Eur Arch Psychiatry Clin Neurosci (2008) 258:489–496 DOI 10.1007/s00406-008-0822-y

ORIGINAL PAPER

Eman Khedr · Sherifa A. Hamed · Esam Elbeih · Hala El-Shereef · Yousreyia Ahmad · Safaa Ahmed

Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment

Received: 5 December 2007 / Accepted: 15 April 2008 / Published online: 20 June 2008

Abstract Many investigators found that iron deficiency anemia (IDA) had a great influence on cognitive functions in infants and children. However, studies of such topic in adults are few and controversial. We prospectively assessed the possible influence of IDA and iron supplementation (for 3 months) on cognitive function and intelligence of 28 young adults with IDA. We used group of hematological, cognitive, neurophysiological tests for assessment including: mini-mental state examination (MMSE), Wechsler memory scale-revised (WMS-R), Wechsler adult intelligence scale-revised (WAIS-R), event-related potentials (ERPs), and electroencephalography (EEG). Compared to controls, patients demonstrated lower scores of different cognitive tests (MMSE, WMS-R, and WAIS-R), which showed significant improvement after treatment. Prolongation of ERPs latencies (N200 and P300) and reduction in their amplitudes (P200 and P300) were identified with significant increase in amplitude occurred after treatment. EEG abnormalities were observed in 55% of patients which showed improvement in 35% after treatment. Positive correlation was identified before and after treatment between hemoglobin levels and MMSE (P = 0.01, 0.05), total verbal (P = 0.04) and performance (P = 0.05, 0.04) IQ scores. Negative correlation was identified between before and after treatment between P300 latency and total IQ of WAIS-R (P = 0.03, 0.008) and hemoglobin level (P = 0.4, 0.01). Positive correlation was found before and after treatment between P300 amplitude and total IQ (P = 0.028, 0.01) and serum iron (P = 0.01, 0.001). In conclusion, IDA is a significant factor in cognitive performance in adult population, which can be partially reversed by treatment.

Key words iron deficiency anemia · cognition · psychometric tests · event related potentials · EEG

Abbreviations IDA: Iron deficiency anemia · ID: Iron deficiency · HB: Hemoglobin · TIBC: Total iron binding capacity · MMSE: Mini-mental state examination · WMS-R: Wechsler Memory Scale · WAIS-R: Wechsler adult intelligence scale-revised · ERPs: Event-related potentials · EEG: Electroencephalography

E. Khedr · Dr. S.A. Hamed, MBBch, MSc, MD (✉)
Department of Neurology and Psychiatry
Assiut University Hospital
P.O. Box 71516, Assiut, Egypt
Tel.: +2-88/237-1820
Fax: +2-88/233-3327
E-Mail: hamed_sherifa@yahoo.com

E. Elbeih · H. El-Shereef · Y. Ahmad · S. Ahmed
Department of Internal Medicine
Assiut University Hospital
Assiut, Egypt

Dr. S.A. Hamed, MBBch, MSc, MD
Children's National Medical Center
Research Center For Genetic Medicine
111 Michigan Avenue, NW
Washington, DC 20010, USA

Introduction

Iron deficiency is a major health problem worldwide affecting more than a quarter of the world's population [7]. Iron deficiency anemia (IDA) is the most common form of anemia affecting 43% of the world's children [9], more than 50% of women of reproductive age [50] and 3% of men [10]. Previous animal and human studies reported that iron deficiency not only manifests as anemia but also adversely effects motor performance, mental development, cognitive and behavioral functions [12, 23, 27, 37, 41]. The adverse effects of iron deficiency on cognition and intelligence are marked at infancy and early childhood, a period of heightened brain growth and development. Iron

deficiency anemia (IDA), during infancy in humans, has been associated with poorer performance on mental and motor measures and behavioral alterations such as wary and hesitant behavior [16, 24]. Iron is an essential component of brain growth, myelination influencing nerve impulse conduction, and is required for cell differentiation, protein synthesis, hormone production and fundamental aspects of cellular energy metabolism and functioning [21, 49]. Iron is also essential for a number of enzymes involved in neurotransmitter synthesis including tryptophan hydroxylase (serotonin) and tyrosine hydroxylase (norepinephrine and dopamine) [49]. Most case-control and longitudinal studies done in countries around the world that assessed overall cognitive functioning revealed that infants with IDA had lowering by 6–15 points in mental development test scores compared to healthy full-term infants with better iron status [25]. Kretch et al. [22] found a significant positive correlation of hemoglobin with mean performance as a measure of sustained attention. Stoltzfus et al. [45] suggested that the long-term effects on IQ are 1.73 points lower for each 1.0-g/dl decrease in hemoglobin. Similarly, Idjaradinata and Pollitt [19] found that iron deficient anemic children scored averages of 88.5 and 88.8% respectively for their motor and mental skills compared with normal children. After iron intervention the scores for the iron-deficient anemic group increased to 112.0 and 108.1 respectively. Animal studies point to the high vulnerability of the developing hippocampus and frontal cortex to early iron deficiency. Iron deficiency mediates metabolic and structural changes in the hippocampus and frontal cortex of developing rats during infancy. The period of infancy is characterized by peak hippocampal and cortical regional development, as well as myelinogenesis, dendritogenesis, and synaptogenesis in the brain and changes in these processes underlie deficits in spatial learning and memory processes [21, 38]. Moreover, the neuronal metabolic marker cytochrome *c* oxidase (CytOx) loss has been found to be marked in the highly metabolic hippocampal areas CA1 and CA3 and in the frontal lobes of the cortex in the iron-deficient rat brain [8]. Furthermore, iron deficiency causes abnormal protein scaffolding for microtubule extension and retraction in brain regions involved in recognition memory processing [21].

Because many of the human studies on cognition, behavior and iron status have focused on infants, preschool and school-age children, it is tempting to assume that there is only a developmental component to this relation of poor iron status and mental performance. Perhaps it is worthwhile to examine this assumption beyond this age group. Biological development and organization of the brain in human is very rapid in-utero and start to slow down in the second year of postnatal life [11]. Although gross organization is nearly complete by 2 or 3 years of age, maturation may continue through adolescence and beyond [20]. As iron plays an important role in oxygen transport and storage, it is also a cofactor in various biochemical processes including mitochondrial electron transport, fundamental aspects of cellular energy metabolism and functioning, it is essential for hormone production, protein synthesis, neurotransmitter synthesis, catecholamine metabolism and DNA synthesis [33]. Hence, a possibility exists that iron deficiency can alter brain metabolism during period of life later than during early growth and development.

Aim of the work

Because reports about the effect of iron deficiency on cognitive abilities in adult group of population are few, controversial and inconclusive, this work aimed to assess cognitive functions in young adult with IDA and the therapeutic effects of iron supplementation on these functions.

Patients and methods

Patients

This study included 28 consecutive adult patients (15 males and 13 females) with iron deficiency anemia (IDA). Patients were recruited from the Clinical Hematology Out-patient clinic, Assiut University Hospital, Egypt. Clinical symptomatology included easy fatigability, reduction in physical work capacity, cold intolerance and problems in concentration. All patients were admitted at the Clinical Hematology Unit for proper diagnosis and start of specific treatment. The exclusion criteria including any medical disease that may affect cognitive function (renal, hepatic, thyroid, connective tissue diseases, neurological diseases, iron-deficiency anemia complicating malignant neoplasm, concomitant presence of other types of anemia e.g. vitamin B12-deficiency), abnormal neuromotor development and use of medications (e.g. major or minor tranquilizer) that may alter cognitive and neurophysiological measures. Twenty-five subjects (12 males and 13 females) healthy non-anemic subjects matched for age, sex, education and socioeconomic conditions were involved as a control group. The protocol of this study was in conformity with the local ethical guidelines and informed written consent was obtained from each participant.

Methods

At the baseline assessment, patients and control subjects were subjected to complete clinical and neurological examination, with special attention to signs of IDA. Blood counts, red cell indices and peripheral hemogram, serum iron and total iron binding capacity (TIBC), urine analysis, stool analysis and liver and kidney function tests were assessed for each subject. Venous blood samples were collected into polypropylene tubes containing EDTA and stored at −20°C. Serum samples were diluted with deionized water (0.5:4.5 v/v). Iron levels were then measured with an atomic absorption/flame emission spectrophotometer (Schimadzu Seisakusho LTD, model AA-630-02, Japan), using an air-acetylene flame and hollow cathode lamps. Standards were obtained from Buck Scientific, NY, USA. The lamp current (mA),

the wave length (nm) and standard concentration (lot number) of 10 mA, 248.3 nm, 1003 μg/ml for Fe in 5% HNO_3 (lot # 9809F).

Neuropsychological assessment

At the initial study after diagnosis of IDA, cognitive function was assessed for each subject using psychometric tests including: (1) mini mental state examination (MMSE) [13]. The MMSE consists of a variety of questions grouped into seven categories, each representing a different cognitive domain or function (orientation to time, orientation to place, repetition of words, attention and calculation, recall of words, language and visual construction) and has a maximum score of 30 points. As most of the subjects of the present study were illiterate, the two points testing reading and writing were excluded and the full score was calculated as 28 instead of 30 points, (2) Wechsler memory scale-revised (WMS-R) [39]: tests included digit forward, digit backward, mental control, logical memory II and II, associate learning and visual reproduction I and II, and (3) Wechsler adult intelligence scale-revised (WAIS-R) [47] in which prorated to estimate verbal and performance intelligence scores.

Neurophysiological assessment

Neurophysiological tests used for assessment included event-related potentials (ERPs), and electroencephalography (EEG).

Event-related potentials (ERPs)

ERPs were elicited with an auditory discrimination task paradigm by presenting a series of binaural 1,000-Hz (standard) Vs 2,000-Hz (target) tones at 70 dB with a 10-ms rise/fall and 40-ms plateau time. Tones were presented at a rate of 1.1 per second, with target tones occurring randomly with a 0.2 probability. Subjects were sitting with their eyes closed and were instructed to mentally count the number of the target but not the frequent tones and then asked to report the number of target tones counted at the end of each run. Potentials were recorded from scalp electrodes placed at Cz and were referred to linked ears. Filter settings were 0.5 and 70 Hz. Separate averages for target and non-target tones were obtained. Responses to 30 target and 120 non-target tones were obtained in each trial. Before recording, subjects were familiarized with the two tones and instructed to press a button when they heard target tones. Latencies of each event-related component (N100, P200, N200 and P300) were measured. P300 latency was measured as the major positive peak after N200, within a range of 250–500 ms. The amplitudes of N200 and P300 were measured peak to peak from the negative component just before the wave to the maximum positive peak of the wave [18].

Electroencephalography

Conventional wakefulness EEGs were obtained using 8-channel Nihon Kohden equipment, employing scalp electrodes placed according to the international 10–20 system with bipolar and referential montages. Hyperventilation for 3 min was used as a provocative test.

Treatment of patients

In all patients, treatment started by treatment of the underlying cause of anemia beside ferrous fumarate 600 mg/day (195 mg elemental iron/day) in three divided doses immediately after meals. Hemoglobin (HB) level assessment was carried out every 20 days till reach to normal values within 3 months. Revaluation of patients was carried out using clinical, laboratory, psychometric and neurophysiological tests after correction of anemia.

Statistical analysis

Data are expressed as means ± SD unless otherwise stated. Calculations were done with the statistical package SPSS for windows, version 14 (SPSS Inc., Chicago, IL, USA). Statistical analyses were performed using student's "*t*" or one way ANOVA tests as appropriate. At the base line assessment the mean values of different psychometric scales between both groups were compared using student's *t*-test for independent samples. Two way ANOVA for repeated measurements analysis for variance was used for statistical analysis. The interaction effect between diagnosis and treatment was significant for some of the cognitive measures could be explained by the possibility that patients increased their performance while controls remained stable. For continuous variables (assumed normal distribution), Pearson's correlation coefficient was utilized while Mann-Whitney *U*-test, non-parametric test, was utilized for variables that had a skewed distribution. The level of significance was set at $P < 0.05$.

Results

Twenty eight patients with IDA were included in this study. Their mean age was 22.00 ± 5.45 years (versus 22.64 ± 4.83 years for control subjects, $P > 0.05$). Patients and controls were divided into 4 groups according to the educational level: (1) illiterate (patients: $n = 14$, 50%; controls: $n = 13$, 52%), (2) can read and write (patients: $n = 4$, 14.3%; controls: $n = 4$, 16%), (3) primary school (patients: $n = 7$, 28%; controls: $n = 6$, 24%), and (4) higher than primary school but less than university (patients: $n = 3$, 10.7%; controls: $n = 2$, 8%). The underlying etiology of iron deficiency in the studied group was parasitic infestations in 14 patients (50%) (11 males and 3 females), heavy menstrual losses in 5 females (17.9%), recurrent bleeding piles in 3 (10.7%) male patients, increased demands due to pregnancy or lactation in 3 (10.7%) patients and recurrent epistaxis in one male patient (3.6%). All patients were receiving good oral feeding and the mean body mass index (BMI) of non-pregnant and lactating patients showed no difference compared to control subjects (25.50 ± 4.10 for patients vs. 26.60 ± 2.80 for controls). Patients with parasitic infestations were treated with antihelminthic medications with reported recovery.

All patients reported amelioration of clinical symptoms of anemia including easy fatigability, reduction in physical work capacity, cold intolerance and problems in concentration. Compared to control subjects, the HB and serum iron of the patients before treatment were significantly lower ($P < 0.001$), while the TIBC was significantly higher ($P < 0.05$). Males demonstrated lower HB, iron and TIBC levels compared to females ($P < 0.05$) (Table 1). Significant improvement of HB and serum iron ($P < 0.001$ and $P < 0.001$ respectively) in patients after treatment compared to pretreatment values, at the same time there was a significant decline of TIBC ($P = 0.005$) (Table 1).

At baseline assessment, patients demonstrated significant lower scores of different psychometric

Table 1 Comparison between hemoglobin, serum iron and total iron binding capacity of the patients before and after treatment and control group

Item	Patients (before treatment)	Patients (after treatment)	Controls (first assessment)	Controls (after 3 months)
HB (g/dL)	6.90 ± 1.26***	13.54 ± 0.88###	13.48 ± 0.94	13.54 ± 0.87
Male	5.90 ± 1.23***, §	12.50 ± 1.50###, §	12.50 ± 0.88§	12.50 ± 0.87§
Female	6.50 ± 1.50***	13.80 ± 1.55###	14.35 ± 0.54	14.35 ± 0.88
Serum iron (μg/dL)	45.50 ± 26.62***	98.25 ± 30.51###	86.16 ± 30.79	89.00 ± 34.7
Male	43.50 ± 25.60***, §	95.25 ± 32.55###, §	83.30 ± 45.66§	86.50 ± 36.53§
Female	45.50 ± 22.30***	98.50 ± 30.35###	86.50 ± 30.75	89.80 ± 22.52
TIBC (μg/dL)	411.40 ± 62.22***	322.40 ± 45.18###	269.16 ± 14.60	275.43 ± 15.67
Male	411.40 ± 62.22***	320.20 ± 59.18###	260.30 ± 15.43§	275.40 ± 15.78§
Female	411.40 ± 62.68***	322.40 ± 30.88###	269.50 ± 14.56	275.88 ± 15.35

Data are expressed as mean ± SD
HB Hemoglobin, *TIBC* total iron binding capacity
***$P < 0.001$: baseline assessment of patients versus controls
###$P < 0.001$: before treatment assessment versus after treatment
§$P < 0.05$: males versus females

tests (MMSE, WMS-R, and WAIS-R). Significant improvements were identified after treatment compared to pretreatment values for MMSE, WMS-R and WAIS-R (Table 2). No gender difference in cognitive testing was identified.

ERPs latencies of the patients before treatment were longer than that of controls (N200 and P300). P200 and P300 amplitudes of the patients before treatment were significantly lower than that of the control group. The differences in latencies remained unchanged after iron intervention while a significant increase was found in P200 and P300 amplitudes ($P = 0.023$, 0.002 respectively) (Table 3). No gender difference in ERPs was identified.

Abnormal EEG findings were observed in 11 (55%) iron deficient anemic patients before treatment. The commonest abnormality was generalized paroxysmal high voltage delta activity, which was observed in 6 patients (30%). Diffuse slowing of background activity was observed in 5 (25%) patients and paroxysmal sharp activity in 4 (20%). EEG abnormalities observed after treatment in 7 patients (35%) who had abnormalities

Table 2 Comparison between patients (before and after treatment) in MMSE, WMS-R and WAIS-R and control group

Cognitive functions	Patients (before treatment)	Patients (after treatment) (mean ± SD)	Controls (first assessment)	Controls (after 3 months)	*P*-value for repeated measurement analysis
MMSE	21.75 ± 1.4**	24.22 ± 1.4	25.72 ± 2.1	26.04 ± 1.9	0.008
WMS-R					
Digit forward	5.35 ± 1.23**	5.90 ± 1.07##	6.36 ± 0.99	6.54 ± 0.98	0.113
Digit backward	2.65 ± 2.11**	3.50 ± 1.7#	4.68 ± 0.99	4.68 ± 0.99	0.017
Mental control	2.50 ± 1.93	2.50 ± 1.93	3.08 ± 1.49	3.09 ± 1.54	0.138
Logical memory I	8.75 ± 2.03***	9.50 ± 2.08##	11.58 ± 2.19	11.43 ± 2.41	0.001
Logical memory II	9.50 ± 1.50***	11.80 ± 1.7##	13.80 ± 2.00	13.50 ± 1.50	0.001
Associate learning	11.94 ± 3.93***	13.43 ± 3.54##	16.72 ± 3.28	17.00 ± 2.11	0.002
Visual reproduction I	3.23 ± 1.19*	3.45 ± 1.14#	3.76 ± 0.44	3.64 ± 0.34	0.47
Visual reproduction II	3.45 ± 1.30*	3.58 ± 1.52#	3.85 ± 0.75	3.73 ± 0.44	0.44
WAIS-R					
Information	5.55 ± 1.96***	6.45 ± 2.19#	11.68 ± 2.70	11.76 ± 3.21	0.05
Comprehension	10.45 ± 3.41**	11.40 ± 2.89	13.80 ± 3.35	13.90 ± 3.52	0.128
Digit span	6.45 ± 3.72**	7.00 ± 4.17	9.40 ± 1.68	9.76 ± 1.32	0.66
Arithmetic	5.15 ± 2.74**	7.25 ± 4.08#	7.71 ± 2.74	7.6 ± 2.3	0.03
Similarities	8.05 ± 1.70***	8.40 ± 2.76	13.12 ± 3.94	13.43 ± 2.34	0.86
Vocabulary	7.45 ± 2.44**	8.20 ± 1.90	10.00 ± 3.87	10.10 ± 2.54	0.08
Total verbal I.Q	78.65 ± 14.26***	85.75 ± 13.99##	109.00 ± 9.92	109.11 ± 9.81	0.005
Picture arrangement	7.80 ± 3.30	8.05 ± 1.96	8.88 ± 1.48	8.70 ± 1.43	0.86
Picture completion	6.65 ± 2.13***	7.00 ± 1.75	11.58 ± 3.05	11.45 ± 3.22	0.72
Block design	7.00 ± 2.0***	7.55 ± 2.14	9.32 ± 2.01	9.32 ± 2.32	0.28
Object assembly	7.75 ± 3.26	7.55 ± 3.65	8.00 ± 2.04	8.4 ± 2.34	0.88
Digit symbol	5.00 ± 2.85***	5.89 ± 2.79#	9.68 ± 3.35	9.88 ± 3.55	0.05
Total performance I.Q	77.00 ± 14.12***	79.75 ± 12.49	102.79 ± 12.60	102.89 ± 12.11	0.09
Total I.Q	77.85 ± 13.01***	82.65 ± 14.05##	106.37 ± 10.61	106.45 ± 11.23	0.001

Data are expressed as mean ± SD
MMSE mini-mental state examination, *WMS-R* Wechsler Memory Scale-Revised, *WAIS-R* Wechsler Adult Intelligence Scale-Revised
***$P < 0.0001$, **$P < 0.01$: baseline assessment patients versus controls
#$P < 0.05$, ##$P < 0.01$: before treatment assessment versus after treatment

Table 3 Event-related potential components in patients and control groups

Item	Patients (before treatment)	Patients (after treatment) (mean ± SD)	Controls (first assessment) group (mean ± SD)	Controls (after 3 months)	*P*-value for repeated measurement analysis
N_{100} latency (ms)	114.45 ± 37.65	119.30 ± 42.29	108.84 ± 12.43	111.34 ± 21.21	0.17
N_{200} latency (ms)	243.85 ± 26.57*	240.65 ± 57.29	226.80 ± 13.59	228.99 ± 34.21	0.67
P_{200} latency (ms)	170.55 ± 31.21	183.60 ± 35.03	163.60 ± 16.19	169.99 ± 20.26	0.23
P_{200} amplitude (mv)	7.59 ± 3.82	11.86 ± 6.90$^{\#}$	9.59 ± 8.57	10.32 ± 11.34	0.023
P_{300} latency (ms)	330.30 ± 34.69*	329.45 ± 29.48	314.80 ± 10.50	320.76 ± 9.76	0.68
P_{300} amplitude (mv)	10.36 ± 5.69*	17.83 ± 8.43$^{\#\#}$	14.99 ± 8.61	16.89 ± 9.76	0.001

Data are expressed as mean ± SD
*$P < 0.05$, **$P < 0.01$: baseline assessment patients versus controls
$^{\#}P < 0.05$, $^{\#\#}P < 0.01$: before treatment assessment versus after treatment

before treatment. The improvement was observed in background and percentage of paroxysmal activity. Overall significant decrease in percentage of EEG abnormalities was observed after treatment (Table 4). While no such changes were found in control group either in the first or second assessment.

Cognitive achievement showed significant correlation with HB level. Significant positive correlation was found between HB levels and MMSE, total verbal IQ and performance IQ (before treatment and after treatment), while no such correlation was observed with serum iron (Table 5). Significant negative correlation were found between P300 latency of the patients before treatment and total IQ of WAIS-R (before treatment: $r = -0.487$, $P = 0.03$, after treatment: $r = -575$, $P = 0.008$) and HB level. A significant positive correlation was found between P300 amplitude of the patients and total IQ (before treatment: $r = 0.491$, $P = 0.028$, after treatment: $r = 680$, $P = 0.01$) and serum iron.

Discussion

Iron deficiency anemia (IDA) is the most common type of anemia and mostly easily treatable. Several clinical studies suggest a significant influence of IDA on dynamic properties and functional features of the central nervous system activity. It has been suggested that cognitive achievement is strongly related to hemoglobin (HB) level in both anemic and non-anemic persons, i.e. higher HB level results in better CNS function [33]. Although, several clinical studies demonstrated significant positive correlation between HB level and various aspects of cognitive function and intellectual performance between anemic and non-anemic children and adolescents [30, 43, 45], however, in adults, clear evidence of an association between anemia and cognitive function have not been found. In the present study, the influences of IDA and iron supplementation on cognitive functions were assessed through different neuropsychological and neurophysiological tests. The results described in this study were rather conclusive and suggested that IDA had great influences on cognitive functions and cerebral activity that iron supplementation improved many of these effects in young adults. These are supported by the following data:

Firstly, patients with IDA suffered from easy fatigability, reduction in physical work capacity, cold intolerance and problems in concentration. Treatment of patients with iron resulted in amelioration of

Table 4 EEG abnormalities in iron deficient anemic patients before and after treatment

Item	Patients (before treatment)	Patients (after treatment)
Normal EEG	9 (45%)	13 (65%)
Abnormal EEG	11 (55%)	7 (35%)
Diffuse slowing of background activity	5 (25%)	2 (10%)
Focal slowing	0	0
Focal sharp or spike activity	0	0
Generalized paroxysmal activity	10 (50%)	6 (30%)
High voltage delta activity	6 (30%)	3 (15%)
Sharp activity	4 (20%)	3 (15%)
Spike wave complex	0	0

Data are expressed as number (%)
EEG Electro-encephalography

Table 5 Significant correlation (*r* and *P*-value) between HB and iron and different psychometric and neurphysiological testing

	HB		Iron	
	r	*P*	*r*	*P*
MMSE				
Before treatment	**0.654**	**0.01**	**0.450**	**0.047**
After treatment	**0.430**	**0.05**	**0.345**	**0.050**
Total verbal IQ score				
Before treatment	**0.462**	**0.04**	0.365	0.07
After treatment	**0.440**	**0.04**	0.250	0.40
Total performance IQ score				
Before treatment	**0.444**	**0.05**	0.350	0.09
After treatment	**0.470**	**0.04**	0.270	0.60
P300 latency				
Before treatment	**−0.46**	**0.04**	−0.450	**0.047**
After treatment	**−0.550**	**0.01**	−0.760	**0.001**
P300 amplitude				
Before treatment	0.264	0.70	0.750	**0.001**
After treatment	0.346	0.09	0.650	**0.01**

HB Haemoglobin
*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$

these symptoms. Ballin and Colleagues [2] found that iron-treated adolescent girls reported decreased lassitude and impairment in mood and ability to concentrate. Nelson et al. [29] suggested that in the short term, the consequences of poor iron status among British adolescent girls may well relate to learning ability and poor physical fitness. Beard et al. [5] reported a strong relation between iron status and depression, stress and cognitive functioning in poor African mothers during the postpartum period. Both studies support the use of iron supplementation. Many treatment trials has showed an association between iron treatment and measures of lassitude, fatigue, ability to concentrate and memory, suggesting that cognitive and behavioral domains do respond directly to an improvement in iron status [2, 5, 6, 17].

Secondly, significant impairments of MMSE and most items of WMS-R, beside the impairment of WAIS-R were found in patients with IDA. These findings provided an evidence of the relationship between IDA and poor cognitive performance related to memory. IDA has deleterious effects not only on memory but also in verbal and performance IQ. These findings strengthen the suggestion that IDA had a great negative effect on intelligence. Iron intervention was associated with partial but significant improvement in most of these items. The partial improvement with iron supplementation could be attributed to the short time follow up in our study and/or the involvement of other nutritional deficiencies or biological alterations associated with the original etiology (e.g. other nutritional or vitamin deficiencies with pregnancy and parasitic infestation, hormonal alteration with pregnancy and lactation and deleterious effect of parasites itself on the brain). Moreover, scores of several measures of psychometric tests (verbal and performance IQ) changed parallel with changes in HB level. However no such correlations were found with serum iron. This mean that the improvement observed in our study is related to the improvement in anemia itself after iron intervention [1]. Pollitt [37] described several studies in Indonesia and Thailand in which iron supplementation for adolescents with poor iron status resulted in significant improvements in tests related to specific components of cognition, for example familiar figures test or general tests of academic ability. In the study of Bruner et al. [6] on 81 non-anaemic iron deficient adolescent girls in high school, iron supplementation was found to improve verbal learning and memory but had no effect on measures of attention. Murray-Kolb and Beard [28] evaluated cognitive abilities in a group of women in their reproductive age having iron deficiency (anemic and non-anemic). The authors found that the severity of iron deficiency is proportionately adversely affects processing speed and accuracy of cognitive function over a broad range of tasks. The authors observed that iron supplementation resulted in improvement by 5–7 folds in cognitive performance assessed by Detterman's Cognitive Abilities Test. They suggested that improvement in HB and serum ferritin was related to improved speed in completing the cognitive tasks. Groner and colleagues [17] did a double blind randomized study on young pregnant women aged 14–24 years attending the hospital for prenatal care, at or before 16 weeks gestation and received vitamins and iron or vitamins alone, and their haematological status, memory and attention tests were assessed at entry and at the end of 1 month's treatment. The authors demonstrated that iron treatment resulted in an improvement in the Digit Symbol, short-term memory and attention span cognitive tests. Beard et al. [5] in their study on poor African mothers during the postpartum period reported that treatment with iron resulted in a significant improvement in previously iron-deficient mothers Raven's progressive matrices test scores as well as their Digit Symbol test scores. These iron-treated mothers had a 25% improvement in scores on the Raven's test and their scores were nearly identical to those of control non-anemic mothers at 9 months.

In contrast to our study, Nelson et al. [31] in their study on 143, 11–12 year-old school children from North London, failed to show significant associations between iron intakes and cognitive function based on the Heims AH4 test or two components digit span and coding of the WAIS-R. Southon et al. [44] in study of 51 children, 13–14 years-old living in Norwich, reported no relationships between initial iron status (based on dietary or biochemical assessments) and results from the WAIS-R tests. They found no effect of multi-vitamin and mineral supplements given daily for 16-weeks. However, in their study no attempt was made to evaluate iron status or iron supplementation on cognitive function. Fordy and Benton [14] in their study in London on a group of young adults (average age 20 years) to determine whether low iron status influences psychological functioning, found that 3.8% of males and 31.8% of females had ferritin levels <12μg/l. However, from the five tests of cognitive function administered there was no significant difference between the anemic and non-anemic groups. It was considered that the presence of ferritin levels below the accepted normal range provided little grounds for concern.

Thirdly, significant impairment of N200 and P300 latencies and P200 and P300 amplitudes of ERPs were identified in patients with IDA with significant improvement after iron supplementation. The hippocampus, thalamus and frontal cortex were considered as possible locations of the P300 generators [15], these structures are important for learning and memory. P300 latency has been found to increase as the dementia symptoms increase [35], while P300 amplitude was depressed overall for all levels of dementia [36]. P300 latency is considered a consequence of attention process, speed of reaction and

immediate memory [34]. The significant correlation between P300 latency and amplitude of the patients before treatment and total IQ suggested that ERPs are sensitive indicators of subtle changes in cognition. The shorter P300 latencies indicate superior mental performance relative to longer latencies while an increase in P300 amplitude is associated with better cognitive performance. A significant positive correlation was found between P300 amplitude of the patients and serum iron may suggest the role of iron in cognitive performance. The significant association between P300 latency of the patients before treatment and HB level suggest that HB level has an important role in attention and recent memory. Shi et al. [42] assessed ERPs in 70 school aged children with asymptomatic IDA based on low HB and either low serum ferritin or high erythrocyte protoporphyrin levels. Children were randomized into treatment and placebo groups of 35 cases each. Pre-treatment, IDA group had prolonged P300 latencies in comparison with non-IDA controls. After treatment, IDA group showed a significant increase in HB levels and shortening in P300 latencies. The authors concluded that nutritional iron deficiency lowers brain iron and interferes with protein synthesis in this organ. Researches in rodent models have found that highest brain concentrations of iron are found in dopaminergic structures and the dopaminergic neurons are co-localized with iron throughout the brain. In iron deficient rats, there was reduction in the number and function of dopamine D_2-receptor, elevation in the extracellular dopamine and norepinephrine in brains and alteration in the density of D_2 and D_1 receptors and dopamine transporters [4]. Intact dopaminergic systems are known to be important in attention and learning processes. Hence, the resultant behavioral changes due to the reduction of dopaminergic activity in iron-deficient animals may go some way to explain the adverse effects of iron deficiency (ID) on cognition, behavioral patterns, learning and attention and changes in ERPs [40, 48].

Fourthly, The percentage of abnormal EEG findings before iron intervention was decreased from 55 to 35% after intervention. These findings suggest that IDA have great influence on brain activity and or a developmental lag as iron is an essential participant in the development of the neuronal dendritic tree [51]. Similar findings were observed by Otero et al. [32] who assessed psychological and electroencephalographic changes in school children with ID and found that the EEG power spectrum of iron deficient children had a slower activity than in iron replete.

The mechanism(s) by which iron deficiency alters cognition and behavior in adults is largely unexplored. All previously mentioned significant abnormalities in different neuropsychological and neurophysiologic tests in IDA and the reversibility of some abnormalities 3 months later, provided strong evidence that there is a relationship between IDA and cognitive defects. Animal studies with adult onset ID were not conclusive regarding neural functioning [4]. Electrophysiologic recordings showed increased asymmetry related to serum ferritin in adults but no relation to anemia per se [46]. Neurotransmitter metabolism was altered in 2 different studies of iron-deficient adult women but the relation to cognition and behavior was not explored [3, 26]. In general, several mechanisms by which iron deficiency may contribute to cognitive impairment have been discussed:

1. Iron may have selective actions on certain brain receptors and interfere with certain brain neurotransmission mechanisms that lead to cognitive impairment, attention and memory processing [33, 49].
2. In advanced stages of ID, the HB is compromised with systemic effects that may impair cognition, e.g., brain hypoxia. Anemia via cerebral hypoxia and other possible mechanisms have been suggested to have great influence on cognition [29].
3. Also, combination of the above-mentioned mechanisms may be a possible mechanism for cognitive deficits caused by iron deficiency anemia.

Conclusions

The importance of iron status in cognition is not limited to the developing brain but it is a significant factor in cognitive performance in different age groups. As IDA is a common treatable condition, further research should be conducted at a community basis to determine the prevalence of cognitive impairment due to IDA in the adult group of population, its functional consequences, risk factors and recommended preventive strategies. It is important to determine and treat IDA to avoid its adverse consequences on cognitive abilities and improve the quality of life in adult group of population.

References

1. Agaoglu L, Torun O, Unuvar E, Sefil Y, Demir D (2007) Effects of iron deficiency anemia on cognitive function in children. Arzneimittelforschung 57(6A):426–30
2. Ballin A, Berar M, Rubinstein U (1992) Iron state in the female adolescent. Am J Dis Child 146:803–805
3. Beard JL, Borel MJ, Derr J (1990) Impaired thermoregulation and thyroid function in iron deficiency anemia. Am J Clin Nutr 52:813–819
4. Beard JL, Connor JR, Jones BC (1993) Iron in the brain. Nutr Rev 51:157–170
5. Beard JL, Hendricks MK, Perez EM, Murray-Kolb LE, Berg A, Vernon-Feagans L, Irlam J, Isaacs W, Sive A, Tomlinson M (2005) Maternal iron deficiency anemia affects postpartum emotions and cognition. J Nutr 135:267–272

6. Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J (1996) Randomized study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. Lancet 348:992–996
7. Cook JD (1995) Iron supplementation: is less better? Lancet 346:587
8. de Deungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK (2000) Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. Pediatr Res 48:169–176
9. De Maeyer E, Adiels-Trasman M (1985) The prevalence of anemia in the world. World Health Stat Q 38:302–316
10. Demetri GD (2001) Anaemia and its functional consequences in cancer patients: current challenges in management and prospects for improving therapy. Brit J Cancer 84(Suppl 1):31–37
11. Dobbing J (1990) Vulnerable periods in developing brain. Brain, behavior, and iron in the infant diet. Springer, London, pp 1–25
12. Felt BT, Beard JL, Schallert T, Shao J, Aldridge JW, Connor JR, Georgieff MK, Lozoff B (2006) Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for perinatal iron deficiency anemia in rats. Brain Behav Res 171(2):261–70
13. Folstein MF, Folstein SE, McHugh PR (1975) 'Mini-Mental State': a practical method for grading the cognitive state of patients for the clinician. J Psychiatry Res 12:189–198
14. Fordy J, Benton D (1994) Does low iron status influence psychological functioning? J Hum Nutr Diet 7:127–133
15. Fukai M, Motomura N, Kobayashi S, Asaba H, Sakai T (1990) Event-related potential (P300) in epilepsy. Acta Neurol Scand 82:197–202
16. Grantham-McGregor S, Ani C (2001) A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr 131:649S–668S
17. Groner NA, Holtzman NA, Charney E, Mellits D (1986) A randomized trial of oral iron on tests of short-term memory and attention span in young pregnant women. J Adolesc Health Care 7:44–48
18. Hansch EC, Syndalko K, Cohen SN, Goldberg ZI, Potvin AR, Touretellotte WW (1982) Cognition in Parkinson's disease: an event related potential prespective. Ann Neurol 11:599–607
19. Idjradinata P, Pollitt E (1993) Reversal of developmental delays in iron-deficient anaemic infants treated with iron. Lancet 341:1–4
20. Jernigan TL, Trauner DA, Hesselink JR, Talla PA (1991) Maturation of the human cerebellum observed in vivo during adolescence. Brain 11:2037–2049
21. Jorgenson LA, Wobken JD, Georgieff MK (2003) Perinatal iron deficiency alters apical dendritic growth in hippocampal CA1 pyramidal neurons. Dev Neurosci 25:412–420
22. Kretch MJ, Fong AK, Green MW, Johnson HL (1998) Cognitive function, iron status, and hemoglobin concentration in obese dieting women. Eur J Clin Nutr 52(7):512–518
23. Lozoff B, Beard J, Connor J, Felt B, Georgieff M, Schallert T (2006) Long-lasting neural and behavioral effects of iron deficiency in infancy. Nutr Rev 64:S34–S43
24. Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, Jimenez R, Mora LA, Gomez I, Krauskoph D (1987) Iron deficiency anemia and iron therapy: effects on infant developmental test performance. Pediatrics 79:981–995
25. Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P (2003) Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. Pediatrics 112:846–854
26. Lukaski HC, Hall CB, Nielsen FH (1992) Thermogenesis and thermoregulatory function of iron-deficient women without anemia. Aviat Space Environ Med 61:913–920
27. McCann JC, Ames BN (2007) An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioral function. Am J Clin Nutr 85(4):931–945
28. Murray-Kolb LE, Beard JL (2007) Iron treatment normalizes cognitive functioning in young women. Am J Clin Nutr 85(3):778–787
29. Nelson CA, Wewerka S, Thomas KM, Tribby-Walbridge S, deRegnier RA, Georgieff M (2000) Neurocognitive sequelae of infants of diabetic mothers. Behav Neurosci 114:950–956
30. Nelson M (1996) Anaemia in adolescent girls: effects on cognitive function and activity. Proc Nutr Soc 55:359–367
31. Nelson M, Naismith DJ, Burley V, Gatenby S, Geddes N (1990) Nutrient intakes, vitamin, mineral supplementation, and intelligence in British school children. Br J Nutr 64:13–22
32. Otero GA, Aguirre DM, Porcayo R, Fernandez T (1999) Psychological and electroencephalographic study in school children with iron deficiency. Int J Neurosci 99(1–4):113–121
33. Petranovic D, Batinac T, Petranovic D, Ruzic A, Ruzic T (2008) Iron deficiency anaemia influences cognitive functions. Med Hypotheses 70(1):70–72
34. Polich J (1991) P300 in the evaluation of aging and dementia. In: Brunia M, Mulder G, Verbaten MN (eds) Event-related brain potential research; EEG supplement 42. Elsevier, Amsterdam
35. Polich J, Ehlers CL, Otis S, Mandell AJ, Bloom FE (1986) P300 latency reflects the degree of cognitive decline in dementing illness. Electroencephalogr Clin Neurophysiol 63:138–144
36. Polich J, Starr A (1984) Evoked potentials in aging. In: Albert ML (ed) Clinical neurology of aging. Oxford University Press, New York, pp 149–177
37. Pollitt E (1993) Iron deficiency and cognitive function. Ann Rev Nutr 13:521–537
38. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK (2003) Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. J Nutr 133:3215–3221
39. Wechsler D (1987) Wechsler memory scales-revised. Psychological Cooperation, New York
40. Schallert T, Petrie BF, Whishaw IQ (1989) Neonatal dopamine depletion: spared and unspared sensorimotor and attentional disorders and effects of further depletion in adulthood. Psychobiology 17:386–396
41. Sen A, Kanani SJ (2006) Deleterious functional impact of anemia on young adolescent school girls. Indian Pediatr 43(3):219–226
42. Shi TH, Yu LF, Huang LZ, Ma XH, Zhu QH (1999) A pilot study on the changes of event-related potentials in school-aged children with iron deficiency anemia. Asia Pacific Journal of Clinical Nutrition 8(2):91–95
43. Soewondo S, Husaini M, Pollitt E (1989) Effects of iron deficiency on attention and learning processes in preschool children: Bandung, Indonesia. Am J Clin Nutr 50:667–674
44. Southon S, Wright AJA, Finglas PM, Bailey AL, Longbridge JM, Walker AD (1994) Dietary intake and micronutrient status of adolescents: effect of vitamin and trace element supplementation on indices of status and performance in tests of verbal and non-verbal intelligence. Br J Nutr 71:897–918
45. Stoltzfus RJ, Mullany L, Black RE (2004) Iron deficiency anaemia. In: Ezzati M, Lopez AD, Rodgers A, Murray CJL (eds) Comparative quantification of health risks: global and regional burden of disease attributable to selected major risk factors, vol 1. World Health Organization, Geneva, Switzerland, pp 163–209
46. Tucker DM, Sandstead HH, Swenson RA, Sawler BG, Penland JG (1982) Longitudinal study of brain function and depletion of iron stores in individual subjects. Physiol Behav 29:737–740
47. WechsLer D (1981) Manual for Wechsler adult intelligence scale-revised. Psychological Cooperation, New York
48. Whishaw IQ, Funk DR, Hawryluk SJ, Karbashewski ED (1987) Absence of sparing of spatial navigation, skilled forelimb and tongue use and limb posture in the rat after neonatal dopamine depletion. Physiol Behav 40:247–253
49. Wigglesworth JM, Baum H (1988) Iron dependent enzymes in the brain. In: Youdim MBH (ed) Brain iron: neurochemical and behavioural aspects. Taylor and Francis, New York, pp 25–66
50. World Health Organization (1992) The prevalence of anemia in women: a tabulation of available information, 2nd edn. WHO/MCH/MSM/92.2
51. Youdim MBH (1985) Brain iron metabolism: biochemical and behavioural aspects in relation to dopaminergic neurotramission. In: Lajtha A (ed) Handbook of neurochemistry, vol 10. Plenum Press, New York, pp 731–756