RESEARCH PAPER

Severity-Related Changes in Inflammatory Markers and Lower Extremity Nerve Conduction in Restless Legs Syndrome

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Received: 29th Sep, 2025; Revised: 8th Nov 2025; Accepted: 16th Nov, 2025; Available Online: 1st December, 2025

ABSTRACT

The pathogenesis of Restless Legs Syndrome (RLS) remains obscure and vague. Emerging evidence suggests that the chronic inflammation and the peripheral nervous system involvement may be associated with disease severity. However, the relationship between inflammatory markers and electrophysiological findings of the lower extremity peripheral nerves in patients with RLS has not been well characterized. The goal of this research was to examine how the severity of restless leg syndrome (RLS), inflammation throughout the body, and electrical activity in the lower extremities' peripheral nerve tissue are related to one another. Seventy patients with clinically diagnosed RLS were evaluated and stratified according to disease severity. Systemic inflammatory and hematologic markers were analyzed, and nerve conduction studies of the lower extremities were performed. Electrophysiological parameters were compared across severity groups, and correlation analyses were conducted between clinical severity, laboratory findings, and nerve conduction parameters. Several systemic inflammatory and hematologic markers differed significantly across RLS severity groups, including C-reactive protein, Sed rate or erythrocyte sedimentation rate (ESR), neutrophil-to-lymphocyte ratio, ferritin, serum iron (serum Fe), and vitamin D levels. Correlation analyses demonstrated crucial associations among inflammatory markers and lower extremity nerve conduction parameters. In addition, RLS severity was significantly correlated with peripheral nerve conduction velocities and amplitudes of the lower extremities. Results showed that greater RLS severity is correlated to greater body-wide inflammation and changes in the electrical conduction properties of lower extremity peripheral nerves. These findings may indicate that inflammation and changes in the electrical activity of the peripheral nervous system may be an additional indicator of severity of RLS. Prospective studies with larger sample sizes and appropriate control groups are warranted to further clarify these associations.

Keywords: Restless legs syndrome (RLS); severity of disease; body-wide inflammation; neutrophil-to-lymphocyte ratio (NLR); C-reactive protein (CRP); vitamin D; nerve conduction study; peripheral nervous system.

How to cite this article: Eliaçık, S., Tutan; D., Severity-Related Changes in Inflammatory Markers and Lower Extremity Nerve Conduction in Restless Legs Syndrome. Int J Drug Deliv Technol. 2026;16(1): 188-198. DOI: 10.25258/ijddt.16.1.20

Source of support: Nil.

Conflict of interest: Nil.

INTRODUCTION

Although it is known that both iron deficiency and abnormalities in dopamine functioning contribute to the development of Restless Legs Syndrome (RLS), it is also unclear how the peripheral nervous system or systemic inflammation may impact developments within this condition [1]. Changes of the peripheral nervous system in individuals with RLS as well as how inflammatory markers and changes occurring within the peripheral nerves evolve throughout the course of RLS disease severity are still not known. When individuals experience symptoms of RLS they often report having an inexplicable need to move their legs due the sensations of burning, tingling, or discomfort, but never due to feelings of pain [2].

RLS is a common neurological disorder experienced throughout the world, with prevalence rates being reported as high as 7.12% globally among adults, and has been reported to occur more often in females than males. RLS can be classified into two; primary and secondary classes. The secondary RLS may happen in association with certain medical conditions or as a consequence of medication use [2, 3, 4, 5].

The present study aims to provide a peripheral nervous system–centered perspective on idiopathic RLS (iRLS), which is traditionally considered a central nervous system disorder, although its etiology has not yet been fully clarified. In this context, changes in systemic inflammatory markers and electrophysiological findings, including nerve conduction studies and electromyography (EMG), were evaluated in relation to increasing disease severity [4].

MATERIALS AND METHODS

Study Design

This research was planned as a prospective observational study. The protocol of this study was approved by the Ethics

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Committee of the XXXX University School of Medicine (approval no: 2023-140). The study was performed according to the protocol of Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement for observational studies (www.strobestatement.org) and the ethical standards of the Declaration of Helsinki. All participants were fully informed about the experimental design, and obtained written informed consent from each participant before inclusion.

Population and Sample

Seventy patients belonging to the age category between 18 and 65 years diagnosed with Restless Legs Syndrome, according to the diagnostic criteria of the International Restless Legs Syndrome Study Group, were included in the study. Patients were classified into four groups according to the acute nature of disease using the International Restless Legs Syndrome Study Group severity rating scale. The secondary causes of RLS were carefully excluded in all participants. Exclusion criteria included pregnant women, iron deficiency anemia, chronic renal failure, peripheral neuropathy, spinocerebellar ataxia, diabetes, and systemic diseases such as hypothyroidism and rheumatoid arthritis conditions. In addition, patients using medications known to be associated with secondary RLS, including corticosteroids, antidepressants, antiepileptics, and dopamine receptor–blocking agents, were excluded from the study.

Data collection

All participants in the four groups stratified according to RLS severity underwent nerve conduction studies in electromyography (EMG) laboratory. Nerve conduction examinations of the bilateral lower extremities, including the tibial, peroneal, and sural nerves, were performed by an examiner who was blinded to disease severity.

Lower extremity nerve conduction velocities and amplitudes were recorded for all participants. In the study population, the mean nerve conduction velocity values were 51.8 ± 9.4 m/s for the peroneal nerve, 48.5 ± 8.1 m/s for the tibial nerve, and 53.2 ± 12.5 m/s for the sural nerve. The mean amplitude values were 9.1 ± 4.4 mV for the peroneal nerve, 8.5 ± 6.2 mV for the tibial nerve, and 17.3 ± 13.3 μV for the sural nerve.

For the evaluation of polyneuropathy, the lower reference limits for motor and sensory nerve conduction velocities were defined as 40 m/s. The lower reference limits for amplitudes were set at 3 mV for motor nerves and 10 μV for the sural sensory nerve.

Venous blood specimens were collected from all patients for the assessment of systemic inflammatory and hematologic markers. The analyzed parameters included the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to-high-density lipoprotein ratio (MHR), C-reactive protein ratio (CAR), C-reactive protein (CRP), ESR, red cell distribution width (RDW), mean platelet volume (MPV), ferritin, serum iron, bilirubin, uric acid, and vitamin D levels.

Statistical analysis

The research was carried out in a prospective manner. All quantitative analyses have been conducted using the IBM SPSS Statistics software, version 25, for Windows (IBM Corporation, Armonk, NY, USA). The count and percentage of categorical data will be presented using descriptive statistics (i.e., tables). Continuously distributed data will be presented using a mean ± standard deviation for normally distributed data and the median (min/max) for non-normally distributed data.

Using the Shapiro-Wilk test, the density of the distribution of data will be evaluated. Correlations between the variables were examined using either Pearson or Spearman's correlation coefficients depending on the results of the Shapiro-Wilk test.

The Kruskal-Wallis test was used to compare continuous dependent variables between groups with differing degrees of severity of RLS (RLS symptom severity, RLS impact on daily living activities, total RLS severity); differences in duration of disease; inflammatory markers (NLR, PLR, MHR, CAR, CRP, ESR, RDW, MPV); biochemical parameters (vitamin D, serum iron, total, direct and indirect bilirubin); electrophysiological parameters. If a statistically significant difference was noted across the four severity groups, pairwise comparison was made with the Mann-Whitney U-test.

The Analysis of variance (ANOVA) and post hoc tests were performed on normally distributed measures of age, vitamin D and ferritin level. Age and vitamin D data were analyzed as continuous variables, but were divided according to gender and participants' medication use and family history. The Chi-square test was used to compare the prevalence of categorical variables by group for both genders, the use of medications, and family history of conditions associated with vitamin D deficiency. Statistical significance was set at $p < 0.05$.

RESULTS AND DISCUSSION

In this research project, we collected data from 70 individuals who satisfied the inclusion criteria: 24 men (34.6%) and 46 women (65.7%). The mean age of the patients was 46.53 ± 10.95 years. Based on the severity of RLS disease, 21 patients (30.0%) had mild disease, 15 (21.4%) had moderate disease, 17 patients (24.3%) had severe disease, and 17 (24.3%) patients had chronic disease. Analysis of sex distribution in the different groups of disease severity showed a significantly higher number of females in the moderate and very severe groups ($p < 0.001$; Tables 1 and 2). In addition, a significant age difference was observed between the moderate and very severe severity groups, with patients in the very severe group being older than those in the moderate group ($p = 0.023$).

Two primary subdomains comprised the International Restless Legs Syndrome Study Group disease severity scale and were evaluated independently, in conjunction with the total severity score. The median RLS symptom severity score for those with mild RLS was 5, which was significantly lower than the scores recorded for those having moderate (8), severe (13), or very severe (16) RLS systems; $p < 0.001$. Additionally, RLS Daily Living

Activities Impact Score (impact score of affected individual's daily living activities) and the total RLS Disease Severity Score were significantly greater among individuals with severe and very severe RLS than among those with mild or moderate RLS.

Pramipexole was the most frequently prescribed medication. The dosage of pramipexole increased with increasing disease severity. Gabapentin monotherapy and combination therapy with pramipexole and gabapentin were more commonly used in the severe and very severe disease groups ($p < 0.001$ and $p < 0.001$, respectively) [Tables 1 and 2].

Variables		All Participants (n=70)	Univariate Analysis - Disease Severity				
			Mild (n=21; 30%)	Moderate (n=15; 21.4%)	Severe (n=17; 24.3%)	Very Severe (n=17; 24.3%)	Statistical Significance
Gender	Male	24 (34.30%)	12 (57.14%)	3 (20.00%)	8 (47.06%)	1 (5.88%)	0.004
	Female	46 (65.70%)	9 (42.86%)	12 (80.00%)	9 (52.94%)	16 (94.12%)	
Age		46.53±10.95	44.33±13.24	41.8±11.45	47.29±7.69	52.65±7.53	0.025
Drug Regimen	No Treatment	11 (15.70%)	4 (19.05%)	5 (33.33%)	1 (5.88%)	1 (5.88%)	<0.001
	0,50 Pramipexole	14 (20.00%)	4 (19.05%)	3 (20.00%)	7 (41.18%)	0 (0.00%)	
	0,75 Pramipexole	18 (25.70%)	0 (0.00%)	1 (6.67%)	6 (35.29%)	11 (64.71%)	
	Pramipexole	20 (28.60%)	13 (61.90%)	6 (40.00%)	1 (5.88%)	0 (0.00%)	
	Gabapentin 1200	2 (2.90%)	0 (0.00%)	0 (0.00%)	1 (5.88%)	1 (5.88%)	
	0,75 Pramipexole + Gabapentin 1200	5 (7.10%)	0 (0.00%)	0 (0.00%)	1 (5.88%)	4 (23.53%)	
RLS Symptom Severity Score		10 (3-20)	5 (3-5)	8 (5-11)	13 (9-15)	16 (15-20)	<0.001
RLS Daily Living Activities Impact Score		10 (3-20)	5 (3-5)	9 (6-12)	13 (10-15)	17 (12-20)	<0.001
RLS Disease Severity Score Total		20 (4-37)	9 (4-10)	18 (12-20)	25 (21-29)	33 (32-37)	<0.001
RLS Duration		24 (8-180)	12 (8-180)	24 (12-60)	36 (12-120)	36 (12-120)	0.022
Family History of RLS		10 (14.29%)	3 (14.29%)	0 (0%)	3 (17.65%)	4 (23.56%)	0.279

RLS Restless Leg Syndrome

Variables		Univariate Analysis of Disease Severity	Post-hoc Analysis					
		Statistical Significance	Mild-Moderate	Mild-Severe	Mild-Very Severe	Moderate-Severe	Moderate-Very Severe	Severe-Very Severe
Gender	Male	0.004	0.041	0.536	0.001	0.147	0.319	0.017
	Female							
Age		0.025	0.927	0.825	0.091	0.413	0.023	0.191
Drug Regimen	No Treatment	<0.001	0.405	<0.001	<0.001	0.020	<0.001	0.046
	0,50 Pramipexole							
	0,75 Pramipexole							
	Pramipexole							
	Gabapentin 1200							
	0,75 Pramipexole + Gabapentin 1200							
RLS Symptom Severity Score		<0.001	0.007	<0.001	<0.001	0.042	<0.001	0.012
RLS Daily Living Activities Impact Score		<0.001	0.006	<0.001	<0.001	0.037	<0.001	0.020
RLS Disease Severity Score Total		<0.001	0.009	<0.001	<0.001	0.026	<0.001	0.015
RLS Duration		0.022	0.219	0.006	0.014	0.173	0.273	0.783
Family History of RLS		0.279						

RLS Restless Leg Syndrome

The duration of RLS symptoms duration broken down by severity (12 months median for mild) is significantly different between groups (with severe being 36 months). Family history is 14.29%, but does not differ significantly among severity groups.

Univariate analysis demonstrated statistically significant differences in several inflammatory and biochemical markers across RLS severity groups. Significant differences were observed for the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), monocyte-to–high-density lipoprotein ratio (MHR), C-reactive protein ratio (CAR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), red cell distribution width (RDW), mean platelet volume (MPV), total bilirubin, indirect bilirubin, direct bilirubin, vitamin D, serum iron levels, and ferritin levels. Among these parameters, the difference in direct bilirubin reached statistical significance at $p = 0.002$, whereas all other markers showed significance at $p < 0.001$ [Table 3].

Table 3.

Variables		Univariate Analysis - Disease Severity

	All Participants (n=70)	Mild (n=21; %30)	Moderate (n=15; 21.4%)	Severe (n=17; 24.3%)	Very Severe (n=17; 24.3%)	Statistical Significance
NLR	2.21 (1.06-7.35)	1.51 (1.06-2.13)	1.89 (1.15-3.67)	2.76 (1.9-5.28)	4.05 (2.16-7.35)	<0.001
PLR	138 (51.21-291.66)	106.72 (51.21-152)	134.73 (98.79-190.22)	159.67 (100-266.66)	212.13 (118.97-291.66)	<0.001
MHR	0.03 (0.004-0.43)	0.01 (0-0.09)	0.03 (0.01-0.17)	0.09 (0.01-0.24)	0.18 (0.01-0.43)	<0.001
CAR	0.19 (0.009-1.11)	0.08 (0.01-0.22)	0.17 (0.06-0.64)	0.23 (0.09-0.6)	0.44 (0.13-1.11)	<0.001
CRP	9 (3-39)	3.19 (3-11.7)	9.2 (3-25)	11 (5-24)	16.3 (6-39)	<0.001
ESR	16 (3-45)	6 (3-16)	13 (5-30)	18 (12-32)	24 (10-45)	<0.001
RDW	13.8 (10-23.1)	12.1 (10-14)	13.8 (10.23-17.2)	14.4 (11.45-17.1)	15.9 (13.3-23.1)	<0.001
MPV	9.9 (8.7-97)	10.9 (9-12.8)	10.06 (9.1-97)	9.6 (8.7-11.21)	9.2 (8.7-11.1)	<0.001
Total Bilirubin	0.77 (0.19-2.13)	0.4 (0.19-1.5)	0.9 (0.53-1.31)	0.85 (0.21-2.13)	0.9 (0.3-1.8)	<0.001
Indirect Bilirubin	0.5 (0.15-1.84)	0.3 (0.15-1)	0.7 (0.4-1.12)	0.55 (0.18-1.84)	0.62 (0.25-1.16)	<0.001
Direct Bilirubin	0.2 (0.03-0.8)	0.1 (0.04-0.5)	0.2 (0.1-0.4)	0.24 (0.03-0.8)	0.2 (0.05-0.8)	0.002
Vitamin D	26.71±19.14	46.07±17.33	29.61±15.56	15.94±8.5	10.99±5.96	<0.001
Serum Iron Levels	95 (50-180)	148 (90-180)	125 (85-150)	76 (55-97)	78 (50-100)	<0.001
Ferritin	95.31±59.14	151.24±57.3	113.93±40.39	58.59±26.32	46.53±21.6	<0.001

Neutrophil/lymphocyte ratio (NLR), PLR (Platelet/Lymphocyte Ratio), MHR (Monocyte/High-Density Lipoprotein Ratio), CAR (C-Reactive Protein/Albumin Ratio), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), Red cell distribution width (RDW), mean platelet volume (MPV)

These analyses indicated a number of statistically significant differences in among many of the inflammatory indicators between the RLS severity groups that were compared. The NLR was significantly different among the vast majority of pairs of severity groups that were examined. There were significant differences between the mild-moderate, mild-severe, mild-very severe, moderate-severe, moderate-very severe, and severe-very severe groups based upon NLR (p-values < 0.001 for all except the mild-severe (p-value = 0.024) and moderate-very severe (p-value = 0.020 groups).

Similarly, the platelet-to-lymphocyte ratio (PLR) differed significantly between multiple severity groups, including mild–moderate, mild–very severe, moderate–severe, moderate–very severe, and severe–very severe groups ($p < 0.001$ for all comparisons except moderate–severe and severe–very severe, with $p = 0.176$ and $p = 0.066$, respectively). In addition, the monocyte-to–high-density lipoprotein ratio (MHR), C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR) demonstrated significant differences across several severity group comparisons ($p < 0.001$ for multiple comparisons) [Table 4].

Ferritin levels differed significantly across RLS severity groups in the overall analysis ($p < 0.001$). Post hoc analyses demonstrated that these differences were present in a specific pairwise comparisons. Significant differences in ferritin levels were observed between the mild to moderate, mild to very severe, moderate to severe, and moderate–very severe groups ($p < 0.001$ for all comparisons except the moderate to severe comparison, where $p = 0.002$). There were no statistically significant differences in the serum

ferritin concentrations between Groups 1 and 2 (p = 0.401) [Table 3 and 4].

Table 4.

Variables	Univariate Analysis - Disease Severity	Post-hoc Analysis					
	Statistical Significance	Mild-Moderate	Mild-Severe	Mild-Very Severe	Moderate-Severe	Moderate-Very Severe	Severe-Very Severe
NLR	<0.001	0.024	<0.001	<0.001	0.020	<0.001	0.131
PLR	<0.001	0.021	<0.001	<0.001	0.176	0.002	0.066
MHR	<0.001	0.029	<0.001	<0.001	0.092	0.016	0.458
CAR	<0.001	0.003	<0.001	<0.001	0.241	0.007	0.115
CRP	<0.001	<0.001	<0.001	<0.001	0.501	0.058	0.207
ESR	<0.001	0.002	<0.001	<0.001	0.156	0.011	0.249
RDW	<0.001	0.001	<0.001	<0.001	0.355	0.012	0.098
MPV	<0.001	0.046	<0.001	<0.001	0.096	0.004	0.210
Total Bilirubin	<0.001	<0.001	<0.001	<0.001	0.718	0.802	0.909
Indirect Bilirubin	<0.001	<0.001	<0.001	<0.001	0.179	0.709	0.316
Direct Bilirubin	0.002	0.025	<0.001	0.012	0.217	0.852	0.279
Vitamin D	<0.001	0.026	<0.001	<0.001	0.03	0.002	0.225
Serum Iron Levels	<0.001	0.296	<0.001	<0.001	<0.001	<0.001	0.903
Ferritin	<0.001	0.191	<0.001	<0.001	0.002	<0.001	0.401

Neutrophil/lymphocyte ratio (NLR), PLR (Platelet/Lymphocyte Ratio), MHR (Monocyte/High-Density Lipoprotein Ratio), CAR (C-Reactive Protein/Albumin Ratio), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), Red cell distribution width (RDW), mean platelet volume (MPV)

Peripheral nerve conduction studies were conducted to assess the lower extremity nerves of patients with Restless Legs Syndrome (RLS) who present with varying degrees of severity. Significant differences were observed when comparing the parameters of the peripheral nerve conduction studies based on the severity level of the disease. On the right side, the tibial nerve conduction velocity differed significantly among the groups (p<0.001), and the tibial nerve from the Group with very severe RLS had the lowest conduction velocity (40.8; [38.9-44.9 m/sec]). The tibial nerve amplitude from the Group with very severe RLS was significantly different from the Groups of lower severity (p<0.001), with the tibial nerve amplitude from this very severe group having the lowest amplitude (6.1; [1.5-10.0 mV]). The left side of the tibial nerve demonstrated the same significant differences in conduction velocity and amplitude across the severity groups (p<0.001). Notably, both left-sided tibial nerves had reduced conduction velocities (41; [40-46 m/sec]) and reduced amplitudes (6; [1.32-9.84 mV]) in the very severe group as compared to the Groups of lower severity [Table 5]. Significant differences also existed for the right-sided peroneal nerve conduction velocity and amplitude among the severity groups (p<0.001) and were lowest in the very severe group (40.9; [39-42.3 m/sec]) and (3; [1.1-4.3 mV]) respectively. The results of the pairwise comparison are presented in Table 6.

The left peroneal nerve conduction velocity and amplitude were found to be significantly different among the groups of varying severities (p < 0.001). The very severe group had the lowest left peroneal nerve conduction velocity (40.1;

37.8–42 m/s) and amplitude (3.56; 1.53–6.54 mV). The right and left sural conduction velocities and amplitudes were also significantly different among the groups of varying severities ($p < 0.001$). The very severe group had the lowest sural conduction velocities (right 40; 35–45 m/s and left 39.7–44.6 m/s) and amplitudes (right 11; 8–20.5 µV and left 12; 9–18.6 µV) (Tables 5 and 6).

Table 5.

Variables	All Participants (n=70)	Univariate Analysis - Disease Severity				
		Mild (n=21; %30)	Moderate (n=15; 21.4%)	Severe (n=17; 24.3%)	Very Severe (n=17; 24.3%)	Statistical Significance
Right Tibial Velocity	44.15 (38.9-59)	51.7 (44.1-59)	44 (39.9-51.4)	41.2 (40-50.01)	40.8 (38.9-44.9)	<0.001
Right Tibial Amplitude	9.1 (1.5-19)	12 (6-19)	10.29 (6.47-18.27)	7.09 (2.69-16)	6.1 (1.5-10)	<0.001
Left Tibial Velocity	43.1 (39-58)	50.5 (42.2-58)	42.6 (40.1-52.1)	43.1 (39-49.3)	41 (40-46)	<0.001
Left Tibial Amplitude	8.82 (1.32-19)	13 (7.12-18.45)	10 (4.8-17.74)	6 (4.1-19)	6 (1.32-9.84)	<0.001
Right Peroneal Velocity	42.1 (37.6-61)	49 (41.9-61)	42.1 (39-53.3)	41 (37.6-50)	40.9 (39-42.3)	<0.001
Right Peroneal Amplitude	5.5 (0.98-19)	9 (3.3-19)	4.9 (2.2-12)	6 (0.98-17)	3 (1.1-4.3)	<0.001
Left Peroneal Velocity	43.25 (37.8-63.2)	48.3 (43.4-63.2)	43 (39-45)	41.3 (38.03-48.3)	40.1 (37.8-42)	<0.001
Left Peroneal Amplitude	5.32 (1.1-18)	11 (3.26-18)	5.1 (3.09-13)	4.55 (1.1-17)	3.56 (1.53-6.54)	<0.001
Right Sural Velocity	41.45 (34.7-68.9)	55 (42.7-68.9)	41 (35.3-64.1)	41 (34.7-53)	40 (35-45)	<0.001
Right Sural Amplitude	15 (8-32)	21 (10.98-32)	14.3 (10-21.1)	13.3 (9-22)	11 (8-20.5)	<0.001
Left Sural Velocity	43 (10-68.9)	52 (44.9-68.9)	41.9 (37.3-56.6)	41.8 (10-47)	40 (39.7-44.6)	<0.001
Left Sural Amplitude	14.85 (8.1-36)	19.5 (9.22-36)	13.9 (9-24.9)	14.5 (8.1-24)	12 (9-18.6)	<0.001

Table 6.

Variables	Univariate Analysis - Disease Severity	Post-hoc Analysis					
	Statistical Significance	Mild-Moderate	Mild-Severe	Mild-Very Severe	Moderate-Severe	Moderate-Very Severe	Severe-Very Severe
Right Tibial Velocity	<0.001	<0.001	<0.001	<0.001	0.359	0.013	0.105
Right Tibial Amplitude	<0.001	0.272	<0.001	<0.001	0.018	<0.001	0.113

Left Tibial Velocity	<0.001	<0.001	<0.001	<0.001	0.731	0.076	0.139
Left Tibial Amplitude	<0.001	0.369	<0.001	<0.001	0.003	<0.001	0.404
Right Peroneal Velocity	<0.001	<0.001	<0.001	<0.001	0.354	0.049	0.284
Right Peroneal Amplitude	<0.001	0.003	<0.001	<0.001	0.761	0.01	0.018
Left Peroneal Velocity	<0.001	<0.001	<0.001	<0.001	0.922	0.029	0.018
Left Peroneal Amplitude	<0.001	0.004	<0.001	<0.001	0.265	0.013	0.161
Right Sural Velocity	<0.001	<0.001	<0.001	<0.001	0.806	0.207	0.294
Right Sural Amplitude	<0.001	<0.001	<0.001	<0.001	0.905	0.124	0.142
Left Sural Velocity	<0.001	<0.001	<0.001	<0.001	0.611	0.248	0.504
Left Sural Amplitude	<0.001	0.002	0.002	<0.001	0.829	0.167	0.099

Correlation analyses showed that there were significant correlations between iRLS severity and many inflammatory markers and peripheral nerve conduction parameters, including but not limited to, iRLS severity and NLR, PLR, MHR, CAR, CRP, ESR, RDW, MPV, and ferritin ($p < 0.001$). Of these, the NLR had the highest positive correlation to RLS severity ($r = 0.833$; $p < 0.001$), followed by PLR ($r = 0.720$; $p < 0.001$), CRP ($r = 0.677$; $p < 0.001$), and MHR ($r = 0.634$). Conversely, the MPV had a significant negative correlation to RLS severity ($r = -0.664$; $p < 0.001$), indicating that as the severity of RLS increased, the MPV value decreased.

All evaluated parameters were significantly associated to the severity of Restless Legs Syndrome (all $p < 0.001$) with lower extremity peripheral nerve conduction velocities and amplitudes. Specifically, the right tibial nerve conduction velocity and the right tibial nerve amplitude were both strongly negatively correlated with the severity of RLS with $r = -0.753$ ($p < 0.001$) and $r = -0.700$ ($p < 0.001$), respectively. Table 7 summarizes the correlation coefficients of these parameters.

Correlation analyses showed additional significant relationships between peripheral nerve conduction parameters and both inflammatory and hematological markers. In fact, significant negative correlations were found between right tibial nerve conduction velocity and NLR ($r = -0.542$, $p < 0.001$), PLR ($r = -0.564$, $p < 0.001$), MHR ($r = -0.516$, $p < 0.001$), CAR ($r = -0.557$, $p < 0.001$), CRP ($r = -0.486$, $p < 0.001$), ESR ($r = -0.544$, $p < 0.001$), and RDW ($r = -0.643$, $p < 0.001$). In contrast, the right tibial velocity was positively correlated with MPV ($r = 0.472$, $p < 0.001$) and ferritin levels ($r = 0.574$, $p < 0.001$).

The right tibial nerve amplitude demonstrated similar significant negative correlations with NLR ($r = -0.674$, $p < 0.001$), PLR ($r = -0.451$, $p < 0.001$), MHR ($r = -0.382$, $p = 0.001$), CAR ($r = -0.516$, $p < 0.001$), CRP ($r = -0.435$, $p < 0.001$), ESR ($r = -0.409$, $p < 0.001$), and RDW ($r = -0.447$, $p < 0.001$) and positive correlations with MPV ($r = 0.424$, $p < 0.001$) and ferritin ($r = 0.469$, $p < 0.001$).

The speed of conduction of nerves in the left tibia exhibited similar results as it had a significant inverse got the NLR ($r = -0.570$, $p < 0.001$), PLR ($r = -0.471$, $p < 0.001$), MHR ($r = -0.427$, $p < 0.001$), CAR ($r = -0.534$, $p < 0.001$), CRP ($r = -0.468$, $p < 0.001$), ESR ($r = -0.499$, $p < 0.001$), and RDW ($r = -0.648$, $p < 0.001$) and there were significant positive associations with MPV ($r = 0.475$, $p < 0.001$) and ferritin levels ($r = 0.531$, $p < 0.001$).

Likewise, the tibial amplitude on the left side of the body was associated with an inverse got the NLR ($r = -0.709$, $p < 0.001$), PLR ($p = -0.475$, $p < 0.001$), MHR ($r = -0.344$, $p = 0.004$), CAR ($r = -0.576$, $p < 0.001$), CRP ($r = -0.479$, $p < 0.001$), ESR ($r = -0.419$, $p < 0.001$) and RDW ($r = -0.424$, $p < 0.001$) and there were significant positive associations with MPV ($r = 0.369$, $p = 0.002$) and ferritin ($r = 0.441$, $p < 0.001$) .

All of the other peripheral nerve conduction parameters for the peroneal / tibial nerves (in the left and right tibia) and the sural nerves (in both feet) showed significant associations (all $p < 0.001$) with inflammatory and hematologic markers. The correlation coefficients are listed in Table 7.

Table 7.

Variables		RLS Disease Severity	NLR	PLR	MHR	CAR	CRP	ESR	RDW	MPV	Ferritin	Vitamin D
NLR	**r**	0.833	--									
	p	<0.001	.									
PLR	**r**	0.720	0.763	--								
	p	<0.001	<0.001	.								
MHR	**r**	0.634	0.431	0.525	--							
	p	<0.001	<0.001	<0.001	.							
CAR	**r**	0.748	0.800	0.732	0.444	--						
	p	<0.001	<0.001	<0.001	<0.001	.						

CRP	**r**	0.677	0.715	0.574	0.284	0.894	--					
	p	<0.001	<0.001	<0.001	0.017	<0.001	.					
ESR	**r**	0.752	0.590	0.563	0.546	0.647	0.591	--				
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.				
RDW	**r**	0.746	0.649	0.542	0.433	0.659	0.643	0.565	--			
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.			
MPV	**r**	-0.664	-0.552	-0.477	-0.449	-0.527	-0.429	-0.530	-0.551	--		
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.		
Ferritin	**r**	-0.767	-0.608	-0.505	-0.408	-0.487	-0.506	-0.527	-0.574	0,403	--	
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.	
Vitamin D	**r**	-0.745	-0.727	-0.521	-0.514	-0.598	-0.525	-0.581	-0.626	0.464	0.572	--
	P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	.
Right Tibial Velocity	**r**	-0.753	-0.542	-0.564	-0.516	-0.557	-0.486	-0.544	-0.643	0.472	0.574	0.548
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Right Tibial Amplitude	**r**	-0.700	-0.674	-0.451	-0.382	-0.516	-0.435	-0.409	-0.447	0.424	0.469	0.762
	p	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Left Tibial Velocity	**r**	-0.682	-0.570	-0.471	-0.427	-0.534	-0.468	-0.499	-0.648	0.475	0.531	0.564
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Left Tibial Amplitude	**r**	-0.688	-0.709	-0.475	-0.344	-0.576	-0.479	-0.419	-0.424	0.369	0.441	0.667
	p	<0.001	<0.001	<0.001	0.004	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
Right Peroneal Velocity	**r**	-0.711	-0.544	-0.486	-0.505	-0.588	-0.508	-0.575	-0.619	0.539	0.424	0.462
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Right Peroneal Amplitude	**r**	-0.699	-0.581	-0.420	-0.383	-0.53	-0.468	-0.556	-0.519	0.427	0.436	0.566
	p	<0.001	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Left Peroneal Velocity	**r**	-0.742	-0.578	-0.529	-0.465	-0.563	-0.574	-0.576	-0.668	0.48	0.548	0.573
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Left Peroneal Amplitude	**r**	-0.711	-0.562	-0.446	-0.449	-0.525	-0.514	-0.526	-0.531	0.457	0.551	0.533
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Right Sural Velocity	**r**	-0.683	-0.65	-0.612	-0.418	-0.617	-0.556	-0.473	-0.617	0.429	0.532	0.506
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Right Sural Amplitude	**r**	-0.613	-0.623	-0.562	-0.348	-0.599	-0.522	-0.389	-0.603	0.351	0.416	0.540
	p	<0.001	<0.001	<0.001	0.003	<0.001	<0.001	0.001	<0.001	0.003	<0.001	<0.001
Left Sural Velocity	**r**	-0.706	-0.628	-0.547	-0.464	-0.602	-0.561	-0.558	-0.729	0.429	0.504	0.558
	p	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Left Sural Amplitude	**r**	-0.557	-0.553	-0.490	-0.319	-0.537	-0.468	-0.367	-0.602	0.281	0.304	0.565
	p	<0.001	<0.001	<0.001	0.007	<0.001	<0.001	0.002	<0.001	0.018	0.010	<0.001

Neutrophil/lymphocyte ratio (NLR), PLR (Platelet/Lymphocyte Ratio), MHR (Monocyte/High-Density Lipoprotein Ratio), CAR (C-Reactive Protein/Albumin Ratio), C reactive protein (CRP), erythrocyte sedimentation rate (ESR), Red cell distribution width (RDW), mean platelet volume (MPV).

The current study has shown that the severity of idiopathic restless legs syndrome (iRLS) is strongly linked to systemic inflammatory activity and progressive changes in peripheral nerve conduction. As the disease progressed, there was a marked increase in the levels of inflammatory and hematological parameters such as neutrophil ratio (NLR), platelet or lymphocyte ratio (PLR), monocyte/high-density lipoprotein ratio (MHR), C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and red cell distribution width (RDW), thereby confirming the increasing body of evidence that inflammation is a pathophysiological mechanism in RLS [6, 7, 8].

Among the inflammatory indices, NLR was found to have the highest positive correlation with the severity of iRLS, followed by PLR, MHR, and CRP. These ratios are increasingly being identified as valid biomarkers of systemic inflammatory burden and immune system dysregulation in neurological disorders [9, 10, 11]. Previous studies have shown that patients with RLS have high levels of NLR and PLR compared to healthy controls, indicating the presence of low-grade systemic inflammation as a contributing factor to the severity of symptoms [12,13].

Iron metabolism indices were also significantly different among the groups of varying disease severity. Ferritin and serum iron concentrations were significantly lower in patients with increasing disease severity, particularly in the early and moderate phases, with no further significant differences between severe and very severe disease. This finding is in line with previous studies indicating that iron deregulation might display a threshold effect in advanced RLS phases [14, 15, 16].

One of the most significant findings of the current study is the demonstration of the severity-dependent abnormalities of peripheral nerve conduction in iRLS. Motor and sensory

nerve conduction velocities and amplitudes of the tibial, peroneal, and sural nerves progressively decreased with increasing disease severity. It is worth noting that these abnormalities were detected despite the exclusion of patients with overt polyneuropathy, indicating subclinical peripheral nerve involvement in iRLS [17, 18, 19]. Previous electrophysiological studies in RLS have yielded inconsistent results; however, more recent data support the existence of subtle peripheral nerve damage, particularly in patients with severe symptoms [20, 21, 22]. These new data further substantiate those previously made by demonstrating a very strong relationship between electrophysiological deficits and severity of disease.

Correlation analyses also supported the relationship between systemic inflammation and peripheral nerve dysfunction. Higher levels of inflammatory biomarkers were consistently associated with decreased nerve conduction velocities and amplitudes, while a positive correlation was found between mean platelet volume and ferritin concentration with electrophysiological measurements. The inflammation-related changes in nerve conduction previously mentioned have been observed in other chronic inflammatory/nervous system diseases and may indicate that there exists a common pathophysiological process between these two groups of disorders [23, 24, 25]. The high degree of correlation between the severity scores from the International RLS Study Group, the levels of inflammatory biomarkers, and the values from the peripheral nerve conduction tests strongly suggests that these physiological alterations have a strong association with the clinical manifestation of the disease and are highly unlikely to be random chance events. The coordinated progression of the clinical symptom severity, inflammation, and electrophysiological dysfunction supports the concept that peripheral nerve damage may contribute to or exacerbate the clinical symptoms of late stage iRLS [26, 27].

These combined data reveal that iRLS must not only be regarded as a disorder of the central nervous system but also as having components of systemic inflammation and subclinical peripheral nerve dysfunction. This provides support for larger future neurophysiological and longitudinal studies to further define the role of inflammation mediated peripheral nerve damage in the pathogenesis and progression of iRLS [28, 29].

CONCLUSION

The present study has demonstrated that Idiopathic RLS is associated with systemic inflammation and subclinical peripheral nerve dysfunction that worsens as the disease progresses. The significant correlation observed between the inflammatory indices and electrophysiological parameters suggests that there may be a relationship between inflammation and peripheral nerve involvement in RLS. This data also supports a multi-dimensional pathophysiological model for iRLS that is larger than just central dopaminergic dysfunction. Future longitudinal studies utilizing greater patient cohorts with advanced neurophysiologic and pathologic assessments will help to further define these processes.

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