Sleep and Breathing
https://doi.org/10.1007/s11325-020-02218-7

NEUROLOGY • ORIGINAL ARTICLE

# Circulating levels of cytokines are increased in restless legs syndrome

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Received: 29 July 2020 / Revised: 4 October 2020 / Accepted: 7 October 2020


## Abstract

**Background** Restless legs syndrome [RLS] is known as a disease of iron and dopaminergic dysregulation but inflammatory processes might also have a role in the pathogenesis. In this study, we compared the circulating levels of hsCRP, IL-1β, IL-6, and TNF-α in patients with primary restless legs syndrome [RLS] and healthy control subjects.

**Methods** We prospectively included 29 patients with primary RLS and 65 healthy controls [HC], all age-sex matched. The diagnosis of RLS was established using international guidelines. IRLSSG Severity Scale was used to evaluate the severity of RLS. Plasma levels of hsCRP, IL-1β, IL-6, and TNF-α were measured in all participants.

**Results** The mean age of patients was 37.8 ± 11.3 and 52% of RLS group were women. Serum IL-1β, IL-6, and TNF-α levels of the patient group were statistically significantly higher compared to HC [$p < 0.001$ for all variables]. Plasma levels of hsCRP did not differ between groups. There were 8 patients with mild RLS [28%], 13 patients with moderate RLS [45%], and 8 patients with severe RLS [28%]. Only IL-6 values were significantly different between the groups. In the severe group, the value of IL-6 was significantly higher than in the other groups [$p$: 0.03].

**Conclusion** These results showing higher circulating levels of inflammatory cytokines in patients with RLS support the notion that inflammation may be involved in the pathogenesis of primary RLS. However, it is necessary to perform further studies to determine if this finding is a cause or an effect.

**Keywords** Restless legs syndrome · Interleukin-6 · Interleukin-1β · TNF-α

## Introduction

Restless legs syndrome [RLS] is a movement disorder which causes a persistent urge to move the legs during the night and interferes with sleep [1]. RLS affects about 5–10% of the adults in North America and Western Europe [2]. Although RLS may occur secondarily to some comorbid conditions such as iron deficiency, uremia, pregnancy, and spinal cord or peripheral nerve injuries, the etiology of "primary RLS" is still not well known [3]. Iron and dopaminergic systems are the most extensively studied systems for understanding the underlying mechanism of RLS [4]. However, other potential pathways might be involved in the pathophysiological process in relation to or separately from these systems. For instance, higher prevalence of RLS in patients with systemic inflammatory diseases such as Crohn's disease, celiac disease, and systemic lupus erythematosus might indicate the role of inflammation in RLS pathophysiology [5–8]. In another study that included patients undergoing hemodialysis, serum levels of high-sensitivity C-reactive protein [hsCRP], interleukin-6 [IL-6], ferritin, and N-terminal pro-Btype natriuretic peptide were found to be higher in patients with RLS compared to the non-RLS subgroup [9]. Another study has reported the association of increased levels of hsCRP with severe periodic leg movement [PLM] of RLS [10]. Cytokines are small regulating proteins that play an important role in inflammation and modulate neuronal activity in the peripheral and central nervous

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Published online: 21 October 2020

system [11]. In one study, polymorphisms of IL-1β and IL-17Alpha genes were found to be associated with RLS [12]. However, the role of circulating cytokines in patients with idiopathic RLS is still unknown. In this current work, we aimed to compare the plasma levels of hsCRP, IL-1β, IL-6, and TNF-α levels of patients with idiopathic RLS and healthy controls. Our hypothesis was that cytokine levels would be higher in patients with RLS favoring their role in the pathogenesis of RLS.

## Materials and methods

Patients with RLS who admitted to the neurology outpatient clinics of Bezmialem Vakıf University between March 1, 2018, and February 28, 2019, were included in the study. Diagnosis of RLS was established according to the last updated version of IRLSSG diagnostic criteria [13]. Age- and sex-matched healthy controls [HC] were selected from hospital employees and medical school students with similar age and gender distribution to the RLS patients. The exclusion criteria were as follows: (1) The use of any medication for RLS; (2) body mass index greater than 25; (3) having a malignancy, genetic, autoimmune, inflammatory, or neurological disease; (4) having a peripheric neuropathy based on a detailed history and neurological examination; (5) history of infectious diseases during the month before enrollment; (6) history of smoking or alcohol use; (7) using any medications including nonsteroidal anti-inflammatory drugs, antioxidants, B, C, D vitamins, selenium and zinc supplements, iron medicine, and oral contraceptive drugs; (8) being pregnant or lactating. Local ethics committee approval was obtained for the study and informed consent was obtained from all participants. HC had no evidence for peripheric neuropathy according to the medical history and neurological examination findings and each participant fulfilled the above-mentioned exclusion criteria. Demographics and laboratory data of the patients were recorded for all participants. The IRLSSG Severity Scale was used to evaluate the severity of RLS. Patients were classified into four groups with scores of mild (0–10), moderate (11–20), severe (21–30), and very severe (31–40) RLS [13]. Venous blood samples were collected between 8 a.m. and 12 p.m., after a 12-h fasting period and they were taken into 10-mL EDTA tubes and then centrifuged, and plasma extracted at 4 °C for 10 min. The plasma was stored at − 80 °C until all participants' samples were collected. The plasma levels of hsCRP, IL-1β, IL-6, and TNF-α levels were studied. Enzyme-linked immunosorbent assays (ELISA) were used to measure serum IL-1β, IL-6, and TNF-α levels.

Statistical analysis descriptive analyses were expressed for all variables. Categorical variables were analyzed using Fisher's exact test, and continuous variables using the independent-samples $t$ test (for normal distributions) and Mann-Whitney $U$ test (for non-normal distributions). Statistical analyses were performed using SPSS (Statistical Package for Social Sciences for Windows Version 22.0, Armonk, NY, IBM Corp). A $p$ value less than 0.05 was considered statistically significant and all significance tests were two tailed.

## Results

There were 130 patients diagnosed with RLS during the study period. Of these, 29 patients were included in the study after the remaining 101 patients were excluded according to the study criteria [Fig. 1]. Fifty-seven of the patients who were excluded were women and the mean age was 60.4 (24–8) years. Patients excluded from the study were significantly older than the patients included in the study [$60.4 \pm 13.8$ vs. $35.4 \pm 11.1$, $p < 0.001$]. The reasons for exclusion from the study are as follows: 34 smoking, 28 use of drugs in the exclusion criteria, 13 diabetes mellitus, 10 anemia, 5 patient unwillingness to participate in the study, 5 chronic kidney failure, 3 thyroid disease, 3 Parkinson's disease, 1 lymphoma. The mean duration of symptoms was $4.3 \pm 4.4$ years in patients with RLS. When RLS

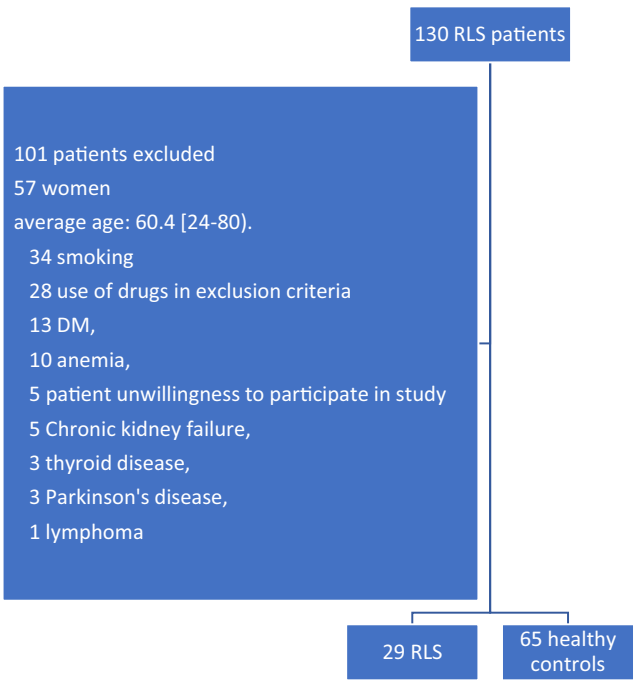


**Fig. 1** Flow chart of patients inclusion/exclusion criteria for the study population

**Table 1** The IL-1β, IL-6, and TNF-α levels in the RLS group and controls

| | RLS | Controls | *p* |
|---|---|---|---|
| Gender | 14 male/15 female | 34 male/35 female | 0.32 |
| Age | 35.4 ± 11.1 | 34.89 ± 7.72 | 0.91 |
| IL-1β | 543.4 ± 54.3 | 302.5 ± 64.2 | < 0.00 |
| IL-6 | 159.8 ± 35.6 | 98.3 ± 25.7 | < 0.00 |
| TNF-α | 320.5 ± 33.9 | 152.8 ± 43.1 | < 0.00 |

**Table 2** Percentage distribution of patients with restless legs syndrome in terms of their severity and cytokine values

| RLS rating scale | Mild [*n*: 8] | Moderate [*n*: 13] | Severe [*n*: 8] | *p* |
|---|---|---|---|---|
| IL-1β | 558,1 ± 60.9 | 527,9 ± 67.2 | 549.5 ± 62.4 | 0.82 |
| IL-6 | 136.2 ± 26.7 | 169.2 ± 28.5 | 192.5 ± 41.7 | 0.03 |
| TNF-α | 301.0 ± 21.2 | 308.0 ± 27.8 | 326.2 ± 35.5 | 0.9 |

patients were compared to the HC [$n = 65$] included in the study, the mean age and sex did not differ [*p*: 0.91 and *p*: 0.32, respectively]. The mean level of hsCRP was 1.1 ± 2.1 [0.02–10] in the RLS group and 1.8 ± 1.7 in the HC group, a difference that was notstatistically different [*p*: 0.18]. The levels of IL-1β, IL-6, and TNF-α were detectable in all participants.

The IL-1β, IL-6, and TNF-α levels in the RLS group were significantly higher compared to the control group [$p < 0.001$ for all variables] [Table 1 and Fig. 2]. There were 8 patients with mild RLS [27.6%], 13 patients with moderate RLS [44.8%], 8 patients with severe RLS [27.6%], and no patients with very severe RLS, based on the IRLSSG rating scale. When the cytokines were evaluated according to severity, the IL-6 values were the only significant difference between the groups. In the severe group, the value of IL-6 was significantly higher than in the other groups [*p*: 0.02]. There was no significant difference between the groups when IL-1β and TNF-α were evaluated [respectively *p* value IL-1β *p*: 0.82, TNF-α *p*: 0.9] [Table 2].

## Discussion

This study is the first to investigate plasma levels of inflammatory cytokines in patients with primary RLS. Our results showed significantly higher circulating levels of IL-1β, IL-6, and TNF-α in RLS patients as compared to non-RLS controls. However, there was no significant difference in the levels of hsCRP between groups. In the literature, there are a few studies evaluating the levels of hsCRP in patients with RLS, with conflicting results. A recent study found higher hsCRP as well as lower albumin levels in patients with RLS as compared to controls [14]. In another general population study of about 2000 participants, hsCRP was not found to be associated with RLS [15]. The mean age in RLS patients in the former study was 52.5 while it was 40 years/old, as in our study, in the latter population-based study. It is speculated that hsCRP might have been associated with RLS in the older age group. Inflammatory cytokines have been frequently investigated in neurodegenerative diseases such as Parkinson's disease, Alzheimer's dementia, and REM Sleep Behavior Disorder and diabetic polyneuropathy [16–18]. Similar to these neurodegenerative diseases, there are many mechanisms in the pathogenesis of RLS, including the possibility of being stimulated by inflammatory and immunological mechanisms. Since a significant number of the medical conditions known to be associated with RLS are inflammatory or infectious conditions, RLS has been thought to be strongly associated with systemic inflammation [19]. Moreover, systemic inflammation was found to be associated with and predictive of cardiovascular disease [10]. In our study, we selected IL1-β, TNF-α, and IL-6 for their pro-inflammatory properties. Of these, IL-6 and TNF-α have been studied in patients with RLS attributed to secondary causes. The comparison of IL-6 levels in RLS and non-RLS groups in patients with

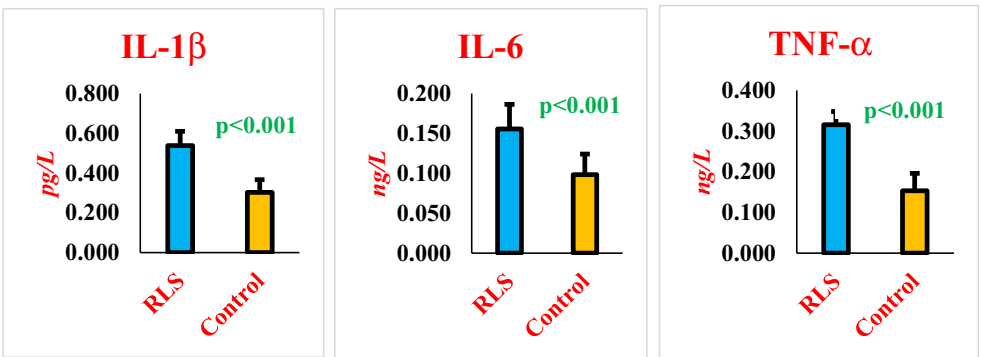


**Fig. 2** The IL-1β, IL-6, and TNF-α levels in the RLS group and the control group

hematological malignancies did not show any significant differences between groups [20, 21]. The levels of IL-6 and TNF-α were also the same in RLS patients with periodic leg movement in a cohort with comorbidities such as diabetes mellitus and smoking [10]. Our study differs from these studies as we only included primary RLS patients who did not have any comorbidities that might cause secondary RLS. Along with the level of IL-1β, IL-6 and TNF-α were also found to be higher in patients with RLS compared to the healthy control group. Since we chose study participants with broad exclusion criteria of any potential secondary causes of RLS, our results support the notion that inflammation plays a role in the pathogenesis of primary RLS. Moreover, our groups were age-sex matched and venous blood was taken in the morning in all participants to prevent any potential interaction of IL-6 and TNF-α with high-fat meals [22–24]. When these parameters were evaluated according to RLS severity, only IL-6 was significantly higher in the severe RLS group. IL-6 is known to be associated with sleep disorders such as obstructive sleep disease, narcolepsy, and insomnia. IL-6 levels have also been shown to correlate with sleep deprivation in healthy people. [25] Considering the quality of sleep of patients with severe RLS, the difference we found was correlated with the expected difference. The study has limitations. Our study sample has a modest size. Nonetheless, our study has a preliminary result showing higher circulating levels of pro-inflammatory cytokines in primary RLS patients. However, since the results in other studies are different, new studies are still warranted to validate the current results as well as to explore the association of plasma cytokines with the severity of RLS symptoms.

**Funding** Financial support was provided by Bezmialem Vakıf University Scientific Research Projects Unit. The sponsor had no role in the design or conduct of this research.

## Compliance with ethical standards

**Ethical approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent** Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article. This article does not contain any studies with animals performed by any of the authors.

**Conflict of interest** The authors declare that they have no conflict of interest.

## References

1. Merlino G, Valente M, Serafini A, Gigli GL (2007) Restless legs syndrome: diagnosis, epidemiology, classification and consequences. Neurol Sci 28(Suppl 1):37–46
2. Ohayon MM, Roth T (2002) Prevalence of restless legs syndrome and periodic limb movement disorder in the general population. J Psychosom Res 53(1):547–554
3. Garcia-Borreguero D, Egatz R, Winkelmann J, Berger K (2006) Epidemiology of restless legs syndrome: the current status. Sleep Med Rev 10(3):153–167
4. Khan FH, Ahlberg CD, Chow CA, Shah DR, Koo BB (2017) Iron, dopamine, genetics, and hormones in the pathophysiology of restless legs syndrome. J Neurol 264(8):1634–1641
5. Hassan N, Pineau CA, Clarke AE, Vinet E, Ng R, Bernatsky S (2011) Systemic lupus and risk of restless legs syndrome. J Rheumatol 38(5):874–876
6. Weinstock LB, Bosworth BP, Scherl EJ, Li E, Iroku U, Munsell MA, Mullen GE, Walters AS (2010) Crohn's disease is associated with restless legs syndrome. Inflamm Bowel Dis 16(2):275–279
7. Weinstock LB, Walters AS, Mullin GE, Duntley SP (2010) Celiac disease is associated with restless legs syndrome. Dig Dis Sci 55(6): 1667–1673
8. Becker J, Berger F, Schindlbeck KA, Poddubnyy D, Koch PM, Preiss JC et al (2018) Restless legs syndrome is a relevant comorbidity in patients with inflammatory bowel disease. Int J Color Dis 33(7):955–962
9. Higuchi T, Abe M, Mizuno M, Yamazaki T, Suzuki H, Moriuchi M, Oikawa O, Okawa E, Ando H, Okada K (2015) Association of restless legs syndrome with oxidative stress and inflammation in patients undergoing hemodialysis. Sleep Med 16(8):941–948
10. Trotti LM, Rye DB, De Staercke C, Hooper WC, Quyyumi A, Bliwise DL (2012) Elevated C-reactive protein is associated with severe periodic leg movements of sleep in patients with restless legs syndrome. Brain Behav Immun 26(8):1239–1243
11. Zhang JM, An J (2007) Cytokines, inflammation, and pain. Int Anesthesiol Clin 45(2):27–37
12. Hennessy MD, Zak RS, Gay CL, Pullinger CR, Lee KA, Aouizerat BE (2014) Polymorphisms of interleukin-1 Beta and interleukin-17Alpha genes are associated with restless legs syndrome. Biol Res Nurs 16(2):143–151
13. Walters AS, LeBrocq C, Dhar A, Hening W, Rosen R, Allen RP, Trenkwalder C (2003) International restless legs syndrome study G. Validation of the international restless legs syndrome study group rating scale for restless legs syndrome. Sleep Med. 4(2):121–132
14. Allen RP, Picchietti DL, Garcia-Borreguero D, Ondo WG, Walters AS, Winkelman JW, Zucconi M, Ferri R, Trenkwalder C, Lee HB (2014) Restless legs syndrome/Willis-Ekbom disease diagnostic criteria: updated International Restless Legs Syndrome Study Group [IRLSSG] consensus criteria–history, rationale, description, and significance. Sleep Med 15(8):860–873
15. Olgun Yazar H, Yazar T, Ozdemir S, Kasko AY (2019) Serum C-reactive protein/albumin ratio and restless legs syndrome. Sleep Med 58:61–65
16. Benediktsdottir B, Janson C, Lindberg E, Arnardottir ES, Olafsson I, Cook E et al (2010) Prevalence of restless legs syndrome among adults in Iceland and Sweden: lung function, comorbidity, ferritin, biomarkers and quality of life. Sleep Med 11(10):1043–1048
17. Kim R, Jun JS, Kim HJ, Jung KY, Shin YW, Yang TW, Kim KT, Kim TJ, Byun JI, Sunwoo JS, Jeon B (2019) Peripheral blood inflammatory cytokines in idiopathic REM sleep behavior disorder. Mov Disord 34(11):1739–1744

18. Karpenko MN, Vasilishina AA, Gromova EA, Muruzheva ZM, Miliukhina IV, Bernadotte A (2018) Interleukin1beta, interleukin-1 receptor antagonist, interleukin-6, interleukin-10, and tumor necrosis factor-alpha levels in CSF and serum in relation to the clinical diversity of Parkinson's disease. Cell Immunol 327:77–82
19. Kim YS, Lee KJ, Kim H (2017) Serum tumour necrosis factor-alpha and interleukin-6 levels in Alzheimer's disease and mild cognitive impairment. Psychogeriatrics. 17(4):224–230
20. Weinstock LB, Walters AS, Paueksakon P (2012) Restless legs syndrome–theoretical roles of inflammatory and immune mechanisms. Sleep Med Rev 16(4):341–354
21. Chandra S, Gupta V, Chandra H, Dhyani M, Kotwal A, Verma SK et al (2019) Serum interleukin-6 is not linked with sleep-quality, restless legs syndrome, and depression, but with 6-month survival in hematological malignancies. J Neurosci Rural Pract 10(1):94–100
22. De Pasquale L, D'Amico A, Verardo M, Petrini S, Bertini E, De Benedetti F (2012) Increased muscle expression of interleukin-17 in Duchenne muscular dystrophy. Neurology. 78(17):1309–1314
23. Ögün M, Karabörk Ş, Önerli M, Türkoğlu Ş, Yildiz S (2019) Cerebrospinal fluid levels of interleukin-17 and interleukin-34 in patients with Neuro-Behcet's syndrome. Neurophysiology. 51(2):83–87
24. Payette C, Blackburn P, Lamarche B, Tremblay A, Bergeron J, Lemieux I, Després JP, Couillard C (2009) Sex differences in postprandial plasma tumor necrosis factor-alpha, interleukin-6, and C-reactive protein concentrations. Metabolism. 58(11):1593–1601
25. Hong S, Mills PJ, Loredo JS, Adler KA, Dimsdale JE (2005) The association between interleukin-6, sleep, and demographic characteristics. Brain Behav Immun 19(2):165–172. https://doi.org/10.1016/j.bbi.2004.07.008