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SCIENTIFIC INVESTIGATIONS

The Association Between Vitamin D Level and Restless Legs Syndrome: A Population-Based Case-Control Study

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Study Objectives: The pathophysiology of restless legs syndrome (RLS) may be related to abnormalities in central dopamine pathways. Vitamin D may play a role in the pathophysiology of RLS by modulating the dopaminergic system. The aim of our study is to examine the possible link between RLS and vitamin D deficiency.

Methods: The total number of subjects enrolled was 201, including 78 patients with RLS based on the International RLS Study Group (IRLSSG) diagnostic criteria and 123 controls. Serum 25-hydroxy vitamin D levels were measured in both groups. RLS severity was assessed in all cases using the IRLSSG symptom severity rating scale.

Results: Fifty-nine patients with RLS (75.6%) and 52 controls (42.3%) had a diagnosis of vitamin D deficiency, $P < .001$. The odds ratio (OR) of the development of RLS was 4.24 for those with a vitamin D level < 50 nmol/L compared to those with a vitamin D level ≥ 50 nmol/L ($P < .001$, 95% confidence interval [CI] 2.3–7.9). After adjusting for all other significant factors in the multivariate logistic model, vitamin D was significantly associated with RLS (OR 3.1, $P < .002$, 95% CI 1.51–6.38). Moreover, a dark or black skin color (OR 3.4, $P < .001$, 95% CI 1.5–6.3) and working as a teacher (OR 8.8, $P < .001$, 95% CI 3.4–23.5) were also independently significantly associated with RLS.

Conclusions: Our study identified an association between vitamin D deficiency and RLS. Consequently, vitamin D deficiency should be considered in the management of RLS. However, further studies are needed to evaluate the causality relationship between vitamin D level and RLS.

Keywords: deficiency, primary, restless legs syndrome, RLS severity, secondary, vitamin D, deficiency

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BRIEF SUMMARY

Current Knowledge/Study Rationale: Most evidence to date suggests that brain dopaminergic dysfunction plays a key role in the development of restless legs syndrome (RLS), and vitamin D is essential for the function of the dopaminergic system. Nonetheless, few studies have examined the relationship between vitamin D and RLS. The current study is the first population-based case-control study to assess the association between RLS and vitamin D levels using an adequate sample size of patients with RLS.

Study Impact: The data show an inverse association between vitamin D levels and RLS. Therefore, vitamin D deficiency should be considered in the management of patients with RLS.

INTRODUCTION

Restless legs syndrome (RLS), also known as Willis-Ekbom disease (WED), is a chronic neurological movement disorder that is characterized by the urge to constantly move the affected body part to stop an uncomfortable sensation. Indeed, the sensation is intensified by relaxation and is temporarily relieved by moving the affected body part. Although RLS most commonly affects the legs, it can also affect other body parts, such as the head, torso, and arms. In 1995, the founding members of the International RLS Study Group (IRLSSG) developed the first "four minimal criteria" for RLS diagnosis.[1] The criteria for RLS/WED were further clarified in 2003 and recently revised in 2014 by the IRLSSG.[2,3]

RLS affects patient quality of life and is comparable to the burden of other chronic disorders.[4] Patients with RLS experience negative effects of the condition with regard to their work, daily living activities, personal relationships, and travel. These patients also report symptoms of depression (17% to 27%) and anxiety (8% to 23%).[5,6] RLS is classified according to its etiology as primary/idiopathic and secondary, although both types share the same clinical presentation. Most cases are primary in origin, and approximately 50% of patients with primary RLS have a family history of the condition.[7,8] Secondary RLS is observed in combination with other conditions, such as iron deficiency anemia, renal failure, diabetes, thyroid disease, pregnancy, and neurological movement disorders or as a side effect of certain medications.[9–13]

Although the pathophysiology of RLS is not completely understood, genetic factors, iron deficiency, and dopaminergic system abnormalities have been proposed as possible mechanisms.[14] Most evidence to date suggests that brain

dopaminergic dysfunction plays a key role in the development of RLS. Dopamine, a neurotransmitter involved in sensory and motor control as well as behavioral modifications, is needed to produce smooth and purposeful muscle activities in the basal ganglia circuits of the brain. Dopaminergic pathway dysfunction frequently results in abnormal involuntary movements, which may explain the mechanism responsible for RLS development.[15,16]

Furthermore, vitamin D is essential for protecting dopaminergic neurons from toxins and increases the levels of dopamine or its metabolites in the nigrostriatal dopaminergic pathway.[17,18] Nevertheless, the role of vitamin D in RLS has not been well studied. Few studies have examined the relationship between vitamin D and RLS, and those that have largely consist of cross-sectional studies or case series involving relatively small samples.[19–22] Therefore, larger and more representative studies are needed to examine the link between RLS and vitamin D levels. To the best of our knowledge, this study is the first population-based case-control study with an adequate sample of RLS cases conducted to examine the relationship between vitamin D levels and RLS and to evaluate the clinical implications of RLS treatments.

METHODS

This case-control study was conducted at Sleep Medicine and Research Center, King Abdul-Aziz University Hospital, Jeddah, Saudi Arabia, between March and November of 2016. The study followed a first-stage cross-sectional survey on the prevalence of obstructive sleep apnea among 2,682 middle-aged individuals in the western region of Saudi Arabia.[23] The cross-sectional survey was conducted from February 2013 to June 2015 in Jeddah, Saudi Arabia, and included teachers, administrative staff, porters, and servants who were employed at government schools. Based on IRLSSG diagnostic criteria, RLS was found in 224 participants (8.4%).[2,23] The RLS cases and controls were consecutively selected from survey study population.

This study conformed to the ethical standards of the Declaration of Helsinki. Ethical approval was obtained from the Research Ethics Committee of King Abdul Aziz University Hospital. An invitation letter with information sheet was provided to the participants, and written consent was obtained from all participants.

Case Selection Using IRLSSG Diagnostic Criteria

In this study, RLS was defined as an urge to move the legs that is usually associated with uncomfortable sensations in the legs, worsening of these symptoms during rest, at least temporary relief provided by activity, and worsening of symptoms in the evening.[2] Conditions that may mimic the symptoms of RLS were clinically excluded to increase the diagnostic specificity of the aforementioned four criteria, as per the latest update of the IRLSSG consensus criteria.[3]

Cases were selected from the 224 participants considered to have RLS in the initial screening performed at the field survey stage.[23] Eligibility was then determined by using the updated diagnostic criteria of RLS, which confirmed the diagnosis of 142 cases.[3] Of those, only 82 subjects were recruited; 38 were unable to be contacted, and 22 refused to participate due to reasons related to moving out of town or social obligations. Four of the recruited cases did not complete their blood tests and hence were excluded. Ultimately, 78 cases were included in the RLS group.

Control Selection

The controls were randomly selected from the remaining 2,458 participants without RLS from the survey population.[23] Of these, 260 were frequently matched to the cases on the basis of a 2:1 ratio for sex and age variables. Because 93 were unable to be contacted and 38 refused to participate due to moving out of town or social obligations, only 129 controls were included. None of the selected controls had RLS according to the IRLSSG criteria. A frequency-matching approach was chosen to provide more flexibility and efficiency. A descriptive flowchart of the study population is presented in **Figure 1**.

Data Collection

Trained physicians interviewed the participants in both groups at the sleep center. A study-specific questionnaire was completed during the interview. The questionnaire collected information regarding relevant sociodemographic variables (age, sex, skin color, marital status, education, occupation, income, family history, history of consanguinity, cigarette and hookah smoking), past medical history (eg, vitamin D deficiency, iron deficiency, varicose veins, diabetes, hypertension, renal disease, thyroid diseases, multiple sclerosis, parkinsonism, epilepsy, schizophrenia, depression, bipolar disorder, habitual foot tapping), medication history (eg, antidopaminergic agents, antipsychotic agents, tricyclic or selective serotonin reuptake inhibitors, antidepressants, beta blockers), RLS symptoms based on diagnostic criteria defined by the IRLSSG, and RLS severity using the IRLSSG rating scale for those with RLS.[24,25] Furthermore, all subjects underwent physical examination including weight and height measurements to the nearest 0.1 cm, and 0.1 kg, body mass index (BMI) calculation and a detailed complete neurological, vascular, musculoskeletal, and rheumatologic examination of the lower limbs.

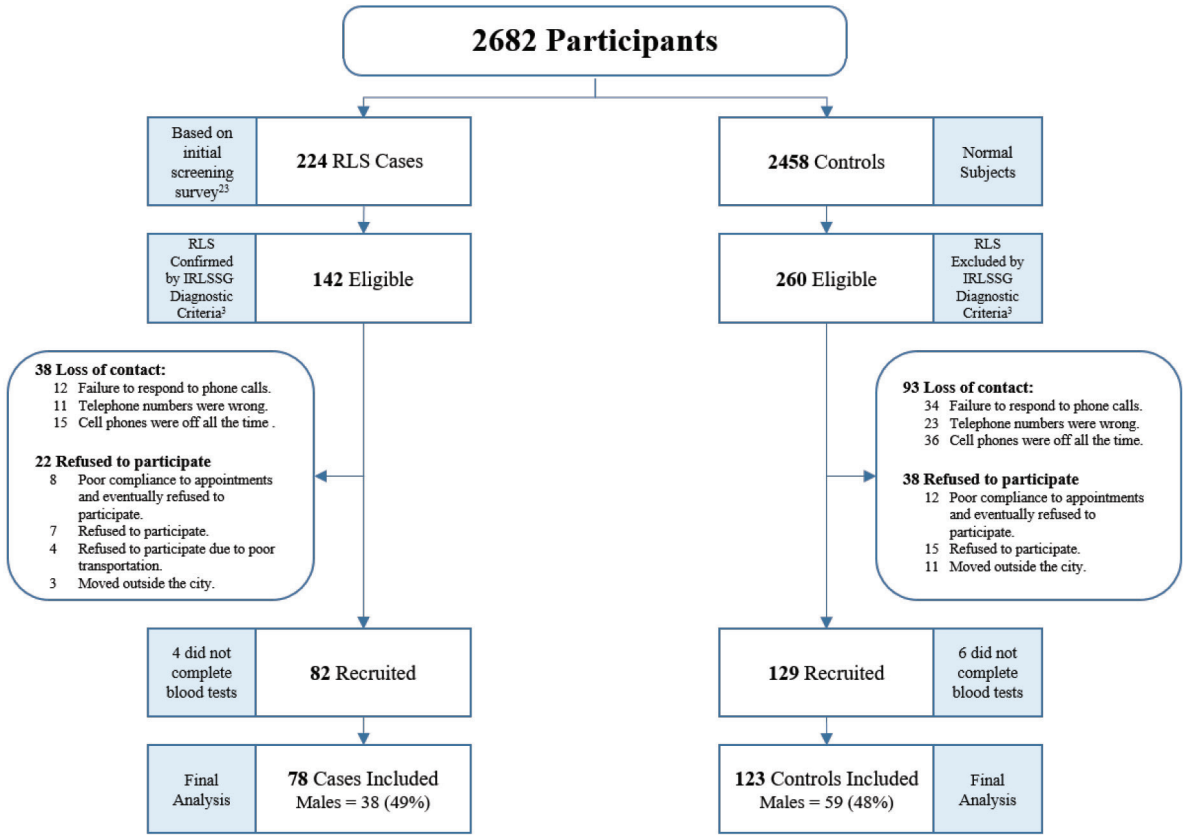
IRLSSG Rating Scale

All patients with RLS completed the 10-item IRLSSG rating scale, which was validated and shown to be reliable for the assessment of RLS severity. The scale consists of five questions regarding the intensity and frequency of RLS symptoms and five questions focused on how those symptoms affect daily life and sleep. Each item was graded on a scale of 0 to 4. Disease severity was classified using the following scale: mild, 0–10 points; moderate, 11–20 points; severe, 21–30 points; and very severe, 31–40 points.[24,25]

Biochemical Measurements

Blood samples were collected from all study participants after the interview and analyzed in the same laboratory; the technicians were blinded to the participants' status in the study. The same procedure was used to collect blood samples from the cases and controls. The blood tests involved analyses of the

Figure 1—Descriptive flowchart of the study population.



IRLSSG = International Restless Legs Syndrome Study Group, RLS = restless legs syndrome.

following parameters: serum hemoglobin (Hgb) level, serum 25-hydroxy vitamin D level, serum iron profile, renal function test, and serum calcium, phosphate, magnesium, vitamin B_{12}, hemoglobin A1c (HgbA1c), and thyroid-stimulating hormone levels.

Serum 25-hydroxy Vitamin D

Vitamin D status is traditionally measured through assays of serum 25-hydroxy vitamin D—25(OH) D—the major circulating form. However, the serum 25(OH) D level that defines vitamin D adequacy, insufficiency, or deficiency or how much of this vitamin is required to sustain its adequate physiological functions and bone health remain debatable. The Institute of Medicine has suggested that approximately 97.5% of the population across all age groups meet the requirements for vitamin D with a serum 25(OH) D value higher than 50 nmol/L.[26,27] In our study, serum 25(OH) D levels were measured as a continuous variable and classified for analysis into two categories: < 50 nmol/L (< 20 ng/mL) for deficiency and ≥ 50 nmol/L (≥ 20 ng/mL) for sufficiency. These values are based on the Institute of Medicine cutoff point and the recommendation of the Standing Committee of Europe Doctors (www.cpme.eu).

Data Analysis

Data were analyzed using Stata Version 13.0. (StataCorp LLC, College Station, Texas, United States). The significance level α at .05 and the 95% confidence interval (CI) were calculated for estimated scores. The sample size was calculated at α = .05 and a power of .80 utilizing the Kelsey and Fleiss formula.[28,29] The total estimated sample size was 144, divided evenly between the two groups. Data were analyzed using Pearson correlations, independent two-sample *t* tests, analyses of variance, and chi-square tests. Stepwise univariate and multivariate logistic regression analysis models were utilized.

RESULTS

A total of 201 participants were enrolled in the final analysis, including 78 patients with RLS and 123 healthy controls (**Figure 1**).

No significant differences in sex, mean age, BMI, smoking habits, or history of consanguinity were observed between the two groups. In contrast, skin color differed significantly between the two groups, as more patients with RLS (53, 68.0%) than controls (41, 33.3%) had dark and black skin ($\chi^2 = 22.9733$, $P < .001$). Consecutively, education and monthly income of the participants varied significantly between the cases and controls. Additionally, there were significantly more teachers in the RLS group compared to the control group, 92.3% versus 57.7% respectively ($P < .001$), and vitamin D deficiency was significantly more prevalent in the RLS teachers (54, 75%)

Table 1—Demographic characteristics of the study population.

	Total (n = 201)	Patients With RLS (n = 78)	Controls (n = 123)	*P*
Age, years, mean ± SD		43.79 ± 6.04	44.75 ± 9.59	.434
Sex, n (%)				.693
Males	97 (48.3)	38 (48.7)	59 (48.0)	
Females	104 (51.7)	40 (51.3)	64 (52.0)	
BMI *, n (%)				.803
Underweight and normal	22 (10.9)	8 (10.3)	14 (11.4)	
Overweight and obese	179 (89.1)	70 (89.7)	109 (88.6)	
Skin color, n (%)				< .001
White	107 (53.2)	25 (32.1)	82 (66.7)	
Dark and black skin	94 (46.8)	53 (67.9)	41 (33.3)	
Education, n (%)				< .001
High school or less	55 (27.3)	9 (11.5)	46 (37.4)	
University/postgraduate	146 (72.6)	69 (88.5)	77 (62.6)	
Occupation, n (%)				< .001
Worker and administrator	58 (28.8)	6 (7.7)	52 (42.3)	
Teacher	143 (71.1)	72 (92.3)	71 (57.7)	
Income, n (%)				< .001
0–10,000 SR	54 (26.8)	10 (12.8)	44 (35.8)	
> 10,000 SR	147 (73.1)	68 (87.2)	79 (64.2)	
Marital status, n (%)				< .001
Single, divorced or widowed	58 (28.8)	10 (12.8)	48 (39.0)	
Married	143 (71.1)	68 (87.2)	75 (61.0)	
Cigarette smoking, n (%)				.092
Never smoked	166 (82.6)	60 (76.9)	106 (86.2)	
Current or former smoker	35 (17.4)	18 (23.1)	17 (13.8)	
Water pipe smoking, n (%)				.324
Never smoked	164 (81.6)	61 (78.2)	103 (83.7)	
Current or former smoker	37 (18.4)	17 (21.8)	20 (16.3)	
Consanguinity, n (%)	17 (8.5)	8 (10.3)	9 (7.3)	.466

P values determined by *t* test for comparisons of two means and chi-square test for categorical variables, statistical significance defined as $P < .05$. * = BMI classification according to the World Health Organization guidelines: underweight < 18, normal 18–24, overweight 25–30, obese > 30 kg/m^2. BMI = body mass index, RLS = restless legs syndrome, SD = standard deviation, SR = Saudi Riyal.

compared to the control teachers (34, 48%) (odds ratio [OR] 3.3, $P < .001$, 95% CI 1.5–7.1). Further analysis of the subgroup of RLS teachers revealed no difference between them and the controls with regard to their smoking status ($P = .304$), BMI ($P = .592$), iron level ($P = .16$), and thyroid hormone level ($P = .156$). **Table 1** lists the basic demographics and clinical characteristics of the participants.

Vitamin D deficiency was found in a higher percentage in cases compared to controls: 59 (75.6%) RLS cases compared to 52 (42.3%) controls ($P < .001$). The risk for the development of RLS in vitamin D deficient cases, ie, < 50 nmol/L (< 20 ng/ml), was higher than for vitamin D sufficient cases, ie, ≥ 50 nmol/L (≥ 20 ng/mL) (OR 4.24, $P < .001$, 95% CI 2.3–7.9). In addition, significantly more patients than controls had a diagnosis of iron deficiency anemia (39, 50.0% versus 43, 35.1%, $P = .034$). Iron deficiency defined as serum ferritin level < 50 ng/mL was marginally significant ($P = .077$). The clinical characteristics of the study participants are presented in **Table 2**.

Furthermore, mean serum 25-hydroxy vitamin D levels were significantly lower in patients with RLS (31.57 ± 17.58 nmol/L or 12.65 ng/mL) compared to controls (65.18 ± 24.64 nmol/L or 26.12 ng/mL) ($t = 10.47$, $P < .001$, 95% CI 27.3–39.9). The biochemical characteristics of the study participants are shown in **Table 3**.

According to univariate logistic regression analysis, vitamin D deficiency, skin color, education, occupation, monthly income, and marital status were significantly associated with RLS. In multivariate logistic regression analysis, only vitamin D deficiency, a dark or black skin color, and working as a teacher remained significantly associated with RLS. The multivariate logistic regression data are presented in **Table 4**.

Among the 78 RLS cases, 50 (64%) were primary and 28 (36%) were secondary. Of the 50 primary RLS cases, 37 (74%) had vitamin D deficiency, whereas only 6 of the 28 secondary RLS cases (21%) had vitamin D deficiency. This difference was statistically significant (OR 10.4, $P < .001$, 95% CI 1.1–37.5).

The mean severity score utilizing the IRLSSG symptom severity rating scale for all RLS cases was 16.6 ± 5.8, which was higher for the RLS vitamin D deficient cases (19.4 ± 5.9) compared to the RLS vitamin D sufficient cases (14.5 ± 4.7) ($P < .002$). Moreover, when comparing the mean vitamin D level at each severity score category, we found that a lower mean vitamin D level was associated with a higher severity score category, though this finding did not reach statistical significance ($P = .6624$).

Table 2—Clinical characteristics of the study participants.

	Patients With RLS (n = 78)	Controls (n = 123)	*P*
Vitamin D deficiency < 50 nmol/L (20 ng/mL)	59 (75.6)	52 (42.3)	< .001
Iron deficiency anemia [a]	39 (50.0)	43 (35.0)	.034
Iron deficiency [b]	48 (61.5)	60 (48.8)	.077
Vitamin B12 deficiency [c]	23 (29.5)	25 (20.3)	.138
Diabetes [d]	15 (19.2)	17 (13.8)	.307
Hypertension [e]	10 (12.8)	16 (13.0)	.969
Thyroid disease [f]			
Hypothyroidism	0 (0.0)	5 (4.1)	.159
Hyperthyroidism	4 (5.1)	14 (11.4)	.204
Arthritis [g]	3 (3.9)	4 (3.3)	.823 *
Varicose veins [g]	5 (6.4)	12 (9.8)	.406
Peripheral neuropathy [g]	3 (3.8)	1 (0.8)	.133 *
Obstructive sleep apnea [e]	1 (1.3)	1 (0.8)	> .999
Chronic obstructive pulmonary disease [e]	8 (10.3)	4 (3.3)	.064
Coronary artery disease [e]	2 (2.6)	0 (0.0)	.149

Values are presented as n (%). *P* values determined by chi-square test for categorical variables or Fisher exact test if followed by asterisk, statistical significance defined as $P < .05$. Superscript letters indicate: a = hemoglobin levels ≤ 12 g/dL with serum ferritin levels < 20 ng/mL, b = serum ferritin < 50 ng/mL, c = serum vitamin B_{12} level < 187 pmol/L, d = diagnosis based on both history and hemoglobin A1c level ≤ 6.5%, e = diagnosis based on history, f = diagnosis based on history and thyroid-stimulating hormone level (hypothyroidism < 0.27 μIU/L, hyperthyroidism > 4.2 μIU/L), g = diagnoses based on history and examinations. RLS = restless legs syndrome.

Table 3—Biochemical characteristics of the study participants.

	Patients with RLS (n = 78)	Controls (n = 123)	*P*
25(OH) vitamin D (nmol/L)	31.58 ± 17.58	65.18 ± 24.64	< .001
Ferritin (ng/mL)	47.89 ± 43.17	71.86 ± 64.95	.004
Serum transferrin (g/L)	2.64 ± 0.39	2.90 ± 2.65	.386
Total iron-binding capacity (μmol/L)	64.64 ± 10.81	66.26 ± 41.57	.736
Folate (nmol/L)	21.87 ± 9.85	23.24 ± 12.60	.418
B12 (pmol/L)	291.60 ± 148.60	277.30 ± 110.60	.438
Blood urea nitrogen (mmol/L)	4.76 ± 4.65	4.13 ± 1.24	.154
Creatinine (μmol/L)	70.94 ± 16.64	73.30 ± 18.68	.365
Calcium (mmol/L)	2.23 ± 0.11	2.20 ± 0.09	.072
Magnesium (mmol/L)	0.82 ± 0.09	0.82 ± 0.08	.857
Hemoglobin (g/dL)	13.25 ± 2.09	13.38 ± 1.81	.646
Hemoglobin A1c (%)	6.07 ± 1.48	6.28 ± 4.28	.678
Thyroid-stimulating hormone (μIU/L)	1.82 ± 1.20	3.27 ± 12.32	.302

Values presented as mean ± standard deviation. Statistical significance defined as $P < .05$. RLS = restless legs syndrome.

Table 4—Results of multivariate logistic regression analysis.

	Odds Ratio	Standard Error	*P*	95% CI
Vitamin D deficiency < 50 nmol/L (20 ng/mL)	3.1	1.14	< .002	1.51–6.38
Dark or black skin	3.4	1.22	< .001	1.72–6.92
Occupation teacher	8.8	4.40	< .001	3.36–23.49

Statistical significance defined as $P < .05$. CI = confidence interval.

DISCUSSION

The risk for the development of RLS was significantly higher in vitamin D deficient cases compared to those who are vitamin D sufficient. Additionally, the mean serum 25(OH) vitamin D level was significantly lower in patients with RLS than in normal controls. The association between vitamin D deficiency and RLS remained significant after adjusting for all other significant factors in the multivariate regression model. Our data also revealed an association between increased IRLSSG

severity rating score and decreased serum vitamin D level. These two findings suggest a link between serum vitamin D levels and RLS.

There is accumulating evidence that the dopaminergic system plays an important role in the pathophysiology of RLS.[14–16] Moreover, vitamin D appears to have a key function in the dopaminergic system; indeed, this vitamin participates in regulating nervous system development and function.[30] Dopamine neurons in the midbrain and their target neurons in the striatum were shown to express vitamin D3 receptor proteins, and Cui et al. confirmed that the vitamin D receptor is present in the nucleus of tyrosine hydroxylase-positive neurons in both the human and rat substantia nigra.[18] Furthermore, Oran et al. observed a dose-responsive increase in the number of rat primary dopaminergic neurons when 1,25-(OH)2 D3 was added to culture media,[20] and these authors concluded that 1,25-(OH)2 D3 may increase the abundance of dopaminergic neurons by upregulating expression of glial-derived neurotrophic factor. In addition, it has been reported that vitamin D affects the nigrostriatal dopaminergic pathway by increasing the levels of dopamine or its metabolites and by protecting dopaminergic neurons against toxins.[31,32] Low doses of 1 alpha, 25-dihydroxyvitamin D3—1, 25-(OH) 2D3—(the hormonally active form of vitamin D) are able to protect mesencephalic dopaminergic neurons against toxins that cause a decrease in the glutathione content, which when decreased may lead to selective dopaminergic neuron death.[33,34] A recent publication also concluded that vitamin D deficiency may lead to an increased risk of central nervous system disease, such as parkinsonism, schizophrenia, and multiple sclerosis.[35] Moreover, an experimental study on rats by Trinko et al. revealed an association between vitamin D deficiency and regulation of the dopamine circuit; animals with vitamin D deficiency had dopaminergic pathway abnormalities, which increased their predisposition toward neuromuscular disorders.[36] Considering these dopaminergic effects of vitamin D, our findings support the hypothesis that decreased vitamin D levels may lead to RLS symptoms.

Our results were also in agreement with several recent studies examining the role of vitamin D in the etiology of RLS. Balaban et al.[22] conducted a hospital-based study and evaluated 25(OH) vitamin D levels in female patients with and without RLS, reporting significantly lower serum levels in the former. However, the study was limited to patients with idiopathic and nonfamilial RLS.[22] The cross-sectional study conducted by Oran et al. examined the prevalence of RLS in subjects with and without vitamin D deficiency; an increased prevalence of RLS was observed in the vitamin D deficiency group, suggesting an association between serum vitamin D level and RLS.[20] Unfortunately, the authors of that study included a convenience sample of patients based on their eligibility criteria, without a calculated sample size.[20] Another cross-sectional study, that by Çakır et al. evaluated the association between the frequency and severity of RLS, sleep quality and vitamin D level,[21] reporting that RLS prevalence and Pittsburgh Sleep Quality Index scores were higher in vitamin D deficient than in vitamin D sufficient subjects. These data further support an association between vitamin D level, sleep disturbances, and RLS. Furthermore, a recently conducted pilot study of 12 patients revealed an improvement in the severity of RLS symptoms upon administration of vitamin D supplements.[37]

In our study, the prevalence of dark and black skin color was significantly higher in patients with RLS than in controls, and this persisted as a risk factor for RLS in the multivariate regression model. However, this finding does not explain the strong association between vitamin D deficiency and RLS, which remained highly significant after adjusting for skin color in multivariate regression analysis. Melanin has been proposed to function as a sun- block in dark-skinned individuals, thereby placing them at a higher risk for vitamin D deficiency. However, a study of Africans from equatorial countries revealed normal vitamin D levels among these individuals. The authors concluded that lower vitamin D levels in dark-skinned individuals residing in northern and high-elevation regions might not be related to their skin pigmentation but instead may be due to other health determinants, such as genetics, diet, and sun exposure.[38] Furthermore, in the study by Alsuwadia et al., the principal causes of low vitamin D levels among the Saudi community were most likely intentional sun and heat avoidance, insufficient dietary intake, and wearing of traditional clothes.[39]

Teaching as an occupation was also significantly related to RLS. Based on our results, 92% of the RLS cases were teachers compared to 58% of the controls ($P < .001$). Furthermore, this occupation persisted as a risk factor for RLS in multivariate regression analysis. Vitamin D deficiency was significantly more frequent among teachers with RLS than control teachers. Considering that there was no difference between the two groups in terms of other known RLS-associated factors (iron level, thyroid hormone level, smoking status, and BMI), this may further support the possible relationship between RLS and vitamin D. This finding also may be associated with the amount of physical activity related to this occupation, as it typically involves a great amount of standing and walking. This finding concurs with previous epidemiological studies reporting a significant link between physical exercise and RLS.[40] In addition, according to one clinical trial, the exercise group exhibited significant improvement in RLS symptoms compared with the control group.[41] Although an interesting observation, due to the nature of the case-control study design being unable to ascertain temporal causality, it remains unclear whether being a teacher increases the risk of RLS or whether more RLS patients become teachers.

A significant association between RLS and BMI was not detected in our study. This finding is consistent with some previous epidemiological studies of RLS.[42,43] Nevertheless, several epidemiological surveys have revealed a role for obesity as an independent risk factor for RLS.[44–46] Additionally, our results did not show a significant link between smoking and RLS, which may be related to the sampled population of school employees who did not smoke due to poor social acceptance in their field. Finally, consanguinity, which is defined as marriage between first-degree cousins, was not associated with RLS in our study. Although this practice is common in certain Saudi families, this finding may be related to the enrollment of an urban population in the city of Jeddah, where the practice may not be as common as in rural regions of the kingdom.

In our study, RLS was categorized into one category, despite the fact that 50 of the 78 cases (64%) had primary RLS and 28 had secondary RLS (36%). This is based on the recently proposed hypothesis that primary and secondary RLS might be a continuous spectrum of one disease that is the consequence of genetic and environmental or comorbid disease interactions and that the classification of primary and secondary forms is likely misleading.[47] A major concern regarding our data is the dramatic differences between the RLS and non-RLS groups regarding socioeconomic level, skin color, professional status, educational level, and marital status, all of which may in fact be related to other factors that are truly predictive of RLS status (e.g., genetics, ethnicity). However, based on the aforementioned hypothesis, these environmental factors may actually trigger the manifestation of RLS symptoms by interacting with genetic makeup in these cases.

In conclusion, the current study is the first population-based case-control study to examine the association between RLS and vitamin D level using an adequate sample size of patients with RLS. The data showed an inverse association between vitamin D level and RLS. Therefore, vitamin D deficiency and insufficiency should be considered in the management of patients with RLS. Well-designed randomized clinical trials or cohort studies are the next logical steps to evaluate the causal relationship between vitamin D level and RLS.

ABBREVIATIONS

CI, confidence interval
Hgb, hemoglobin
HgbA1c, hemoglobin A1c
IRLSSG, International Restless Legs Syndrome Study Group
RLS, restless legs syndrome
TSH, thyroid-stimulating hormone
WED, Willis-Ekbom disease

REFERENCES

1. Walters AS. Toward a better definition of the restless legs syndrome. The International Restless Legs Syndrome Study Group. *Mov Disord.* 1995;10(5):634–642.
2. Allen RP, Picchietti D, Hening WA, et al. Restless legs syndrome: diagnostic criteria, special considerations, and epidemiology. A report from the restless legs syndrome diagnosis and epidemiology workshop at the National Institutes of Health. *Sleep Med.* 2003;4(2):101–119.
3. Allen RP, Picchietti DL, Garcia-Borreguero D, et al. 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.* 2014;15(8):860–873.
4. Abetz L, Allen R, Follet A, et al. Evaluating the quality of life of patients with restless legs syndrome. *Clin Ther.* 2004;26(6):925–935.
5. Hening W, Walters AS, Allen RP, Montplaisir J, Myers A, Ferini-Strambi L. Impact, diagnosis and treatment of restless legs syndrome (RLS) in a primary care population: the REST (RLS epidemiology, symptoms, and treatment) primary care study. *Sleep Med.* 2004;5(3):237–246.
6. Winkelmann J, Prager M, Lieb R, et al. "Anxietas tibiarum". depression and anxiety disorders in patients with restless legs syndrome. *J Neurol.* 2005;252(1):67–71.
7. Winkelmann J, Muller-Myhsok B, Wittchen HU, et al. Complex segregation analysis of restless legs syndrome provides evidence for an autosomal dominant mode of inheritance in early age at onset families. *Ann Neurol.* 2002;52(3):297–302.
8. Winkelmann J, Wetter TC, Collado-Seidel V, et al. Clinical characteristics and frequency of the hereditary restless legs syndrome in a population of 300 patients. *Sleep.* 2000;23(5):597–602.
9. Winkelman JW. Considering the causes of RLS. *Eur J Neurol.* 2006;13 (Suppl. 3):8–14.
10. Karroum E, Konofal E, Arnulf I. [Restless-legs syndrome]. *Rev Neurol.* 2008;164(8–9):701–721.
11. Allen RP, Earley CJ. Restless legs syndrome: a review of clinical and pathophysiologic features. *J Clin Neurophysiol.* 2001;18(2):128–147.
12. Bachmann CG, Rolke R, Scheidt U, et al. Thermal hypoaesthesia differentiates secondary restless legs syndrome associated with small fibre neuropathy from primary restless legs syndrome. *Brain.* 2010;133(3):762–770.
13. Uglane MT, Westad S, Backe B. Restless legs syndrome in pregnancy is a frequent disorder with a good prognosis. *Acta Obstet Gynecol Scand.* 2011;90(9):1046–1048.
14. Trotti LM, Bhadriraju S, Rye DB. An update on the pathophysiology and genetics of restless legs syndrome. *Curr Neurol Neurosci Rep.* 2008;8(4):281–287.
15. Connor JR, Wang XS, Allen RP, et al. Altered dopaminergic profile in the putamen and substantia nigra in restless leg syndrome. *Brain.* 2009;132(9):2403–2412.
16. Turjanski N, Lees AJ, Brooks DJ. Striatal dopaminergic function in restless legs syndrome: 18F-dopa and 11C-raclopride PET studies. *Neurology.* 1999;52(5):932–937.
17. Ibi M, Sawada H, Nakanishi M, et al. Protective effects of 1 alpha,25-(OH)(2) D(3) against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology.* 2001;40(6):761–771.
18. Cui X, Pelekanos M, Liu PY, Burne TH, McGrath JJ, Eyles DW. The vitamin D receptor in dopamine neurons; its presence in human substantia nigra and its ontogenesis in rat midbrain. *Neuroscience.* 2013;236:77–87.
19. Prakash S, Bhanvadia RJ, Shah ND. Restless legs syndrome with carbamazepine-induced osteomalacia: causal or casual association. *Gen Hosp Psychiatry.* 2010;32(2):228.e1–228.e3.
20. Oran M, Unsal C, Albayrak Y, et al. Possible association between vitamin D deficiency and restless legs syndrome. *Neuropsych Dis Treat.* 2014;10:953–958.
21. Çakır T, Doğan G, Subaşı V, et al. An evaluation of sleep quality and the prevalence of restless leg syndrome in vitamin D deficiency. *Acta Neurol Belg.* 2015;115(4):623–627.
22. Balaban H, Yıldız ÖK, Çil G, et al. Serum 25-hydroxyvitamin D levels in restless legs syndrome patients. *Sleep Med.* 2012;13(7):953–957.
23. Wali SO, Abaalkhail B. Prevalence of restless legs syndrome and associated risk factors among middle-aged Saudi population. *Ann Thorac Med.* 2015;10(3):193–198.
24. Walters AS, LeBrocq C, Dhar A, et al. Validation of the International Restless Legs Syndrome Study Group rating scale for restless legs syndrome. *Sleep Med.* 2003;4(2):121–132.
25. Abetz L, Arbuckle R, Allen RP, et al. The reliability, validity and responsiveness of the International Restless Legs Syndrome Study Group rating scale and subscales in a clinical-trial setting. *Sleep Med.* 2006;7(4):340–349.
26. van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab.* 2011;25(4):671–680.
27. Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol.* 2004;89–90(1–5):611–614.
28. Fleiss JL. *Statistical Methods for Rates and Proportions.* 2nd ed. New York, NY: John Wiley & Sons; 1981.
29. Kelsey JL, Whittemore AS, Evans AS, et al. *Methods in Observational Epidemiology.* Oxford, United Kingdom: Oxford University Press; 1996.
30. Wrzosek M, Lukaszkiewicz J, Wrzosek M, et al. Vitamin D and the central nervous system. *Pharmacol Rep.* 2013;65(2):271–278.

31. Ibia M, Sawadab H, Nakanishia M, et al. Protective effects of 1,25-(OH)2D3 against the neurotoxicity of glutamate and reactive oxygen species in mesencephalic culture. *Neuropharmacology*. 2001;40:761–771.
32. Shinpo K, Kikuchi S, Sasaki H, Moriwaka F, Tashiro K. Effect of 1,25-dihydroxyvitamin D(3) on cultured mesencephalic dopaminergic neurons to the combined toxicity caused by L-buthionine sulfoximine and 1-methyl-4-phenylpyridine. *J Neurosci Res*. 2000;62(3):374–382.
33. Nakamura K, Wang W, Kang UJ. The role of glutathione in dopaminergic neuronal survival. *J Neurochem*. 1997;69(5):1850–1858.
34. Evatt ML, Delong MR, Khazai N, Rosen A, Triche S, Tangpricha V. Prevalence of vitamin d insufficiency in patients with Parkinson disease and Alzheimer disease. *Arch Neurol*. 2008;65(10):1348–1352.
35. Newmark HL, Newmark J. Vitamin D and Parkinson's disease—a hypothesis. *Mov Disord*. 2007;22(4):461–468.
36. Trinko JR, Land BB, Solecki WB, et al. Vitamin D3: a role in dopamine circuit regulation, diet-induced obesity, and drug consumption. *eNeuro*. 2016;3(2).
37. Wali S, Shukr A, Boudal A, Alsaiari A, Krayem A. The effect of vitamin D supplements on the severity of restless legs syndrome. *Sleep Breath*. 2015;19(2):579–583.
38. Prentice A, Schoenmakers I, Jones KS, Jarjou LM, Goldberg GR. Vitamin D deficiency and its health consequences in Africa. *Clin Rev Bone Miner Metab*. 2009;7:94–106.
39. Alsuwadia AO, Farag YM, Al Sayyari AA, et al. Prevalence of vitamin D deficiency in Saudi adults. *Saudi Med J*. 2013;34(8):814–818.
40. Phillips B, Young T, Finn L, Asher K, Hening WA, Purvis C. Epidemiology of restless legs symptoms in adults. *Arch Intern Med*. 2000;160(14):2137–2141.
41. Aukerman MM, Aukerman D, Bayard M, Tudiver F, Thorp L, Bailey B. Exercise and restless legs syndrome: a randomized controlled trial. *J Am Board Fam Med*. 2006;19(5):487–493.
42. Celle S, Roche F, Kerleroux J, et al. Prevalence and clinical correlates of restless legs syndrome in an elderly French population: the synapse study. *J Gerontol A Biol Sci Med Sci*. 2010;65(2):167–173.
43. Winkelman JW, Redline S, Baldwin CM, Resnick HE, Newman AB, Gottlieb DJ. Polysomnographic and health-related quality of life correlates of restless legs syndrome in the Sleep Heart Health Study. *Sleep*. 2009;32(6):772–778.
44. Gao X, Schwarzschild MA, Wang H, Ascherio A. Obesity and restless legs syndrome in men and women. *Neurology*. 2009;72(14):1255–1261.
45. Ohayon MM, Roth T. Prevalence of restless legs syndrome and periodic limb movement disorder in the general population. *J Psychosom Res*. 2002;53(1):547–554.
46. Kim J, Choi C, Shin K, et al. Prevalence of restless legs syndrome and associated factors in the Korean adult population: the Korean Health and Genome Study. *Psychiatry Clin Neurosci*. 2005;59(3):350–353.
47. Trenkwalder C, Allen R, Högl B, Paulus W, Winkelmann J. Restless legs syndrome associated with major diseases: a systematic review and new concept. *Neurology*. 2016;86(14):1336–1343.

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