	0.045±0.024	0.040 (0.010-0.120)	U=791	0.346
MicroR(%)	2.393±2.272	1.800 (0.400-13.200)	3.855±5.370	1.900 (0.500-27.400)	U=768	0.259
MacroR(%)	3.964±0.414	3.900 (3.100-5.600)	3.955±0.460	3.900 (2.900-5.100)	U=881.5	0.880
NLR	1.853±0.715	1.740 (0.610-3.600)	1.956±0.822	1.750 (0.870-4.320)	U=866.5	0.780
PLR	116.77±52.87	103.47 (55.14-387.56)	118.45±30.58	109.50 (67.26-210.32)	U=763	0.242
MLR	0.256±0.096	0.245 (0.130-0.600)	0.253±0.101	0.230 (0.120-0.530)	U=869	0.796
SII	521.41±373.68	445.04 (97.66-2464.91)	518.79±214.21	465.24 (188.33-1144.13)	U=787.5	0.337
SIRI	1.202±1.029	0.920 (0.270-7.120)	1.166±0.738	0.950 (0.360-3.230)	U=886	0.911

t: Independent two sample t test statistic, U: Mann-Whitney U test statistic

RLS patients and healthy controls. Our investigation did not reveal a significant link between NLR and RLS. We attributed this to the fact that, as the neutrophil count rises in RLS patients, the lymphocyte count also rises.

Several studies indicated a link between neurological diseases and PLR. PLR was exhibited to be useful in predicting the prognosis and mortality of neurological illnesses.[14] Furthermore, in individuals with epilepsy, PLR level has been found to be connected to seizures.[15] Ozdemir et al.[16] observed that patients with Guillain Barre syndrome (GBS) had a high PLT level, which is associated with inflammation. Consistent with the literature, we observed that PLR levels were lower in RLS patients than in healthy controls. This was explained by the patient population's notably elevated lymphocyte count.

SII has been demonstrated to be helpful in predicting prognosis and mortality in a variety of neurological illnesses. High SII was found to be substantially related to poor outcomes in stroke patients.[17] Furthermore, Liu et al.[18] found that high SII levels were related to a poor prognosis in GBS patients. Additionally, it was determined that increased SII was an independent predictor of stenosis severity in carotid artery stenosis.[19] To our knowledge, no studies have been performed within the literature to investigate the link between RLS and SII. However, our investigation showed no significant difference in SII levels between RLS patients and the healthy control group.

The association between SIRI, an inflammatory parameter, and some neurological disorders has been studied. Han et al.[20] observed that SIRI predicted worse functional outcomes in ischemic stroke patients. Furthermore, a study showed that SIRI was linked to respiratory failure in GBS patients.[21] In our study, SIRI levels were significantly higher in RLS patients than in the healthy control group.

The microR and macroR parameters are indices of red blood cells that allow for a thorough morphological evaluation of erythrocytes. MicroR represents the percentage of microcytic RBC with a volume less than 60 fL, while macroR represents the percentage of macrocytic RBC with a volume greater

than 120 fL.[22] MicroR and macroR levels have not received significant consideration in studies. To our knowledge, there are very few investigations on this topic in the literature. Çığrı et al.[23] observed microR to be linked with newborn sepsis. In the present research, the microR value was significantly lower than the healthy control groups, but there was no significant difference in the macroR value.

Limitations

However, some limitations regarding our study should be mentioned. Several variables can influence the hemogram parameters. As a result, a thorough analysis is required. Second, the sample size was relatively small. More multi-center research with more participants are needed on this topic in the future.

CONCLUSION

In the present investigation, the inflammatory variables PLR, MLR, SIRI, and microR distinguished significantly from healthy control groups, suggesting that inflammation plays a key role in RLS.

ETHICAL DECLARATIONS

Ethics Committee Approval

The study was carried out with the permission of Kastamonu University Clinical Researches Ethics Committee (Date: 12.06.2023, Decision No:2023-KAEK-156).

Informed Consent

All patients signed and free and informed consent form.

Referee Evaluation Process

Externally peer-reviewed.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Financial Disclosure

The authors declared that this study has received no financial support.

Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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