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CLINICAL STUDY

Neutrophil-to-lymphocyte ratio as a marker of inflammation in restless legs syndrome during pregnancy

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ABSTRACT

PURPOSE: The pregnancy is accepted as an independent risk factor for restless legs syndrome/Willis–Ekbom disease (RLS/WED). The neutrophil-to-lymphocyte ratio (NLR) was recently reported in the pathophysiology of RLS/WED. In this report, we investigated the relationship between the presence of RLS/WED and the levels of NLR in pregnancy.

METHODS: We included 268 pregnant women attending routine prenatal visits; 148 women had RLS/WED, and 120 women without RLS/WED were the control group. A pre-formed questionnaire was administered to all participants regarding demographic characteristics, habitual behaviors, detailed medical history and questions about RLS/WED including disease duration, severity, and family history. Laboratory investigations were performed in all participants regarding the complete blood count, NLR, fasting blood glucose, blood urea nitrogen, creatinine, ferritin, and total iron binding capacity.

RESULTS: NLR was significantly higher in pregnant women with RLS/WED as compared to those without it (3.9±0.9 versus 3.5±1.1, p=0.039). Smoking was also significantly more common in pregnant women with RLS/WED (p=0.022). NLR significantly increased as the gestational period progressed, even after the adjustments for age, BMI, and smoking (p=0.035). Higher NLR in pregnant women with RLS/WED was especially prominent in the 3rd trimester, although the difference was not significant.

CONCLUSION: These results may suggest that an increased inflammation demonstrated by the increased NLR, may, in part, play a role in higher prevalence of RLS/WED in pregnancy, especially in late gestational weeks *(Tab. 2, Fig. 1, Ref. 34)*. Text in PDF *www.elis.sk*

KEY WORDS: neutrophil-to-lymphocyte ratio, restless legs syndrome/Willis-Ekbom disease, pregnancy, inflammation.

Introduction

Restless legs syndrome/Willis Ekbom disease (RLS/WED) is a common sleep-related movement disorder, characterized by an urge to move usually accompanied by a subjective unpleasant sensation in the legs, which occurs in a circadian pattern, and is relieved by the movement (1). The prevalence of RLS/WED has been reported to vary between 2 and 15 %, mainly depending on geographical populations (2). It is almost a constant observation worldwide that there is a female preponderance in RLS/WED, with a female-to-male ratio of 2 : 1 (3). This gender difference in the prevalence is especially significant around the 3rd decade of life, which was attributed to the secondary cases of RLS/WED, being secondary to either decreased serum ferritin levels or pregnancy (4). The pregnancy is accepted as an independent risk factor for RLS/WED, which may account for the observed gender differences (5). While the prevalence rates of RLS/WED in men and nulliparous women are similar, multiparity increases the risk of RLS/WED gradually later in life (5, 6). The prevalence of RLS/WED is reported to vary between 13 % and 34 % during pregnancy, with an overall prevalence of RLS/WED across all trimesters as 21 % (7, 8, 9, 10).

The hypotheses explaining the higher prevalence of RLS/WED in pregnancy mainly focus on the increased sex hormone levels and iron deficiency during pregnancy (11). However, contradictory results have also been reported in studies failing to show significant differences in estrogen levels in pregnancy with and without RLS/WED (7, 12). Although more consistent results have been obtained for the iron deficiency in pregnancy secondary to the increased demand by the fetus, an increased vulnerability for developing and/or having RLS/WED later in life despite corrected iron status requires further explanation (6).

Recent studies on the pathophysiology of RLS/WED have defined possible new interactions between the iron metabolism and hypoxia pathways. Brain iron deficiency (BID), due to either inadequate iron supply or abnormalities in iron metabolism, leads to a hypoxic state via disturbed cellular functioning (13). This, in turn, causes the activation of hypoxic pathways through

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hypoxia-inducible factor 1 alpha (HIF- α) (14). The relationship between HIF-1α and inflammation provides new insights into the role of inflammation in RLS/WED (15, 16).

The neutrophil-to-lymphocyte ratio (NLR), which is a marker of systemic inflammation, was first introduced by Zahorec in 2001 (17). Since then, NLR is widely used across almost all medical disciplines as a reliable and easily available marker of immune response to various infectious and non-infectious stimuli (18). In a study examining comparing NLR between patients with RLS/WED and healthy subjects, there was not any statistically significance between groups (19, 20). On the other hand, Varim et al observed significant differences in NLR between patients with RLS/WED and healthy, suggestive of chronic systemic inflammation in RLS/WED patients (21).

The aforementioned biological changes which are triggered by the disturbed iron metabolism and resulted in the activation of inflammatory pathways may explain the higher prevalence of RLS/WED during and after pregnancy. On this basis, we designed a study to investigate the relationship between the presence of RLS/WED and levels of NLR in pregnancy.

Patients and methods

The study population was formed by pregnant women attending routine prenatal visits at Zonguldak Obstetrics−Gynecology and Children's Hospital in Zonguldak city and at Istanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty, Department of Obstetrics and Gynecology in İstanbul city, Turkey. All pregnant women attending the outpatient clinics within the study period were evaluated and those being older than 18 years of age at any gestational week, and those who agreed to participate into the study were prospectively recruited. Pregnant women with any gestational complications, maternal or fetal risks, and any disease/condition that might interfere with RLS/WED symptoms were excluded. Pregnant women without regular follow up controls and those who were not under regular iron and folate replacement therapy were also excluded in order to achieve a homogenous study population. Last, but not least, pregnant women with a total white cell count below 4.5×10^9/L or over 11.0×10^9/L were also excluded. The study was approved by the Local Ethical Committee in Zonguldak Bülent Ecevit University Medical Faculty. All participants were informed about the study, and a written informed consent was obtained from every participant.

A pre-formed questionnaire was administered to all participants during face-to-face interviews by the physicians. Each interview lasted for about 30 minutes, and all problems regarding the diagnosis of RLS/WED were discussed with the senior author in order to confirm the definitive diagnosis (GBS). The clinical-diagnostic interview was conducted in accordance with the criteria defined by the International RLS Study Group (IRLSSG), and the severity of RLS/WED was assessed by using the IRLSSG criteria (1, 22).

The questionnaire consisted of 50 questions including: (1) demographic characteristics such as age, body mass index (BMI), education, income, number of previous pregnancies, gestational week of the current pregnancy; (2) habitual behaviors such as current or past history of smoking, alcohol intake, use of iron or other vitamin supplements; (3) detailed medical history; and (4) questions on the subject of RLS/WED, and if present, detailed characteristics of RLS/WED (including disease duration, severity, and family history). All pregnant women at antenatal clinics were consecutively evaluated, and subsequently assigned into patient or control group upon recruitment of the participants depending on the inclusion and exclusion criteria.

Laboratory investigations were performed in all participants, and the following parameters were noted: complete blood count, NLR, fasting blood glucose, blood urea nitrogen, creatinine, ferritin, iron and total iron binding capacity. Blood samples were obtained early in the morning after one-night fasting within three days of clinical assessment, and evaluations were made blind to the clinical data (whether pregnant women had RLS/WED or not).

Statistical analysis

The IBM SPSS (Statistical Package for the Social Sciences, V21) program was used for the statistical analysis. Kolmogorov–Smirnov test was performed to test normality. Chi-square test was used for the nominal parameters; Mann–Whitney U or Kruskal–Wallis tests were used for the non-normally distributed continuous data. In correlation analysis, Pearson correlation, logistic correlation and multivariate regression analysis were used. The false discovery rate for multiple between-group comparisons was corrected by using the Benjamin–Hochberg procedure, with a false discovery rate of $q = 0.05$.

Results

A total of 268 pregnant women participated into our study; 148 pregnant women had RLS/WED, and 120 pregnant women without RLS/WED constituted the control group. Among the pregnant women with RLS/WED, only 12.2 % (n = 18) had an onset of RLS/WED symptomatology before the pregnancy. It was observed that the severity of RLS/WED was moderate in most of the pregnant women (38.4 %) on the basis of IRLSSG rating scale, followed by mild symptomatology in 24.7 %, severe symptomatology in 13.7 % and very severe symptomatology in 9.6 % of the patients. A majority of the patients (64.4 %) stated that their complaints were related to the legs only, while 8.2 % stated that both the legs and the arms were involved, and 2.7 % reported the involvement of the body, as well.

The comparison of demographic parameters revealed that the mean age, BMI and the gestational week in pregnant women with and without RLS/WED were similar (Tab. 1). Smoking, on the other hand, was significantly more common in pregnant women with RLS/WED ($p = 0.022$) (Tab. 1). The patients with RLS/WED were mostly in the 1st trimester (n = 15/26 women; 57.6 %), and in the 3rd trimester (n = 113/207 women; 54.6 %) as compared to those in the 2nd trimester (n = 17/35 women; 48.6 %), although the difference was not significant ($p = 0.288$). While 46.4 % of the primiparous women were diagnosed as having RLS/WED, 57.1 % of the multiparous women, and 75.3 % of the pregnant women with a history of three or more pregnancies had RLS/WED ($p = 0.056$). A positive family history for RLS/WED was significantly

Tab. 1. Demographical and clinical data of pregnant women with and without RLS/WED.

Parameters	RLS/WED (+) (n=148)	RLS/WED (-) (n=120)	p
	Mean±SD	Mean±SD	
Age (years)	30.1±5.8	29.1±5.9	0.160
BMI (kg/m2)	27.3±6.6	26±8.6	0.483
Gestational week	28.5±11.0	29.4±8.4	0.740
Total number of pregnancies	2.3±1.2	2.1±1.1	0.271
	n (%)	n (%)	
Smoking	19 (12.8)	6 (5)	0.022*
Alcohol intake	1 (0.7)	0	0.552
Education			
None	5 (3.4)	1 (0.8)	
Primary school	46 (31.1)	24 (20)	
Elementary school	26 (17.6)	24 (20)	0.171
High school	38 (25.7)	41 (34.2)	
University	33 (22.3)	30 (25)	
Parity			
Primiparous	85 (57.5)	77 (64.2)	0.147
Multiparous	63 (42.6)	43 (35.8)	
Chronic diseases			
Hypothyroidism	14 (9.5)	16 (13.3)	
Renal disease	1 (0.7)	5 (4.2)	
Heart disease	3 (2.0)	1 (0.8)	0.270
Hypertension	2 (1.4)	1 (0.8)	
Diabetes mellitus	2 (1.4)	1 (0.8)	

RLS/WED: restless legs syndrome/ Willis Ekbom disease; BMI: body mass index; SD: standard deviation; n: number, *Statistically significant

Tab. 2. Biochemical parameters of pregnant women with and without RLS/WED.

Laboratory data	RLS/WED (+) (n=148)	RLS/WED (–) (n=120)	p
Hemoglobin (g/dL)	11.2±2.2	11.2±2.0	0.528
Hematocrit (%)	34.3±3.1	35.7±6.6	0.285
Neutrophils (/mcL of blood)	7.6±1.8	7.02±1.8	0.076
Lymphocytes (/mcL of blood)	2±0.5	2.1±0.8	0.626
NLR	3.9±0.9	3.5±1.1	0.039*
Fasting blood glucose (mg/dL)	93.2±12.2	92.3±11.5	0.404
Blood urea nitrogen (mg/dL)	12.5±3.0	14.05±4.64	0.194
Creatinine (mg/dL)	0.44±0.12	0.47±0.14	0.117
Ferritin (ng/mL)	22.1±16.6	20.4±15.4	0.530
Iron (mcg/dL)	82.2±49.8	73±36.9	0.563
TIBC (%)	375.4±97.9	394±110.5	0.590

RLS/WED: restless legs syndrome/ Willis Ekbom disease; NLR: neutrophils-to-lymphocytes ratio; TIBC: total iron binding capacity; n: number, *Statistically significant

higher in pregnant women with RLS/WED (9.5 %) as compared to those without it (3.3 %, p = 0.038).

The laboratory investigations of the pregnant women with and without RLS/WED are given in Table 2. We observed that the neutrophil count was higher in pregnant women with RLS/WED than in those without it, although the difference did not reach a statistically significant level (p = 0.076). Yet, NLR was significantly higher in pregnant women with RLS/WED as compared to those without it (p = 0.039). The other parameters, such as ferritin, TIBC or creatinine failed to show significant differences between two groups (Tab. 2).

In correlation analyses, no significant correlation was observed between NLR and age (p = 0.371) or BMI (p = 0.083) of

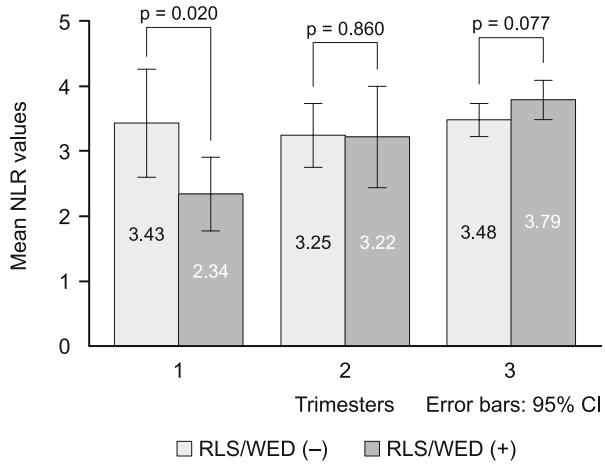

Fig. 1. The comparison of NLR values between pregnant women (in 1st, 2nd and 3rd trimesters) with and without RLS/WED.

the pregnant women with RLS/WED. The correlation analyses of NLR and smoking (p = 0.539), glucose (p = 0.916), creatinine (p = 0.271), ferritin (p = 0.383), TIBC (p = 0.585), hemoglobin (p = 0.171) or hematocrit levels (p = 0.898) also failed to show significant correlations. The mean NLR was 3.0 ± 1.2 among smoking pregnant women, and 3.6 ± 1.3 among non-smokers (p = 0.063). The higher the total number of the pregnancies, the higher the NLR, although not significantly (p = 0.226). On the other hand, the NLR was significantly increased as the gestational period progressed (t = 2.749; p = 0.012), even after the adjustments for age, BMI, and smoking (p = 0.035). The mean NLR was 3.6 ± 1.3 in pregnant women in the 3rd trimester, 3.2 ± 1.1 in those in the 2nd trimester, and 2.7 ± 0.9 in those in the 1st trimester (p = 0.008). The mean NLR in pregnant women with and without RLS/WED are shown per trimester in Figure 1. We observed that the higher NLR in pregnant women with RLS/WED as compared to those without it was especially prominent in the 3rd trimester, although the difference was not found to be significant.

Discussion

Here we demonstrated that NLR was significantly higher in pregnant women with RLS/WED and it increased as pregnancy progressed, whereas pregnant women without RLS/WED had relatively stable NLR throughout their pregnancy. On this basis, we may suggest that an increased inflammation demonstrated by the increased NLR may in part play an important role in high prevalence of RLS/WED in pregnancy, especially in late gestational weeks.

The prevalence of RLS/WED is well-known to be higher in pregnancy as compared to general population (7). We observed that the prevalence of RLS/WED increased, as the total number of previous pregnancies increased, being almost significantly higher in those with the history of three or more pregnancies. The number of t previous pregnancies was reported as an important risk factor in many studies in the literature (12, 5, 23, 24). Among other important risk factors suggested for the development of RLS/

WED in pregnancy (5, 12, 23, 24), the age and BMI did not show significant association in our study cohort. On the other hand, we demonstrated that smoking during pregnancy was significantly higher in women with RLS/WED, which was reported in some studies (5, 24). The family history for RLS/WED, as a well-known important risk factor (5, 12, 24, 25), was also observed to be more common in pregnant women with RLS/WED.

The underlying pathophysiology of RLS/WED during pregnancy has not been delineated yet, probably because it is complex and multifactorial. The most widely reported factors are the brain iron deficiency, and increased sex hormone levels during pregnancy (5, 10, 11). In this study, we failed to show significant differences in serum ferritin levels between pregnant women with and without RLS/WED. Although similar results have been reported, this may also be explained by the low number of pregnant women in the 1st and 2nd trimesters (the majority being in the 3rd trimester), and by the low ferritin levels in both groups. Indeed, the demand by the fetus increases as the gestational week progresses, which worsens the brain iron deficiency in pregnant women and triggers the development of RLS/WED or increases its severity (5, 12, 26).

Besides brain iron deficiency, hormonal factors have been suggested to play an important role in the pathophysiology of RLS/WED during pregnancy (5, 6, 11). It has been suggested that the increased levels of estradiol, progesterone and prolactin may trigger or augment the RLS/WED during pregnancy. Nevertheless, contradictory results have also been reported in the literature (24), particularly also in the previous study of the authors (12). The causal association between hormonal factors and RLS/WED during pregnancy needs to be demonstrated by more convincing evidence. Nevertheless, it is a limitation of our study that it lacks the measurements of estradiol or other pregnancy hormones.

In the current literature, newer hypotheses have been suggested in the pathophysiology of RLS/WED, including hypoxia and inflammation (27–29). Hypoxia was shown to stimulate the production of HIF-1α in *substantia nigra*, and hence to lead to iron deficiency (29). The bio-informatics analysis has identified that several other molecules were involved in the pathophysiology of RLS/WED, which represented the cellular pathways and networks in the brain iron deficiency, hypoxia, and inflammation (13). Changes in the inflammation markers have been increasingly reported in mediating the pathophysiologic mechanism in RLS/WED, including increased levels of NLR (17–21). Here we demonstrated for the first time in the literature that the higher NLR was significantly associated with the RLS/WED in pregnancy, especially in the 3rd trimester. Indeed, the NLR, together with the platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) were reported to increase during with the progress of gestational weeks, while reflecting the subclinical chronic increase in the inflammatory burden (31). We may therefore suggest that the pathophysiology underlying the RLS/WED during pregnancy may show peculiar differences, and the inflammation, as shown by the increased NLR, may be responsible for the development and/or augmentation of RLS/WED in the 3rd trimester. From another point of the view, it is also shown that stress-related hormones such as catecholamine and cortisol are related to an increase in NLR (32). Considering that nocturnal overactivity of the hypothalamic-pituitary-adrenal system is linked to RLS/WED and related disturbed sleep, it might be a factor that contributes to increased NLR in pregnant women with RLS/WED, but further studies are needed for this conclusion (33), Also, an increase in NLR and PLR was associated with smoking (34), which was suggested to be used as a systemic inflammatory biomarker to indicate the deleterious effects of smoking. In this term, the mechanisms by which smoking increases the risk of RLS/WED during pregnancy may be explained at least in part by inflammation.

The major limitation of our study is the lack of the analysis of other inflammation-related biomarkers since NLR is a non-specific indicator of inflammation, as well as of physiological stress. Although NLR values were still within normal range, they were higher in pregnant women with RLS/WED as compared to those without it. While this difference may also be explained by other factors than RLS/WED, the exclusion criteria were set to exclude as many confounding factors as possible. Another limitation of our study is the lack of the analysis of some other factors playing role in the development of RLS/WED in pregnancy, including hormonal factors, folate, or vitamin D. The other metabolic factors such as hypothyroidism or hyperparathyroidism, or other metabolites/molecules in the pathways of iron, hypoxia and inflammation were not investigated, either. The lack of difference in concentration of serum iron levels between patient groups with and without RLS/WED is another limitation, which may be explained by the iron replacement therapy. It is worth mentioning that anemia is another factor linked to increased NLR. However, because we aimed to homogenize the study groups in respect of pregnancy complications such as anemia and iron replacement therapy, we could not make a reliable conclusion regarding this correlation. Also, the majority of the study population was in the 3rd trimester, which hinders drawing clear conclusions about NLR in regard to different trimesters. Last, but not least, the results of our study should be replicated in larger cohorts with additional biomarkers of inflammation.

Learning points

- The neutrophil-to-lymphocyte ratio is significantly increased in pregnant women with RLS/WED as compared to those without it.
- The neutrophil-to-lymphocyte ratio is significantly increased with the progression of pregnancy.
- Increased inflammation, as demonstrated by the increased neutrophil-to-lymphocyte ratio, may explain the high prevalence of RLS/WED during pregnancy. Data from some of the participants from Istanbul University-Cerrahpasa, Cerrahpasa Faculty of Medicine, were partly used in the previous study of the authors (12).

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