Pediatric RESEARCH

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CLINICAL RESEARCH ARTICLE

Hypoferritinemia and iron deficiency in youth with pediatric acute-onset neuropsychiatric syndrome

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BACKGROUND: Pediatric acute-onset neuropsychiatric syndrome (PANS) is an abrupt debilitating psychiatric illness. We anecdotally observed hypoferritinemia and iron deficiency in a subset of patients with PANS, prompting this study.
METHODS: In this IRB-approved prospective cohort study, we included patients seen at the Stanford PANS Clinic who met study criteria. The prevalence of hypoferritinemia (using cut-offs of 7 ng/ml in children ≤ 15 years and 18 ng/ml in adolescents > 15 years) and iron deficiency was estimated. Differences in patients with and without hypoferritinemia during PANS flare were explored.
RESULTS: Seventy-nine subjects (mean age of PANS onset of 8.7 years) met study criteria. Hypoferritinemia was observed in 27% and three quarters occurred during a PANS flare. Compared to patients without hypoferritinemia during PANS flare, patients with hypoferritinemia had worse global impairment, more comorbid inflammatory diseases, and exhibited a chronic course of PANS illness. The estimated prevalence of iron deficiency was 3–8% in the PANS cohort, 1.4–2.0-fold higher than in the age- and sex-matched U.S. population. More stringent ferritin level cut-offs than the comparison CDC dataset were used.
CONCLUSION: Hypoferritinemia and iron deficiency appear to be more common in PANS patients. More research is needed to confirm and understand this association.

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IMPACT:

- Our study suggests hypoferritinemia and iron deficiency are more common in patients with pediatric acute-onset neuropsychiatric syndrome (PANS) than in the sex- and age-matched US population.
- Hypoferritinemia was commonly observed during a disease flare but not associated with dietary or demographic factors. In patients with PANS and iron deficiency, clinicians should consider possibility of inflammation as the cause especially if iron deficiency cannot be explained by diet and blood loss.
- Future research should include larger cohorts to corroborate our study findings and consider examining the iron dynamics on MRI brain imaging in order to better understand the pathophysiology of PANS.

INTRODUCTION

Pediatric acute-onset neuropsychiatric syndrome (PANS) is a psychiatric disorder characterized by an abrupt onset of obsessive compulsive symptoms and/or severe eating restrictions along with at least two other severely debilitating cognitive, behavioral, or neurological symptoms.[1,2] PANS has been reported and characterized by different centers around the world.[3–8] A recent MRI study from our institution showed microstructural changes throughout the brain as indicated by increased median diffusivity in patients with PANS compared to controls.[9] The deep gray matter (e.g., the thalamus, basal ganglia, and amygdala) demonstrated the most profound increases in diffusivity consistent with the cardinal clinical symptoms. These findings go along with previous imaging studies and suggest inflammation in these regions.[10,11] A growing body of evidence supports the role of inflammation in PANS.[10–20]

Iron deficiency remains the most common childhood nutritional deficiency in the United States.[21,22] Low dietary iron intake, poor absorption of iron, chronic diseases, and chronic blood loss are causes of iron deficiency and iron deficiency anemia.[23,24] Children with anxiety, depression, tic disorders, attention-deficit/hyperactivity disorder, febrile seizures, breath holding spells, and fibromyalgia are more likely iron deficient.[25–34] The medical literature supports the role of iron deficiency contributing to the development of neurologic issues, through a negative effect on neurodevelopment in children.[35–37] Iron deficiency occurring in pregnant mother and fetus may even impact prenatal neurodevelopment, and the neurological impact may last even after iron repletion.[38–40]

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The total body iron stores are estimated by measuring serum ferritin levels. Ferritin varies less than serum iron and is more sensitive to iron store depletion.[41] It is also a well-known acute phase reactant, and nonspecific elevations occur in infections, inflammation, hematochromatosis, and malignancy.[41–43] During infection, increased ferritin levels play a role in host defense against bacterial growth and may also modulate inflammation.[43] While evidence suggests the association between PANS, inflammation, and streptococcal infections,[8–11,44,45] patients seen in our PANS clinic appeared to have hypoferritinemia (low serum ferritin levels) on laboratory workup which is usually ordered during their PANS flares (abrupt worsening of neuropsychiatric symptoms) or during the phase following flares which are characterized by fatigue and cognitive difficulties.[46] This paradoxical observation in PANS, particularly during flares, remains unexplained.

To confirm our clinical observation, we performed this study with the objective to examine the prevalence of iron deficiency in patients with PANS, and compare it to a national cohort from the Centers for Disease Control and Prevention (CDC).[22] We examined the likelihood of hypoferritinemia coinciding with a PANS flare by comparing the groups of patients with and without hypoferritinemia during a PANS flare with regard to demographics, diets, and clinical characteristics. We also explored the potential association between hypoferritinemia and neuropsychiatric symptoms over time.

METHODS

Study design, source of data, and participants

The Stanford Institutional Review Board approved this study (IRB#26922). Consent was obtained from parents of minors and adult patients, and assent obtained from patients aged 7–17 years. Subjects were recruited from the Stanford PANS/Immune Behavioral Health Clinic. Questionnaires were given to patients/chief caregivers prior to each clinic visit inquiring about medical conditions, psychiatric symptoms, and treatments. In addition, patients completed a demographic and dietary questionnaire during the study period (September 3, 2012 to March 30, 2018). Laboratory work to evaluate iron stores and anemia was requested on all new patients to our clinic during the above study period. However, we were unable to obtain these studies in some patients for the following reasons: patient unwilling or too anxious to do a laboratory draw, or need to prioritize other laboratory tests. To supplement data from the patient questionnaire, we also reviewed electronic medical records for psychiatric symptoms at clinic entry and for history of eating restriction.

For this study, we included consecutive patients who met strict PANS criteria.[1,2] We excluded patients who declined research, had <3 clinic visits, and never had ferritin levels checked. To avoid potential referral bias, we restricted our study cohort to the community patients living within 90 miles from our clinic and the seven surrounding counties.

Definitions of variables

The first serum ferritin test result available after PANS onset was included for analysis. Hypoferritinemia was defined by serum ferritin levels <7 ng/ml in children and youth aged ≤ 15 years, and < 18 ng/ml in adolescents aged > 15 years.[47] A more stringent criterion was used in our study, compared to the WHO (< 15 ng/ml) and CDC reports (< 9 ng/ml), because some publications report serum ferritin levels can be as low as 6–7 ng/ml in healthy people.[48,49] This strategy helps avoid overestimation of iron deficiency in our sample.

We defined iron deficiency by the presence of hypoferritinemia and concurrently either of the following: (a) a low transferrin saturation, or (b) a high total iron binding capacity (TIBC). Iron deficiency anemia was diagnosed when a patient had iron deficiency and a concurrent low hemoglobin per laboratory refernece range.

Eating restriction was diagnosed if the following two criteria were met: (a) the chart mentioned restricted food intake (reduced appetite, restriction due to fear of choking, etc.), distress or resistance with food, and (b) this new eating restriction resulted in weight loss of more than two pounds documented in the chart.

A PANS flare was defined by neuropsychiatric deterioration reported by parents, teachers, and patients together with the worsening of psychometric test scores on the questionnaire and confirmed by clinical interview.

Statistical analysis

We calculated the proportion of patients that had hypoferritinemia and iron deficiency with and without anemia. Since adolescent females are at an elevated risk for iron deficiency,[22,50] we checked the robustness of our result by excluding females over 12 years old at the time of ferritin tests, in order to minimize the confounding effect by periodic menstrual blood loss. For the fact that some patients did not have ferritin or iron workup, we performed a series of sensitivity analyses in order to ascertain the range of uncertainty of iron deficiency in patients with PANS. We made three separate assumptions for the risk of iron deficiency in patients who did not have the full iron workup (untested patients). We first assumed untested patients came from a random sample of our PANS population; second, we assumed untested patients had the same risk of iron deficiency as the general population matched for age and sex; last, we assumed none of our untested patients were iron deficient. Under each assumption, we recalculated the prevalence rate, and compared it to the age- and sex-matched prevalence estimates of iron deficiency in the general population.[22] Odds ratio and 95% confidence interval were reported.

Prior studies have suggested hypoferritinemia can coincide with psychiatric symptoms in other disorders;[25–31] thus, we compared characteristics of patients with and without hypoferritinemia during a PANS flare, using chi-square or Fisher's exact tests for categorical variables, and two-sample *t* tests or Mann–Whitney *U* test, whenever appropriate. We chose to restrict the comparison to "flare state" since the disease state itself can be a confounder. Factors to be examined included demographics, socioeconomic status, dietary factors, body mass, psychiatric symptom severity, and caregiver burden. We used maternal education and annual household income as the proxy of socioeconomic status.[51]

To explore the association between changes in serum ferritin levels over time and psychiatric disease severity, we performed mixed models with random time. Outcome measures were patient's global impairment (GI) and caregiver burden inventory (CGBI) scores, both of which are rated by parents before each clinic visit. Global impairment score is a parent-rated scale ranging from 0 to 100 (0 = no impairment, 100 = worst impairment), and was validated in our PANS population.[52] Caregiver burden inventory score is a measure of caregiver burden.[53] Its use is considered valid and reliable in the PANS population.[46,54] These two scores within 10 days of ferritin tests were collected. They were used as an outcome measure in separate mixed models while hypoferritinemia (yes/no) was a time-varying variable, and other covariates in the model included time-in-clinic, sex, PANS flare (defined as an abrupt worsening of neuropsychiatric symptoms; yes/no), and chronic state of illness (defined as ongoing neuropsychiatric symptoms for at least 9 months continuously; yes/no).[46]

Statistical analysis was performed using Statistical Analysis Systems software program (SAS® University Edition, the United States). All statistical tests were considered statistically significant if two-sided *p* value was <0.05.

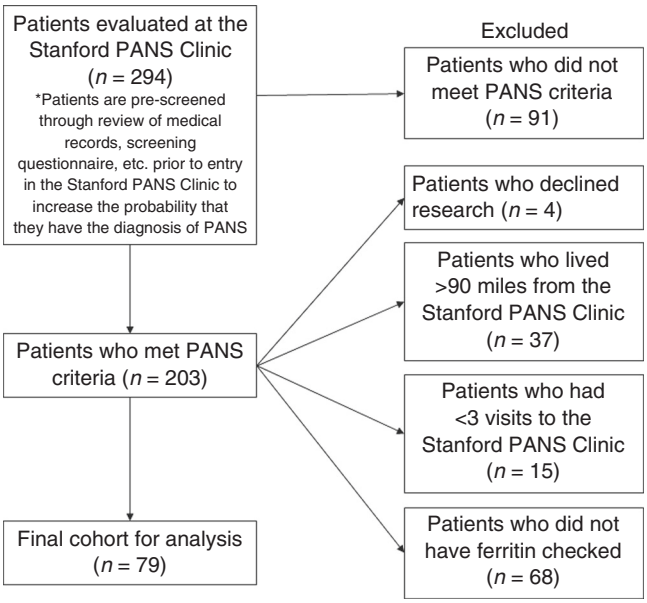

Fig. 1 A total of 294 patients was evaluated during the study period. After exclusion for several reasons, our final cohort included 79 patients for analysis. Inclusion and exclusion flowchart to study ferritin levels in patients with pediatric acute-onset neuropsychiatric syndrome (PANS).

RESULTS

Our study cohort included 79 consecutive community patients with PANS and available ferritin test results (Fig. 1). The median time ± interquartile range (IQR) between PANS onset and the first ferritin tests was 3.1 ± 4.8 years. The mean age ± standard deviation (SD) of PANS onset was 8.7 ± 3.8 years (Table 1). The majority of our patients were male (63%) and non-Hispanic White (94%). The socioeconomic status was high as reflected by a low percentage (3%) of families having annual household income <US$50,000 and a high percentage (78%) of mothers attaining college education or above. At clinic presentation, common symptoms reported by our study patients included obsessive compulsive symptoms, anxiety, irritability, emotional lability, motor and somatic symptoms.

Based on our definition of hypoferritinemia, 21/79 (27%) of our study cohort had hypoferritinemia (Table 2). This number dropped to 21% after exclusion of girls older than 12 years of age. Three quarters of hypoferritinemic cases were observed during a PANS flare. The prevalence of iron deficiency in our study cohort of patients who had iron workup was 14%, and about half of patients with iron deficiency also had anemia. After excluding girls older than 12 years, prevalences of hypoferritinemia, iron deficiency, and iron deficiency anemia dropped slightly.

Our sensitivity analyses under the first two assumptions of iron deficiency prevalence in 96 patients who did not have ferritin ($n = 68$) and iron workup ($n = 28$) showed that our patient sample had a higher odds of iron deficiency by 1.4–2.0-fold than the sex- and age-matched population in the United States (Table 3).[22] The last assumption, which was the most restrictive one and eliminated the background risk by assuming all untested patients were not iron deficient, gave a lower odds of iron deficiency than the sex- and age-matched population (odds ratio 0.7, 95% CI 0.4–1.1, $p = 0.11$).

Table 4 shows a comparison between patients with and without hypoferritinemia observed during a PANS flare. The latter group included patients with normal serum ferritin levels during a PANS flare ($n = 49$) and cases at remission ($n = 14$).

Table 1. Clinical characteristics of 79 consecutive patients with pediatric acute-onset neuropsychiatric syndrome (PANS) who met the study entry criteria.

Age of PANS onset, years	8.7 ± 3.8
Age at the first clinic visit, years	11.3 ± 4.1
Male gender	50 (63%)
Non-Hispanic White	74 (94%)
Low annual household income[a]	2/58 (3%)
High maternal education[b]	53/68 (78%)
Psychiatric symptoms at the initial clinic presentation	
Obsessive compulsive symptoms	61 (77%)
Eating restriction	32 (41%)
Anxiety	60 (76%)
Emotional lability and/or depression	47 (59%)
Irritability, aggression and/or severely oppositional behaviors	46 (58%)
Behavioral/developmental regression	27 (34%)
Cognitive impairment	37 (47%)
Sensory issues	28 (35%)
Motor issues	42 (53%)
Urinary symptoms	2 (3%)
Sleep disturbances	45 (60%)
Psychometric impairment scores at clinic entry	
Global impairment[c]	48.6 ± 26.0
Caregiver burden inventory[d]	36.4 ± 21.8

Data are presented in number (percentage), or mean ± SD unless specified.
[a]Low annual household income was defined as the annual household income <US$50,000.
[b]Mothers attained college education or above. This variable serves as a proxy for lower socioeconomic status.[51]
[c]Global impairment score is a parent-rated score of global functioning, and ranges from 0 to 100 (the higher the worse). It has been validated in patients with PANS.[52]
[d]Caregiver burden inventory is a parent-rated score of caregiver burden, and ranges from 0 to 96 (the higher the worse). A cut-off point of 36 represents respite care.[41] It has been validated in patients with PANS.[53]

There was no evidence of demographic differences (including age of PANS onset, sex, race/ethnicity, annual household income, and maternal education) between the two groups. Vegetarian and "no red meat" diets were common and comparable in both groups. At clinic entry, the hypoferritinemic group were older and had worse GI scores ($p < 0.01$) than the other group (Table 4). Body mass index (BMI), eating restriction, and socioeconomic status (reflected by annual household income and maternal education) were similar. However, at the time of ferritin tests, the hypoferritinemic group had a higher rate of chronic PANS illness (69% vs 46%) and higher GI scores (63.8 vs 43.9, $p < 0.01$) when compared to the other group. At the time of ferritin tests, GI and CGBI scores were both higher in the hypoferritinemic group, which is compatible with the definition of flares, in which psychometric impairment scores would be increasing. Comorbid inflammatory diseases were also more commonly seen in the hypoferritinemic group. As expected, low hemoglobin (Hb), low mean corpuscular volume (MCV), and high red blood cell distribution width (RDW) were also more common in the hypoferritinemic group.

The mixed models showed that hypoferritinemia at different time points were not significantly associated with psychometric impairment scores such as GI (GI 4.84, SE 3.93, $p = 0.22$) and CGBI (CGBI 4.67, SE 5.66, $p = 0.39$). In contrast, the disease state (flare or not, chronic illness or not) was associated with

Table 2. Ferritin and iron profiles in 79 consecutive patients with pediatric acute-onset neuropsychiatric syndrome (PANS) who met study criteria.

	Whole PANS study cohort	PANS study cohort after excluding females ≥12 years old
Hypoferritinemia[a]	21/79 (27%)	14/66 (21%)
Hypoferritinemia observed during a PANS flare	16/21 (76%)	11/14 (79%)
Iron deficiency[b]	7/51 (14%)	3/39 (8%)
Iron deficiency and anemia[c]	3/51 (6%)	2/39 (5%)

The denominator indicates the number of patients who had the laboratory tests performed.
[a]Hypoferritinemia was defined by serum ferritin level <7 ng/ml in children and youth aged ≤15 years and <18 ng/ml in adolescents aged >15 years.
[b]Iron deficiency was defined by hypoferritinemia in conjunction with either a low transferrin saturation or a high total iron binding capacity (TIBC) as designated by the respective laboratory.
[c]Anemia was defined by lower hemoglobin level than the reference range by the laboratory.

Table 3. Iron deficiency prevalence in a cohort of consecutive patients with pediatric acute-onset neuropsychiatric syndrome (PANS) and the general population in the United States.

Assumption about the patients with PANS who did not have ferritin and iron workup ($n = 96$)[a]	Projected prevalence of iron deficiency (in 100) in our study cohort ($n = 147$)[b]	Prevalence of iron deficiency (in 100) in age- and sex-matched general population[c]	Odds ratio (95% CI)	p value
Prevalence of iron deficiency is the same as patients with iron workup.	7.8 (2.2–18.9)	4.0 (2.0–9.0)	2.0 (1.4–3.0)	<0.001
Prevalence of iron deficiency is the same as the age- and sex-matched general population.	5.4 (2.4–10.4)		1.4 (0.9–2.1)	0.14
Prevalence of iron deficiency is zero (i.e., none of these patients have iron deficiency).	2.7 (0.7–6.8)		0.7 (0.4–1.1)	0.11

[a]This includes 68 patients who did not have serum ferritin levels checked and were excluded from the study, and 28 patients who had ferritin level checked, without iron workup.
[b]This number is the sum of 79 patients included in the study and 68 patients excluded from the study due to absence of ferritin tests.
[c]Using the CDC iron deficiency report in 2002.[21]

these psychometric impairment scores with statistical significance ($p < 0.01$).

DISCUSSION

In our PANS study cohort, a quarter (27%) of tested patients had hypoferritinemia. If we expand the denominator and include untested patients (i.e., no ferritin test obtained), the rate was halved (14%). In patients who had an iron workup, 14% (7/51) had iron deficiency and 6% (3/51) had iron deficiency anemia. The odds of iron deficiency in our patients was 1.4–2.0-fold higher than in the age- and sex-matched general population in the United States;[22] this finding is especially remarkable since we used more stringent cut-off levels for ferritin than the CDC comparison population. Three quarters of hypoferritinemic cases were observed during a PANS flare. When comparing the two groups of patients with and without hypoferritinemia during a PANS flare, we did not observe differences in demographics or dietary factors. However, hypoferritinemia during a PANS flare was usually associated with chronic PANS state and higher global impairment and caregiver burden at the time of ferritin tests.

An increased rate of hypoferritinemia and iron deficiency in our PANS cohort does not appear to be associated with typical demographic factors linked to iron deficiency. In our community PANS cohort, only 3% had annual household income <US$50,000; 78% of patients' mothers attained college education or above; and 94% of patients were non-Hispanic White (Table 1); these factors are known to be associated with adequate dietary intake and normal iron levels.[50] Eating restriction and vegetarian diets were found in roughly one third of our study patients. However, these patients were usually fed with other iron-rich food, such as green leafy vegetables, white meat, or iron fortified products, according to the parents.

Other factors could potentially explain a higher rate of hypoferritinemia and iron deficiency seen in our PANS cohort. First, a subgroup of PANS, called pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections (PANDAS), has been associated with Streptococcal infections.[55,56] Extracellular bacterial infections such as streptococcal, promote the expression of hepcidin, which acts to block iron exit from enterocytes in the duodenum and iron-recycling macrophages.[57] This reduces iron delivery to the circulation and subsequently results in anemia. Since the gut plays a role in iron metabolism, microbiome shift in the gut may be part of the picture. A recent study has also showed that gut microbiome changes in patients with PANS/PANDAS.[58] Use of medications and varying eating patterns during PANS flares may further complicate the picture.

Accumulating evidence points to a neuroinflammatory process as a cause of neuropsychiatric symptoms in PANS/PANDAS,[9–20] but the role of iron has not yet been understood. In PANS/PANDAS, the inflammatory processes are more pronounced in certain structures, e.g., basal ganglia, thalamus, amygdala.[9–11] In murine models, proinflammatory stimuli such as lipopolysaccharide or β-amyloid (Aβ) enhance brain microglia to preferentially take up the non-transferrin bound iron, therefore increasing iron deposition in inflamed regions.[59] The excessive iron in the brain then releases radical oxygen species, and other proinflammatory factors, further exacerbating neuroinflammation, apoptosis, and brain injury. This process might be similar to the iron accumulation and brain injury involved in many neurodegenerative diseases

Table 4. Comparison of demographic and clinical characteristics in 79 study patients with and without hypoferritinemia during a pediatric acute-onset neuropsychiatric syndrome (PANS) flare.

Characteristic	Patients with hypoferritinemia during a flare[a] ($N = 16$)	Patients without hypoferritinemia during a flare[a] ($N = 63$)	p value[b]
Demographics			
Age of PANS onset, years	9.5 ± 4.0	8.5 ± 3.7	0.34
Age at the first clinic visit, years	12.7 ± 4.0	10.9 ± 4.1	0.12
Male gender	8 (50%)	42 (67%)	0.22
Non-Hispanic White	15 (94%)	59 (94%)	1.00
Low annual household income[c]	0/11	2/47 (4%)	1.00
High maternal education[d]	12/14 (86%)	41/54 (76%)	0.72
Dietary factors and body mass			
Vegetarian diet	5/14 (36%)	10/61 (16%)	0.14
No red meat	5/12 (58%)	37/51 (73%)	0.08
Eating restriction			
At clinic entry	5 (31%)	23 (37%)	0.69
At the time of ferritin test	4 (25%)	19 (30%)	0.77
Body mass index, kg/m^2, median ± IQR			
At clinic entry	18.8 ± 6.6	18.0 ± 5.7	0.61
At the time of ferritin test	19.5 ± 7.6	18.9 ± 6.6	0.61
Disease status/psychometric impairment scores			
Chronic PANS disease state at the time of ferritin tests	11 (69%)	29 (46%)	0.10
Global impairment score[e]			
At clinic entry	63.8 ± 19.3	43.9 ± 25.2	<0.01
At the time of ferritin test	39.9 ± 19.9	38.0 ± 25.3	0.78
Caregiver burden inventory[f]			
At clinic entry	34.6 ± 21.4	36.8 ± 22.2	0.81
At the time of ferritin test	40.5 ± 18.0	32.6 ± 20.4	0.16
Comorbid inflammatory diseases[g]	10 (63%)	30 (48%)	0.29
Hematologic measures at the time of ferritin tests[h]			
Low Hb	7/15 (47%)	11/61 (18%)	0.02
Low MCV	4/15 (27%)	7/61 (11%)	0.13
High RDW	4/15 (27%)	6/61 (10%)	0.84

Data are presented as number (percentage) or mean ± standard deviation.
PANS pediatric acute-onset neuropsychiatric syndrome, *Hb* hemoglobin, *IQR* interquartile range, *MCV* mean corpuscular volume, *RDW* red blood cell distribution width.
[a]The presence of a denominator means only a portion of data was available. Otherwise, the percentage was calculated with respect to the whole study cohort ($n = 79$).
[b]p value was calculated by chi-square or Fisher's exact tests for categorical variables, and two-sample t tests or Mann–Whitney U test, whenever appropriate.
[c]Low annual household income was defined as the annual household income <US$50,000.
[d]Mothers attained college education or above. This variable serves as a proxy for lower socioeconomic status.[51]
[e]Global impairment score is a parent-rated score of global functioning and ranges from 0 to 100 (the higher, the worse). It has been validated in patients with PANS.[52]
[f]Caregiver burden inventory is a parent-rated score of caregiver burden and ranges from 0 to 96 (the higher, the worse). A cut-off point of 36 represents respite care.[45] It has been validated in patients with PANS.[54]
[g]This group of diseases includes celiac disease, autoimmune thyroiditis, inflammatory bowel disease, psoriasis, eosinophilic esophagitis, antiphospholipid syndrome, chronic arthritis, and chronic urticaria.
[h]Abnormalities were defined by values out of the reference ranges suggested by the laboratory.

such as multiple sclerosis, pantothenate kinase-associated neurodegeneration, Alzheimer's disease, and Parkinson's disease where increased basal ganglion iron has been observed on brain imaging.[60–63] Reduced iron delivery from the gut and macrophages as a result of increased hepcidin expression coupled with increased iron delivery to the inflamed brain are possible explanations for a higher rate of hypoferritinemia in our PANS patients, especially during a PANS flare.

Hypoferritinemia is not only commonly seen in patients with PANS but it has also been observed in Behcet's disease which causes inflammation in blood vessels (vasculitis) and neuropsychiatric disease. One study of Behcet's disease reported low serum ferritin in 15% of patients.[64] Interestingly, the same HLA epitope (HLA-Bw4) associated with Behcet's disease has also been shown to be strongly associated with PANS compared to healthy controls.[65,66] These similarities in patients with PANS and Behcet's disease may suggest similar underlying inflammatory processes in these diseases.

By the end of the study period, most of the hypoferritinemic patients (11/16, 69%) had normalization of ferritin levels after

receiving either IVIG ($n = 6$, with average of 2.8 infusions preceding the resolution), methylprednisolone ($n = 5$), prednisone bursts ($n = 6$), NSAID ($n = 8$), or a combination of these therapies. Only three of these patients took concurrent iron supplements. Of the remaining 5/16 patients, three did not have ferritin levels rechecked by the end of the study, and two failed to normalize ferritin levels before the end of this study. Further studies on the effects of immunomodulation on hypoferritinemia and iron deficiency are required to guide clinicians in treating patients with hypoferritinemia during PANS flares.

Study strengths and limitations

To our best knowledge, this study is the first study to describe the prevalence of hypoferritinemia and iron deficiency in a cohort of patients with PANS. We used a stringent cut-off value for hypoferritinemia to estimate its prevalence and the prevalence of iron deficiency. The present conservative estimate, which may or may not be underestimated, was still higher than the general population.

Our study has several limitations. First, while we intended to systematically measure ferritin levels in every patient, various factors impeded this evaluation, including behavioral/anxiety reactions to blood draws, and phlebotomy blood volume limits to prevent excessive phlebotomy in small children. Post-hoc comparative analysis of the included and excluded patients showed that patients excluded for missing ferritin levels (but otherwise would meet inclusion criteria) from the study cohort were younger at their first clinic visit (9.9 years in the excluded vs 11.4 years in the included, $p = 0.03$). They did not differ in sex ratio, race/ethnicity, or symptoms at clinic presentation. Thus, our results are only generalizable to older children at this time. Second, for the estimation of iron deficiency prevalence, we matched patients by age and sex, but due to limitations in the CDC data available to us, we were unable to match 1:1 for race and ethnicity. However, we compared our PANS cohort (94% were non-Hispanic White) to the CDC non-Hispanic White data; the difference is likely minimal. Third, our data about socioeconomic status and diet preference were cross-sectional at the clinic entry, and we did not have follow-up data to address possible changes over time. We collected data regarding eating restriction and body mass at the time of ferritin tests through chart review and patient questionnaires in order to delineate any significant changes in these parameters that may potentially provide information about food supply and intake. Last, our cohort size was small; thus, our study results need to be reproduced in larger cohorts and preferably with systematic acquisition of ferritin and iron tests, although this will likely always be a challenge in young children with anxiety-related disorders.

Future directions

Given accumulating research evidence on the inflammatory processes underlying PANS, it would be informative to perform serial measurements of serum iron, ferritin, hepcidin, and erythrocyte kinetics in patients with PANS, particularly during flare vs remission states, in order to elucidate a potential role of iron homeostasis in this illness. In addition, using MRI techniques to quantify and map iron deposition in the brain, especially the thalamus, basal ganglia, and amygdala, will provide more information for understanding the pathology of PANS illness with relation to iron dysregulation.[67,68]

CONCLUSION

Our study shows an increased rate of hypoferritinemia and iron deficiency among a community cohort of youth with PANS. The odds of iron deficiency in our study patients was 1.4–2.0-fold higher than in the age- and sex-matched general population in the United States. Three quarters of hypoferritinemic cases were observed during a PANS flare, and when compared to patients without hypoferritinemia during a PANS flare, these cases presented with worse global impairment at clinic entry. At the time of ferritin tests, these patients were more likely to have chronic PANS illness and a comorbid inflammatory disease. A larger study cohort with routine evaluation of ferritin and status of iron stores in patients with PANS is needed to corroborate our findings and examine the relationship with neuropsychiatric symptom severity. MRI brain techniques to quantify and map the iron deposition in brain structures will provide additional information about iron hemostasis.

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AUTHOR CONTRIBUTIONS

J.F. conceptualized and designed the study, supervised research assistants, interpreted data, and provided intellectual review of the manuscript. M.J. provided expertise regarding ferritin, anemia and study design, and revised the manuscript. H. K. conceptualized using the CDC data as a comparison dataset and performed the initial data analysis. Both H.K. and E.S. collected data and prepared the first draft of the manuscript. A.C. contributed to further data acquisition, data analysis and interpretation, and manuscript writing. M.T., T.W., and B.F., FNP-c, provided expert advice on chart reviews/data collection, and revised the manuscript. All authors approved the final version of the manuscript.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

Consent from research subjects: Consent was obtained from parents of minors and adult patients, and assent obtained from patients aged 7–17 years.

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REFERENCES

1. Chang, K. et al. Clinical evaluation of youth with Pediatric Acute-Onset Neuropsychiatric Syndrome (PANS): recommendations from the 2013 PANS Consensus Conference. *J. Child Adolesc. Psychopharmacol.* **25**, 3–13 (2015).
2. Swedo, S. E., Leckman, J. F. & Rose, N. R. From research subgroup to clinical syndrome: modifying the PANDAS criteria to describe PANS (Pediatric Acute-onset Neuropsychiatric Syndrome). *Pediatr. Ther.* **2**, 1–8 (2012).
3. Frankovich, J. et al. Multidisciplinary clinic dedicated to treating youth with pediatric acute-onset neuropsychiatric syndrome: presenting characteristics of the first 47 consecutive patients. *J. Child Adolesc. Psychopharmacol.* **25**, 38–47 (2015).
4. Calaprice, D., Tona, J., Parker-Athill, E. C. & Murphy, T. K. A survey of pediatric acute-onset neuropsychiatric syndrome characteristics and course. *J. Child Adolesc. Psychopharmacol.* **27**, 607–618 (2017).
5. Gromark, C. et al. Establishing a pediatric acute-onset neuropsychiatric syndrome clinic: baseline clinical features of the pediatric acute-onset neuropsychiatric syndrome cohort at Karolinska Institutet. *J. Child Adolesc. Psychopharmacol.* **29**, 625–633 (2019).
6. Hesselmark, E. & Bejerot, S. Clinical features of paediatric acute-onset neuropsychiatric syndrome: findings from a case–control study. *Br. J. Psychiatry Open* **5**, e25 (2019).

7. Gamucci, A. et al. PANDAS and PANS: clinical, neuropsychological, and biological characterization of a monocentric series of patients and proposal for a diagnostic protocol. *J. Child Adolesc. Psychopharmacol.* **29**, 305–312 (2019).
8. Johnson, M. et al. Paediatric acute-onset neuropsychiatric syndrome in children and adolescents: an observational cohort study. *Lancet Child Adolesc. Health* **3**, 175–180 (2019).
9. Zheng, J. et al. Association of pediatric acute-onset neuropsychiatric syndrome with microstructural differences in the deep grey matter. *JAMA Netw. Open* **3**, e204063 (2020).
10. Giedd, J. N., Rapoport, J. L., Garvey, M. A., Perlmutter, S. & Swedo, S. E. MRI assessment of children with obsessive-compulsive disorder or tics associated with streptococcal infection. *Am. J. Psychiatry* **157**, 281–283 (2000).
11. Kumar, A., Williams, M. T. & Chugani, H. T. Evaluation of basal ganglia and thalamic inflammation in children with pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection and tourette syndrome: a positron emission tomographic (PET) study using 11C-[R]-PK11195. *J. Child Neurol.* **30**, 749–756 (2015).
12. Dale, R. C. & Brilot, F. Autoimmune basal ganglia disorders. *J. Child Neurol.* **27**, 1470–1481 (2012).
13. Cutforth, T., DeMille, M. M., Agalliu, I. & Agalliu, D. CNS autoimmune disease after Streptococcus pyogenes infections: animal models, cellular mechanisms and genetic factors. *Future Neurol.* **11**, 63–76 (2016).
14. Platt, M. P. et al. Th17 lymphocytes drive vascular and neuronal deficits in a mouse model of postinfectious autoimmune encephalitis. *Proc. Natl Acad. Sci. USA* **117**, 6708–6716 (2020).
15. Brimberg, L. et al. Behavioral, pharmacological, and immunological abnormalities after streptococcal exposure: a novel rat model of Sydenham chorea and related neuropsychiatric disorders. *Neuropsychopharmacology* **37**, 2076–2087 (2012).
16. Frick, L. & Pittenger, C. Microglial dysregulation in OCD, Tourette Syndrome, and PANDAS. *J. Immunol. Res.* **2016**, 8606057 (2016).
17. Lotan, D. et al. Behavioral and neural effects of intra-striatal infusion of anti-streptococcal antibodies in rats. *Brain Behav. Immun.* **38**, 249–262 (2014).
18. Xu, J. et al. Antibodies from children with PANDAS bind specifically to striatal cholinergic interneurons and alter their activity. *Am. J. Psychiatry*. (2020). https://doi.org/10.1176/appi.ajp.2020.19070698. [ahead of print].
19. Yaddanapudi, K. et al. Passive transfer of streptococcus-induced antibodies reproduces behavioral disturbances in a mouse model of pediatric autoimmune neuropsychiatric disorders associated with streptococcal infection. *Mol. Psychiatry* **15**, 712–726 (2010).
20. Dileepan, T. et al. Group A Streptococcus intranasal infection promotes CNS infiltration by streptococcal-specific Th17 cells. *J. Clin. Invest.* **126**, 303–317 (2016).
21. Suskind, D. L. Nutritional deficiencies during normal growth. *Pediatr. Clin. North Am.* **56**, 1035–1053 (2009).
22. CDC. Iron deficiency—United States, 1999−2000. *JAMA* **288**, 2114–2116 (2002).
23. Camaschella, C. Iron-deficiency anemia. *N. Engl. J. Med.* **372**, 1832–1843 (2015).
24. Lopez, A., Cacoub, P., Macdougall, L. C. & Peyrin-Biroulet, L. Iron deficiency anaemia. *Lancet* **387**, 907–916 (2016).
25. Avrahami, M., Barzilay, R., HarGil, M., Weizman, A. & Watemberg, N. Serum ferritin levels are lower in children with tic disorders compared with children without tics: a cross-sectional study. *J. Child Adolesc. Psychopharmacol.* **27**, 192–195 (2016).
26. Gorman, D. A., Zhu, H., Anderson, G. M., Davies, M. & Peterson, B. S. Ferritin levels and their association with regional brain volumes in Tourette's syndrome. *Am. J. Psychiatry* **163**, 1264–1272 (2006).
27. Ghosh, D. & Burkman, E. Relationship of serum ferritin level and tic severity in children with Tourette syndrome. *Childs Nerv. Syst.* **33**, 1373–1378 (2017).
28. Mader, R., Koton, Y., Buskila, D., Herer, P. & Elias, M. Serum iron and iron stores in non-anemic patients with fibromyalgia. *Clin. Rheumatol.* **31**, 595–599 (2012).
29. Ortancil, O., Sanli, A., Eryuksel, R., Basaran, A. & Ankarali, H. Association between serum ferritin level and fibromyalgia syndrome. *Eur. J. Clin. Nutr.* **64**, 308–312 (2010).
30. Shariatpanaahi, M. V., Shariatpanaahi, Z. V., Moshtaaghi, M., Shahbaazi, S. H. & Abadi, A. The relationship between depression and serum ferritin level. *Eur. J. Clin. Nutr.* **61**, 532–535 (2007).
31. Gottfried, R. J., Gerring, J. P., MacHell, K., Yenokyan, G. & Riddle, M. A. The iron status of children and youth in a community mental health clinic is lower than that of a national sample. *J. Child Adolesc. Psychopharmacol.* **23**, 91–100 (2013).
32. Chen, M. H. et al. Association between psychiatric disorders and iron deficiency anemia among children and adolescents: a nationwide population-based study. *BMC Psychiatry* **13**, 161 (2013).
33. Kwak, B. O., Kim, S. N. & Lee, R. Relationship between iron deficiency anemia and febrile seizures in children: a systematic review and meta-analysis. *Seizure* **52**, 27–34 (2017).
34. Tomoum, H., Habeeb, N., Elagouza, I. & Mobarez, H. Paediatric breath-holding spells are associated with autonomic dysfunction and iron deficiency may play a role. *Acta Paediatr. Int. J. Paediatr.* **107**, 653–657 (2018).
35. Georgieff, M. K. Iron assessment to protect the developing brain. *Am. J. Clin. Nutr.* **106**, 1588S–1593S (2017).
36. Barks, A., Fretham, S. J. B., Georgieff, M. K. & Tran, P. V. Early-life neuronal-specific iron deficiency alters the adult mouse hippocampal transcriptome. *J. Nutr.* **148**, 1521–1528 (2018).
37. Leyshon, B. J., Radlowski, E. C., Mudd, A. T., Steelman, A. J. & Johnson, R. W. Postnatal iron deficiency alters brain development in piglets. *J. Nutr.* **146**, 1420–1427 (2016).
38. Wiegersma, A. M., Dalman, C., Lee, B. K., Karlsson, H. & Gardner, R. M. Association of prenatal maternal anemia with neurodevelopmental disorders. *JAMA Psychiatry* **76**, 1–12 (2019).
39. Georgieff, M. K. The role of iron in neurodevelopment: fetal iron deficiency and the developing hippocampus. *Biochem. Soc. Trans.* **36**, 1267–1271 (2008).
40. Berglund, S. K. et al. The impacts of maternal iron deficiency and being overweight during pregnancy on neurodevelopment of the offspring. *Br. J. Nutr.* **118**, 533–540 (2017).
41. Wang, W., Knovich, M. A., Coffman, L. G., Torti, F. M. & Torti, S. V. Serum ferritin: past, present and future. *Biochim. Biophys. Acta* **1800**, 760–769 (2010).
42. Alkhateeb, A. A. & Connor, J. R. The significance of ferritin in cancer: anti-oxidation, inflammation and tumorigenesis. *Biochim. Biophys. Acta* **1836**, 245–254 (2013).
43. Kernan, K. F. & Carcillo, J. A. Hyperferritinemia and inflammation. *Int. Immunol.* **29**, 401–409 (2017).
44. Chan, A. L. & Frankovich, J. Infections, antibiotics, and mental health deteriorations. *J. Child Adolesc. Psychopharmacol.* **29**, 647–648 (2019).
45. Orefici, G, Cardona, F, Cox, C. J. & Cunningham, M. W. in *Streptococcus pyogenes: Basic Biology to Clinical Manifestations [Internet]*. (eds. Ferretti, J, Stevens, D, & Fischetti, V.) (University of Oklahoma Health Sciences Center, Oklahoma City, OK, 2016).
46. Frankovich, J. et al. The burden of caring for a child or adolescent with pediatric acute-onset neuropsychiatric syndrome (PANS): an observational longitudinal study. *J. Clin. Psychiatry* **80**, 17m12091 (2018).
47. Agha, F., Akhter, P. & Khan, R. A. Serum ferritin levels in apparently healthy subjects. *J. Pak. Med. Assoc.* **37**, 63–66 (1987).
48. Jacobs, A., Miller, F., Worwood, M., Beamish, M. R. & Wardrop, C. A. J. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Br. Med. J.* **4**, 206–208 (1972).
49. Siimes, M. A., Addiego, J. E. & Dallman, P. R. Ferritin in serum: diagnosis of iron deficiency and iron overload in infants and children. *Blood* **43**, 581–590 (1974).
50. Centers for Disease Control and Prevention. Second National Report on Biochemical Indicators of Diet and Nutrition in the US Population [Internet]. https://www.cdc.gov/nutritionreport/pdf/Nutrition_Book_complete508_final.pdf (2012).
51. Vollmer, S., Bommer, C., Krishna, A., Harttgen, K. & Subramanian, S. V. The association of parental education with childhood undernutrition in low- and middle-income countries: comparing the role of paternal and maternal education. *Int. J. Epidemiol.* **16**, 312–323 (2017).
52. Leibold, C., Thienemann, M., Farhadian, B., Willett, T. & Frankovich, J. Psychometric properties of the pediatric acute-onset neuropsychiatric syndrome global impairment score in children and adolescents with pediatric acute-onset neuropsychiatric syndrome. *J. Child Adolesc. Psychopharmacol.* **29**, 41–49 (2018).
53. Novak, M. & Guest, C. Application of a multidimensional caregiver burden inventory. *Gerontologist* **29**, 798–803 (1989).
54. Farmer, C. et al. Psychometric evaluation of the caregiver burden inventory in children and adolescents with PANS. *J. Pediatr. Psychol.* **43**, 749–757 (2018).
55. Swedo, S. E. et al. Pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections: clinical description of the first 50 cases. *Am. J. Psychiatry* **155**, 264–271 (1998).
56. Orlovska, S. et al. Association of streptococcal throat infection with mental disorders: testing key aspects of the PANDAS hypothesis in a nationwide study. *JAMA Psychiatry* **74**, 740–746 (2017).
57. Nairz, M. et al. Iron and innate antimicrobial immunity—depriving the pathogen, defending the host. *J. Trace Elem. Med. Biol.* **48**, 118–133 (2018).
58. Quagliariello, A. et al. Gut microbiota profiling and gut-brain crosstalk in children affected by pediatric acute-onset neuropsychiatric syndrome and pediatric autoimmune neuropsychiatric disorders associated with streptococcal infections. *Front. Microbiol.* **9**, 675 (2018).
59. McCarthy, R. C. et al. Inflammation-induced iron transport and metabolism by brain microglia. *J. Biol. Chem.* **293**, 7853–7863 (2018).
60. Lane, D. J. R., Ayton, S. & Bush, A. I. Iron and Alzheimer's disease: an update on emerging mechanisms. *J. Alzheimers Dis.* **64**, S379–S395 (2018).

61. Bartzokis, G., Tishler, T. A., Shin, I. S., Lu, P. H. & Cummings, J. L. Brain ferritin iron as a risk factor for age at onset in neurodegenerative diseases. *Ann. N. Y. Acad. Sci.* **1012**, 224–236 (2004).
62. Ropele, S., Enzinger, C. & Fazekas, F. Iron mapping in multiple sclerosis. *Neuroimaging Clin. N. Am.* **27**, 335–342 (2017).
63. Hayflick, S. J., Kurian, M. A. & Hogarth, P. Neurodegeneration with brain iron accumulation. *Handb. Clin. Neurol.* **147**, 293–305 (2018).
64. Challacombe, S. J., Scully, C., Keevil, B. & Lehner, T. Serum ferritin in recurrent oral ulceration. *J. Oral. Pathol. Med.* **12**, 290–299 (1983).
65. Frankovich, J. et al. HLA findings in youth with pediatric acute-onset neuropsychiatric syndrome (PANS). In *CARRA Annual Meeting* 614399 (2019).
66. Mohammad-Ebrahim, H. et al. Association of killer cell immunoglobulin-like receptor (KIR) genes and their HLA ligands with susceptibility to Behçet's disease. *Scand. J. Rheumatol.* **47**, 155–163 (2018).
67. Langkammer, C. et al. Quantitative susceptibility mapping in Parkinson's disease. *PLoS ONE* **11**, e0162460 (2016).
68. Langkammer, C., Ropele, S., Pirpamer, L., Fazekas, F. & Schmidt, R. MRI for iron mapping in Alzheimer's disease. *Neurodegener. Dis.* **13**, 189–191 (2014).