Suggestive evidence of erythropoietin level abnormality in patients with sporadic and familial cases of the restless legs syndrome

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Abstract

BACKGROUND: The restless legs syndrome (RLS) is divided into two forms: idiopathic and secondary. About half the cases of the former are found to have a positive family history. The lack of objective and quantitative parameters in familial RLS also represents a drawback for genetic studies. We tried to find a feature distinguishing the sporadic from the familial forms of the RLS.
METHODS: RLS patients were examined for clinical picture and laboratory markers including erythropoietin levels. Patients with a priori known causes of secondary RLS, were excluded. All biochemical and hematological parameters were standardized for sex and age groups relative to the population mean and standard deviation.
RESULTS: In our set of 311 patients (65.3% women, mean age 54.6 years, SD 14.7 years) 96 reported positive family history (64.6% women, mean age 53.1 years, SD 15.8 years). We found a significantly lower age at the onset of RLS symptoms in familial cases (mean 29.3 vs. 44.0, Z 5.9, $p<0.0001$), and, in sporadic cases, a significantly lower absolute count of red blood cells (Z –2.02, $p=0.043$ respectively). Erythropoietin levels in the familial cases were significantly lower than in the reference population (median –2.26 SDs from the mean). None of the other parameters were significantly different between the groups.
CONCLUSIONS: Our results confirm previously published lower age at symptom onset in familial RLS and provide the first evidence of lower erythropoietin levels in RLS patients. These observations might help to identify the specific phenotype for genetic association studies.

ORIGINAL ARTICLE

INTRODUCTION

The restless legs syndrome (RLS) is a sensorimotor neurological disorder characterized by an urge to move legs, usually accompanied by unpleasant sensation in the affected extremities. This disease is currently classified among sleep related movement disorders. The symptoms of RLS appear during physical or mental inactivity and are typically improved by movement, at least as long as the activity lasts. These symptoms also display circadian rhythm, with a maximum in the evening and the beginning of the night. In its fully developed form, RLS significantly interferes with normal sleep initiation and maintenance due to the urge to move legs in the night hours [1]. In respect of the 10–12% prevalence of RLS in western countries, it is the most common cause of organic insomnia, reducing significantly the quality of life [4,11]. About half the cases have a positive family history [2].

RLS is diagnosed according to its clinical mandatory criteria. Additional clinical features are sleep disturbance and its consequences, involuntary movements – periodic leg movements in sleep and involuntary limb movements while awake and at rest, positive family history and good therapeutic response to dopaminergic agents. RLS is further divided into two forms: idiopathic and secondary, the latter occurring mainly in sideropenic states, during pregnancy, in end-stage renal disease and metabolic neuropathies [1].

In this study, we tried to find a feature distinguishing the sporadic from the familial forms of the RLS in laboratory biochemical and haematological tests of peripheral blood samples using, among others, erythropoietin levels. Erythropoietin (EPO) is produced primarily by the kidneys and promotes the growth, differentiation and survival of erythroid progenitors apart from supporting the viability of erythroid cells during maturation [14]. The EPO receptor is a member of the cytokine receptor family as found in the hippocampus, temporal cortex, amygdala and cerebellum of human, monkey and rodent brains [7,9]. The observation that EPO is produced by astrocytes, and binds to EPO receptors on adjacent neurons, suggests that EPO has a paracrine action on neurons, independent of its endocrine role in the erythropoietic system [5,13]. Systemically administered EPO crosses the blood–brain barrier and is present in human cerebrospinal fluid.

The administration of human recombinant EPO (rHuEPO) has become an important clinical therapeutic adjunct to the treatment of patients with chronic anaemia or renal dysfunction. The majority of patients receiving this drug respond satisfactorily with a significant rise in hemoglobin concentrations, and with improved health-related quality of life.

PATIENTS AND METHODS

We have retrospectively analyzed the records of RLS patients treated at our center for sleep disorders. Laboratory tests of peripheral blood were designed to establish: complete blood count, EPO level (radioimmunoassay, manually), ferritin (chemiluminiscence imunoanalysis,

Table 1. The results of all tested biochemical parameters, standardized using population mean and standard deviation. The individual values of familial and sporadic cases were compared using Mann-Whitney test (M-W test).

	Familial RLS				Sporadic RLS				M-W test
	Mean	SD	No.	Median	Mean	SD	No.	Median	p-value
Age at onset of RLS symptoms	29.25	16.75	80	30.00	44.03	16.28	148	45.00	**3.08E–09**
IRLSSGRS	21.88	7.97	72	22.00	21.15	7.30	89	20.00	0.4758
Erythropoietin	–1.69	1.86	63	**–2.26**	–1.50	2.20	97	–1.92	0.3703
Soluble transferrin receptor	0.47	2.13	37	–0.08	–0.18	1.55	72	–0.02	0.4615
Transferin	0.99	1.29	65	0.80	1.13	1.42	113	0.92	0.5447
Ferritin	–0.37	4.79	67	–1.30	1.45	12.70	116	–1.02	0.1495
Total Iron	–0.03	1.17	68	–0.08	–0.15	1.04	116	–0.13	0.5141
Iron binding capacity	–0.14	1.97	64	–0.43	–0.21	2.17	96	–0.14	0.7198
Red blood cells	–0.04	1.09	60	0.02	–0.32	0.94	111	–0.30	**0.0431**
Total hemoglobin	–0.52	1.17	60	–0.38	–0.76	1.09	111	–0.64	0.0652
Hematocrite	–0.34	1.08	60	–0.20	–0.55	1.02	111	–0.38	0.1715
Erytrocyte volume	–0.41	1.21	60	–0.16	–0.26	1.01	111	–0.12	0.5364
Urea	0.47	1.32	35	0.30	0.45	1.61	62	0.02	0.5963
Creatinine	0.09	0.68	35	0.20	0.55	1.26	62	0.34	0.1667
Vitamin B12	–1.16	2.68	43	–1.72	–1.21	1.89	54	–1.56	0.3303
Folates	–0.80	1.13	55	–1.06	–0.54	1.47	92	–0.80	0.4773

ADVIA:Centaur, Bayer), transferrin (turbidimetry), total serum iron (photometry using ferrozin) and soluble transferrin receptor levels (imunoturbidimetry), using an automatic Modular (Roche) analyzer. The samples were drawn at early morning in fasting patients. Clinically, RLS severity was measured using the standard 40-point rating scale; family history and age at the onset of RLS symptoms were other parameters under study. All these examinations were carried out as routine procedures.

Patients with a priori known causes of secondary RLS were excluded (such as pregnant women or patients with end-stage renal disease). All biochemical and hematological parameters were standardized for sex and age groups relative to the population mean and standard deviation. The difference between the population mean and actual value was divided by the population standard deviation, higher values than the mean were given positive sign, lower were assigned a negative. Since most parameters were not normally distributed, we have used a non-parametric Man-Whitney test for group comparisons. The non-parametric Spearman test was employed for correlation analysis, the resulting p-values were corrected for multiple testing using Bonferroni's algorithm at the α=0.05 significance level.

RESULTS

Our cohort consisted of 311 patients (65.3% women, mean age 54.6 years, SD 14.7 years). 96 reported positive family history (64.6% women, mean age 53.1 years, SD 15.8 years). In 228 patients, data on the age of onset RLS symptoms were available. It was found significantly lower in the familial than in the sporadic forms (mean 29.3 vs. 44.0, Z 5.9, p<0.0001). The results of laboratory tests were available in 183. A significantly lower absolute red blood cell count was seen in the sporadic cases. (Z –2.02, p=0.043). Table 1 summarizes the clinical, biochemical and haematological results. EPO levels below 2 SD of the population mean were observed in 51% of the familial RLS subgroup and in 46% of the sporadic RLS patients. There was, however, no significant difference between the two. 87% of our RLS patients had EPO levels below the reference population mean. There was no significant age- or sex-related difference in any of the analysed parameters, suggesting these subgroups were properly sex and age matched.

The clinical parameters were tested for correlation with the biochemical ones, but no significant results were found after correction for multiple testing, except for the correlation of age and urea levels, which we regard as a physiological sign of aging, see Table 2.

DISCUSSION

Confirming previously reported lower age at symptom onset in familial RLS [12,16], our results provide the first evidence of lower EPO levels in RLS patients generally, more prominent in familial cases (even though the difference between sporadic a familiar cases was not significant). These observations might help establish

Table 2. Summary of correlation of selected parameters, non-parametric Spearman correlation was computed. (N – number of observations, R – Spearman's correlation coefficient). The p-values by-passing the correction for multiple comparisons are marked with **bold**.

	Age			Age at onset of RLS			IRLSSGRS		
	N	R	p-value	N	R	p-value	N	R	p-value
Erythropoietin	160	0.12164	0.07375	133	0.01181	0.88065	113	–0.15130	0.10968
Soluble transferrin receptor	109	–0.06169	0.52399	81	0.05753	0.60997	73	0.01403	0.90620
Transferin	178	–0.21206	0.00449	138	–0.15231	0.07452	112	0.08134	0.39392
Ferritin	183	0.26864	0.00024	143	0.13504	0.10783	116	0.06939	0.45920
Total Iron	184	–0.08533	0.24948	143	–0.11513	0.17094	116	–0.03399	0.71718
Iron binding capacity	160	–0.17367	0.02807	125	–0.08096	0.36941	103	0.01052	0.91599
Red blood cells	171	–0.16433	0.03173	137	–0.14757	0.08527	104	0.11601	0.24089
Total hemoglobin	171	–0.16285	0.03333	137	–0.13637	0.11206	104	–0.04349	0.66112
Hematocrite	171	–0.07926	0.30281	137	–0.03217	0.70902	104	0.03252	0.74313
Erytrocyte volume	171	0.12920	0.09213	137	0.19827	0.02020	104	–0.08077	0.41502
Urea	97	0.61672	**1.75E–11**	78	0.31031	0.00569	56	0.27649	0.03913
Creatinine	97	0.28231	0.00508	78	0.01454	0.89947	56	0.10215	0.45377
Vitamin B12	97	–0.04174	0.68479	80	0.09012	0.42664	62	–0.17003	0.18644
Folates	147	0.05747	0.48929	118	0.06569	0.47975	85	–0.00119	0.99135

the specific phenotype for genetic association studies. The possible explanation is that, the low brain EPO levels may account for a worse sleep quality, as shown in rats. Moreover, as EPO intraventricularly given to rats is known to prolong sleep, it might also be involved in sleep-wake cycle regulation [10].

A circadian rhythm of EPO plasma levels was shown in healthy volunteers, with peak after midnight and nadir in the early afternoon [6]. This oscillation of EPO levels might be a predisposing factor for the development of PLMS, conceivably associated with typical circadian rhythm of RLS symptoms.

Sleep, in patients with end-stage renal disease as determined by subjective assessment, improves after rHuEPO treatment [17]. Furthermore, rHuEPO given to patients with periodic limb movements in sleep (PLMS) reduces both sleep fragmentation and the total number of PLMS-induced arousals, thereby improving the quality of sleep and daytime alertness [3].

The primary role of EPO in the brain corresponds to the neurotrophic factor – enhancing the survival of neurons in oxidative stress [8], preventing neurodegeneration, and regulating the growth and maturation of neuronal structures, but also neurovascular protection and cerebral angiogenesis [15]. Therefore, its lower levels might reflect, to some extent, signs of neurodegeneration in specific areas of the brain.

To corroborate our hypotheses, further research is necessary. We also believe that estimation of EPO levels in the cerebrospinal fluid and its correlation to the plasma levels and RLS-related clinical parameters will clarify this possible etiopathogenetic aspect of RLS.

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