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Evaluation of Periodic Limb Movements in Sleep and Iron Status in Children with Autism

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Abstract

Background—Recent data suggest that both disordered sleep and low serum iron occur more frequently in children with autism compared to children with typical development. Iron deficiency has been linked to specific sleep disorders.

Basic Procedures—The goal of the current study was to evaluate periodic limb movements in sleep and iron status in a group of children with autism compared with typically developing children and children with non-autism developmental delay to determine if iron status was observed to correlate with polysomnographic measures of latency and continuity and periodic limb movements in sleep. 102 children (68 autism, 18 typically developing, 16 developmental delay) aged 2 to 7 years underwent a one-night modified polysomnography study and phlebotomy at the National Institutes of Health to measure serum markers of iron status (ferritin, iron, transferrin, percent transferrin saturation).

Main Findings—No serum iron marker was associated with periodic limb movements of sleep or any other sleep parameter, and this did not differ among the diagnostic groups. No significant differences among groups were observed on serum iron markers or most polysomnogram parameters: periodic limb movements in sleep, periodic limb movements index, wake after sleep onset, or sleep efficiency. Children in the autism group had significantly less total sleep time. Serum ferritin was uniformly low across groups.

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Principal Conclusions—This study found no evidence that serum ferritin is associated with polysomnogram measures of latency or sleep continuity or that young children with autism are at increased risk for higher periodic limb movements index compared with typically developing and developmental delay peers.

Clinical Trials Registration—Clinical and Immunological Investigations of Subtypes of Autism, NCT00298246: <http://www.clinicaltrials.gov/show/NCT00298246>. Minocycline to Treat Childhood Regressive Autism, NCT00409747: <http://clinicaltrials.gov/show/NCT00409747>.

Keywords

autism; ferritin; iron; periodic leg movements

Introduction

Iron is well-known to be important to cognitive, behavioral and motor development.¹ Serum ferritin level is often chosen as a marker of iron status because it is the storage protein for iron and the first value to decrease in the setting of deficiency.² While serum ferritin level is the most specific measure of iron status, it is also an acute phase reactant and its level may be affected by inflammation making serum iron, transferrin and total iron binding capacity additionally useful in measuring iron status. According to the World Health Organization, iron deficiency is the most common micronutrient deficiency in the world, prevalent in both developing and industrialized countries.³

In addition to its prevalence in the general population, it has been proposed that iron deficiency is even more common in childhood developmental disorders such as attention deficit hyperactivity disorder, bipolar disorder, tic disorder, and autism spectrum disorder (ASD).⁴ Reported rates of iron deficiency in ASD range from 8.3% to 52%.^{1,5–7} The lowest estimate was found in a small sample of 12 toddlers,⁵ and the highest estimate was from a modest sample of 23 children across the broad age range of 19 months to 8 years, 5 months.⁶ However, the latter study defined iron deficiency generally at <12µg/L rather than using the established Centers for Disease Control and Prevention age-dependent standards (10µg/L for children under 6 years and 12µg/L for children 6 years or older).⁸ Using these standards, two Turkish studies estimated the rate of iron deficiency in ASD to be about 32%.^{1,7}

While these studies suggest that relative to normative data iron deficiency is common in ASD, other studies have focused on comparing serum ferritin levels between ASD and typically developing cohorts. One study found no significant difference in mean serum ferritin among 69 patients with ASD, 14 patients with developmental delay, and 37 typically developing children.⁹ In contrast, Youssef et al.¹⁰ reported significantly lower mean serum ferritin in a sample of 53 children with ASD than in 53 healthy controls. While it appears that iron deficiency as measured by serum ferritin levels may be common in ASD patients, whether they differ in this respect from the general population remains an open question.

Children with ASD are also characterized by high prevalence of sleep disorders.¹¹ It is estimated that 44–83% of children with ASD experience sleep problems.¹² Specifically, several studies have found higher than expected rates of periodic limb movement in sleep

(PLMS) in children with ASD compared to healthy controls.^{10,13,14} A pair of studies report increased prevalence of restless leg syndrome in children with ASD compared to typically developing peers.^{15,16}

Dosman et al. hypothesized that the poor sleep often found in children with autism and the high prevalence of iron deficiency might be related.¹⁷ In a pilot study, they investigated the effect of oral iron supplementation on restless sleep in children with ASD and found that this treatment improved both serum ferritin levels and restless sleep, as measured by parent report.¹⁷ In this study, we evaluated 102 children with polysomnography (PSG) to explore the relationship between PLMS and serum iron measurements in children with ASD compared to children with developmental delay and typically developing children.

Methods

Patient Population

Participants were drawn from two National Institutes of Health IRB-approved studies of ASD at the National Institute of Mental Health (06-M-0102, 07-M-0024). Recruitment targeted children suspected of having an ASD diagnosis, as well as control and comparison children for whom social-communication symptoms were not a concern. Children between the ages of 1 and 6 at the initial evaluation were eligible, and enrolled participants were evaluated at 6-month or 1-year intervals, depending on the age of the child. Preexisting sleep concerns or iron deficiency were not requirements for study inclusion or specified in recruitment/eligibility materials.

Following diagnostic evaluation, participants were designated as DSM-IV-TR defined Autistic Disorder, non-ASD developmental disorder, or typically developing. The diagnostic evaluation consisted of the Autism Diagnostic Observation Schedule (ADOS),¹⁸ a clinician-administered structured play interview designed to elicit behaviors relevant to a diagnosis of autism, and the Autism Diagnostic Interview-Revised (ADI-R),¹⁹ a semi-structured parent interview concerning all domains of impairment in autism spectrum disorders. The Social Communication Questionnaire was substituted for the ADI-R at the initial assessment for some typically developing children.²⁰ Research categorization of autism was based on these measures and clinical judgment. ASD was not a presenting concern and was ruled out for all children in the typically developing and developmental delay groups.

The inclusion criteria for the current analyses were successful completion of an overnight electroencephalogram (EEG) recording with electro-oculogram, electrocardiogram, and surface chin and anterior tibialis electromyogram (EMG), and blood specimen collection. For participants in the longitudinal study (06-M-0102), data from the first visit that met these criteria were used. In the majority (n=109, 91%) of cases, this was the baseline visit. Out of the 188 subjects enrolled in the longitudinal study, data from 120 children (autism, n=78; typically developing, n=23; developmental delay, n=19) met the inclusion criteria.

Polysomnography

Continuous overnight modified Polysomnography (PSG) recordings and clinical readings were performed as previously described by Buckley et al.²¹ Children were admitted for a

continuous overnight recording that included a referential, 21-lead electroencephalogram montage, electro-oculogram, electrocardiogram, and surface electromyogram (chin, anterior tibialis). Respiratory parameters were not measured. Lights out approximated the child's actual bedtime. All recordings were videotaped and ended at a median wake up time of 07:00 for all groups.

The clinical readings were completed by the EEG section of the National Institute of Neurological Disorders, using National Institute of Neurological Disorders and Stroke electroencephalogram laboratory standards for sleep architecture. A second, blinded reading for sleep architecture and leg movements was done using Grass telefactor software (Grass Technologies, West Warwick, Rhode Island) by a different neurologist, board certified in neurology, neurophysiology, and sleep medicine (A.J.R.). Wake/sleep was subdivided into 30-second epochs and scored according to American Academy of Sleep Medicine guidelines.²² The following sleep variables are reported: total sleep time (TST; the entire sleep period minus the time spent in wakefulness after sleep onset), wakefulness after sleep onset (minutes), sleep efficiency index (total sleep time divided by time in bed $\times$ 100), periodic limb movements in sleep (PLMS), and periodic limb movement index (PLMI; PLMS per hour).

Laboratory testing

All participants underwent a baseline medical visit that included phlebotomy. Attempts were made to collect and analyze blood specimens during the same visit as the sleep study. When this was not possible, specimens were used if obtained within 28 days of the sleep study. All specimens were analyzed for serum ferritin. A subset of specimens were flash frozen on dry ice and stored in 0.1 cc eppendorf tubes at -80°C prior to lab processing. These frozen samples were analyzed for serum ferritin, iron, transferrin, and percent transferrin saturation.

In order to assess iron status, we used current recommendations from the World Health Organization. Children under 5 years of age with serum ferritin less than $12\mu\text{g/L}$ and children 5 years of age or older with serum ferritin less than $15\mu\text{g/L}$ were considered to have low ferritin, and thus be at a risk of iron deficiency.²³

Statistical Analysis

Categorical variables were compared between groups using chi-squared tests. Continuous variables were compared using general linear models (most commonly one-way ANOVA) with planned post-hoc tests for significant group effects. Pearson correlations were used to assess relationships between continuous variables. Significance was set at $p < .01$ to allow partial control for multiple comparisons. SAS/STAT Version 9.3 was used for all analyses.

Results

Serum Ferritin and Polysomnography

Of the 102 participants, the groups (68 autism, 18 typically developing, 16 developmental delay) were comparable with respect to age (52.9 ± 16.2 ; 48.5 ± 9.4 ; 47.8 ± 19.1 months for autism, typically developing, and developmental delay respectively) [$F(2, 99) = 1.04$, $p = .36$].

As expected, the autism group was predominately male (78%), compared to 75% in the developmental delay group and 61% in the typically developing group. Data were visually inspected for outliers; one participant in the developmental delay group had a serum ferritin of 200µg/L. Exclusion of this data point did not change the significance of the results, so this participant's data were included in the final analyses.

For 81% of the sample, phlebotomy occurred within 2 days of the sleep study; the maximum lag was 28 days (mean time: 3.9 ± 6.8 days). Table 1 shows the average serum ferritin and sleep parameter measurements for each diagnostic group. The mean serum ferritin level did not differ significantly among groups. Participants were classified as having low iron stores using World Health Organization guidelines, such that values of less than 12 µg/L for children under 5 years and 15 µg/L for children 5 years and older were considered low. These cutoffs described similar proportions in each of the diagnostic groups (autism: $n=11$, 16%, typically developing: $n=3$, 17%, developmental delay: $n=3$, 19%; $\chi^2=0.06$, $p=.97$). Of note, only six subjects (6%) in this study had ferritin levels above the commonly used clinical threshold for intervention of 50 µg/L. The serum ferritin results in this subgroup of 102 participants with a sleep study did not differ from those found in the larger study's full sample of 188 patients (data available upon request).

Controlling for age and sex, the diagnostic groups did not differ on any sleep parameter except for TST; the autism group had significantly lower TST than the typically developing group (Table 1). Overall, 33% (34/102) of the children had an abnormally elevated PLMI of five or more. This did not differ across diagnostic groups ($\chi^2=3.1$, $p=.21$ [35% autism, 44% developmental delay, 17% typically developing]).

Given that the groups did not differ on serum ferritin levels, and that ANCOVA tests showed no significant interaction between diagnosis and serum ferritin in predicting the measured sleep parameters (all $p>.05$), the three groups were combined for the correlational analyses. Figure 1 shows the correlations between serum ferritin and the PSG recording variables total PLMS, PLMI, wake after sleep onset, total sleep time, and sleep efficiency. Serum ferritin was not significantly correlated with any sleep parameter. Additional iron status markers (serum iron, transferrin, and percent transferrin saturation) were available for a subset of 60 children (65% of autism, 56% of typically developing, 38% of developmental delay). No significant correlations were observed between these additional markers and sleep parameters (see Figure 1).

Discussion and Limitations

This study assessed the relationship between observed measures of sleep duration and continuity using a modified PSG and serum iron status in children with autism compared to samples with developmental delay and typical development. Children with autism in this study were no more likely than the comparators to have decreased serum ferritin. Data analyzed from the 2003–2006 National Health and Nutrition Examination Survey found 5% of children ages 3–5 years to be iron deficient based on serum ferritin, erythrocyte protoporphyrin, and transferrin saturation.²⁴ Our observed rate of 17% of children with autism with low serum ferritin levels falls in the range of previously reported iron deficiency

rates for this group. Youseff et al. recently reported lower levels of serum ferritin in subjects with ASD (27 ng/ml) versus healthy controls (86 ng/ml) in a retrospective case/control report of 53 children with ASD with a mean age of 8 years.¹⁰ The higher values in their control population are contrasted by our findings, which revealed that children with typical development were as likely as those with autism to have a serum ferritin level below 50 ng/ml, the level often used in clinical settings as the threshold for iron supplementation.

This is the largest study to measure PLMI in a cohort of children with autism and we found no difference in PLMI between our diagnostic groups, though there was a relatively high rate of abnormally high PLMI across groups (33%). A PLMI of at least five has been reported in 5–12% of community-based pediatric samples and is considered clinically relevant.^{25,26} This is an important finding for the evaluation and treatment of sleep disorders in children with ASD who may not be able to describe clinical symptoms of sleep disorders.

In addition to being important to many enzymatic reactions, iron acts as a regulator of dopamine signaling in the brain. Animal studies have shown that iron deficiency decreases D1 receptor, D2 receptor and dopamine transporter density in the striatum.^{27,28} Furthermore, iron-deficient rats have been shown to have increased extracellular dopamine in the striatum.²⁹ Altered dopamine signaling is thought to be integral to the occurrence of restless leg syndrome (RLS), as dopamine-antagonists precipitate symptoms indistinguishable from RLS, and pharmacological enhancement of dopamine signaling reliably reduces RLS symptoms.^{30,31} Similarly, dopamine agonists have been shown to reduce PLMS in a double-blind placebo-controlled trial.³²

PLMS are characterized by periodic episodes of involuntary, stereotyped limb movements during sleep and are generated by repetitive contraction of the anterior tibialis.³³ Periodic limb movement disorder (PLMD) occurs when patients with PLMS either have insomnia or disturbed sleep that cannot be explained by other sleep disorders.³³ PLMD is distinct from RLS, a sensorimotor disorder characterized by an uncomfortable sensation in the legs that occurs during the waking state, usually in the evening hours, that gives the patient an uncontrollable impulse to move his legs.³⁰ Although PLMS are commonly found in RLS and patients with RLS may have PLMD, most patients with PLMD will not have RLS.³⁰

It has long been recognized that iron deficiency is a common occurrence in RLS and both RLS and PLMS have been associated with this condition.³⁴ Brain imaging studies of patients with RLS have shown local changes in brain iron levels, particularly in the substantia nigra and striatum, associated with RLS.³⁰ For example, there is markedly decreased iron in the substantia nigra of patients with early onset RLS and the degree of iron deficiency correlates with symptom severity.³⁵ These local changes in iron concentration in the brain are important. Low iron is common among RLS patients but only about 30% of patients with iron deficiency have RLS, suggesting that RLS may be determined more by how well iron is retained and utilized by specific brain regions than by systemic iron levels.³⁰ Further support for a difference between central and peripheral iron regulation in RLS comes from a study comparing iron status between patients with RLS and healthy volunteers that found reduced cerebral spinal fluid ferritin and elevated transferrin in RLS patients despite finding no difference in serum iron markers.³⁶ Autopsy studies provide

further evidence of altered central nervous system iron regulation in RLS patients reporting decreased iron in the substantia nigra as well as a redistribution of L-ferritin from oligodendrocytes to astrocytes and decreased transferrin receptor density.³⁵ Furthermore, iron supplementation has been effective in alleviating symptoms of RLS, even in patients who had normal serum iron levels before treatment.³⁰

Similar to RLS, there is evidence for an association between iron deficiency and PLMS. Genome wide association studies have identified a genetic variant in the intron of BTBD9 that is associated specifically with susceptibility to PLMD and also correlated with iron deficiency³⁷. Furthermore, Bakkola et al. reports decreased serum ferritin levels in children with increased PLMS and Simakajornboon shows both low serum ferritin and low serum iron in children with PLMS.^{38,39} In the latter study, severity of symptoms correlated with serum iron levels, even when they were above the threshold for iron deficiency anemia, again suggesting that serum iron levels may not correlate *directly* with iron levels in specific brain regions, which may be more sensitive to changes in iron status.³⁹ Moreover iron supplementation improved both PLM Index and PLMS symptoms in these children.

The precise contribution of iron to the pathophysiology of RLS and PLMD is not well understood. However, it is clear that supplemental iron can provide improvement in symptoms suggesting a role for iron in the pathophysiology of these disorders and perhaps an approach to alleviating insomnia in sufferers.^{39,40} A study of preschoolers who had previously been iron deficient as infants found that they were more likely to have increased leg movements during active sleep throughout the day compared to peers without a history of iron deficiency as an infant. No differences in leg movements were noted during nighttime.⁴¹ In contrast, an association between higher serum ferritin levels and PLMI has been observed in children with sickle cell disease.⁴² While the mechanism is unclear the authors of that study suggested that this might merely be a surrogate for more frequent transfusions. In the present study, we did not find differences in serum ferritin levels among the diagnostic groups, nor a correlation between PLMI and iron status. Both imaging and autopsy studies have shown decreased iron levels in the brains of adult RLS patients and other studies have shown that cerebral spinal fluid ferritin is decreased in adult RLS patients compared with controls.^{30,43,44} These investigations suggest that any associations between RLS/PLMD and iron status may lie within the central nervous system and are thus indiscernible from serum analyses alone. Peirano et al. found increased PLMI during non-rapid eye movement sleep in children who had previously been iron deficient during infancy, suggesting that sleep disturbances due to iron deficiency may in fact be established early in life.⁴⁵

Both low serum iron levels and sleep disorder rates higher than average have been reported in children with autism and at least one treatment study has attempted to connect these phenomena. Dosman et al. treated young children with ASD, low serum ferritin, and restless sleep with supplemental iron at 6 mg elemental iron/kg/day in an 8-week open-label study and reported that twenty-nine percent of 24 participants showed an improvement in sleep quality, as measured by parent report of restlessness.¹⁷ Although we did not find increased rates of either periodic leg movements or low levels of serum ferritin in children with autism as compared to controls with non-ASD developmental delay or typical development, it is

possible that children with ASD may be less able to communicate symptoms of PLMD/RLS to parents and that the functional role of iron may be different in special populations.

There are several important limitations to our study. Picchiatti et al. reported that the number of PLMS experienced night to night by children varies,⁴⁶ so it is important to consider that the one-night sleep study represents only a selection of the child's sleep behavior. Our average PLMI for all groups was higher than that described by Montgomery-Downs et al. This may be due to the fact that we performed a modified sleep study, in which respiratory parameters were not measured.⁴⁷ Current scoring guidelines indicate that leg movements following certain respiratory events should not be scored.⁴⁸ Therefore, our PLMI may be higher than expected if respiratory parameters had been measured. Finally, due to small numbers of subjects with serum ferritin >50 µg/L we were not able to do analyses using higher levels as a cut-off and thus we were unable to comment on the relationship between PLMI and serum ferritin levels across a wider range of values.

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Figure 1.

No significant Pearson correlations observed between serum iron markers and PSG parameters

Note: PLMS=total period leg movements of sleep; PLM Index=periodic leg movement index; WASO=wake after sleep onset. Additional iron markers available for a subset of the sample, n=60 (65% of AUT, 56% of TD, 38% of DD). All Pearson correlations $r < .25$ and $p > .01$.

Table 1

Serum ferritin and PSG parameter descriptives

	Autism	Typical	Developmental Delay	Full Sample	F (p) [#]
<i>n</i>	68	18	16	102	--
Serum Ferritin (µg/L)	23.74 ± 13.48	19.89 ± 9.04	36.25 ± 46.57	25.02 ± 21.96	2.80 (.07)
PLMS	40.04 ± 54.57	22.61 ± 32.95	40.38 ± 30.21	37.02 ± 48.36	0.97 (.38)
PLMI Index	5.19 ± 7.04	2.79 ± 3.86	4.59 ± 3.42	4.67 ± 6.16	1.09 (.34)
Wake After Sleep Onset (min)	96.9 ± 122.12	70.44 ± 54.09	50.27 ± 29.22	84.92 ± 104.1	1.53 (.22)
Total Sleep Time (min)	443.58 ± 108.41	505.61 ± 74.25	517.41 ± 58	466.1 ± 101.3	5.56 (.005)*
Sleep Efficiency (%)	77.36 ± 16.19	83.27 ± 7.95	84.23 ± 6.13	79.48 ± 14.11	2.38 (.10)

PSG = polysomnography, PLMS = total period leg movements of sleep, PLMI = periodic leg movement index, WASO = wake after sleep onset, TST = total sleep time, SE = sleep efficiency (%).

[#] ANOVA testing difference between diagnostic groups, *df*(2,99).

* Tukey HSD: Autism vs. Typical ($p < .01$); Autism vs. Developmental Delay ($p < .01$); Typical vs. Developmental Delay (n.s.).