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Brain Derived Neurotrophic Growth Factor and Cognitive Function in Children with Iron Deficiency Anemia

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Authors' contributions

This work was carried out in collaboration between all authors. Author MFN designed the study, supervised its execution, performed the data interpretation, and wrote final manuscript. Author NTY wrote the protocol, supervised the whole steps of the study and helped revising the manuscript. Author JFN was responsible for performing and interpreting the cognitive function test and editing the relevant part in the manuscript. Author SEEA did the laboratory assessment and wrote the relevant part in the manuscript. Author BMM enrolled the patients, executed the study procedures and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Background: The negative effects of iron deficiency anemia (IDA) on cognition are well documented in previous studies; however, distinct mechanisms of these effects are still a matter of debate. Brain derived neurotrophic factor (BDNF) has an important role in brain functions specially memory and learning and was reported to be low in IDA in animal studies.
Objective: This study was thus designed to estimate BDNF serum level in patients suffering from IDA and correlate its level to their cognitive function.
Study Design: Cross sectional case-control study.
Place and Duration of the Study: The Outpatient Clinic in the Children`s Hospital, Ain Shams University, between May, 2009 and March, 2010.

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Methods: The study was conducted on 27 child suffering from IDA (hemoglobin <11gm/dl and serum ferritin<12ng/ml) with a mean age of 7.96±3.06 years. Full history taking and thorough clinical examination were done. Complete blood count, serum ferritin and BDNF were measured. Cognitive assessment using Wechsler intelligence test was performed for enrolled children. Fifty healthy age and sex matched children were enrolled as controls.
Results: Wechsler intelligence test showed significantly lower verbal, performance and total IQ values among IDA patients compared to controls. Total IQ score showed significant positive correlation with hemoglobin level and significant negative correlation with red cell width (RDW). Although BDNF level was not significantly lower in IDA patients, it showed a significant positive correlation with object assembly. Multiple regression analysis using total IQ as a dependent variable showed that RDW was the most determinant factor that affected IQ scores.
Conclusion: Wechsler IQ test results were adversely affected in IDA patients. BDNF level was not significantly lower in IDA children but it showed a significant positive correlation with one of Wechsler IQ test items in such patients. Larger scale studies are recommended to further investigate BDNF as a possible mediator that disturb cognitive functions in IDA and explore other mediators.

Keywords: BDNF; cognition; iron deficiency anemia; Wechsler intelligence test.

1. INTRODUCTION

A systematic analysis of global anemia burden from 1990 to 2010 for 187 countries, both sexes, and 20 age groups showed global anemia prevalence of 32.9% in 2010 and that iron deficiency anemia (IDA) was the top cause globally [1].

Iron deficiency (ID) is the most common and widespread nutritional disorder in the world. As well as affecting a large number of children and women in developing countries, it is the only nutrient deficiency which is also significantly prevalent in Industrialized Countries. In developing countries about 40% of preschool children are estimated to be anemic [2]. According to the Egyptian Health and Demographic surveys, between 2000 and 2005 the prevalence of anemia increased from 37.04% to over 52% among 12 to 36 months old Egyptian children [3].

Iron exerts its heaviest overall toll in terms of ill-health, premature death and lost earnings. It reduces the work capacity of individuals and entire populations, bringing serious economic consequences and obstacles to national development [2]. Several studies in animals and humans have clearly demonstrated the effect of ID on development, cognition, behavior and neurophysiology. ID has repercussions in the perinatal period, infancy and childhood. Some effects are irreversible while other defects may be corrected, making timing of ID in a child critical [4-6].

Specific growth factors, such as IGF-I/II and brain-derived neurotrophic factor (BDNF), have an essential role in cognition, particularly in processes involving learning and memory, by the activation of intracellular-signaling pathways involved in cell proliferation, differentiation, and survival [7]. BDNF also regulates plasticity-related processes underlying memory and learning [8]. It is known that nutritional deficiencies promote reductions in systemic and CNS concentrations of growth factors, such as IGF-I/II and BDNF and that altered expression of these molecules and their receptors in the CNS leads to psychomotor and developmental

deficits [7]. Animal studies previously reported that IDA lowers BDNF function and impairs neuronal differentiation in the hippocampus [9].

The present study was designed to estimate BDNF serum levels in children suffering from IDA. A secondary objective was to explore its relation to the cognitive status of those children.

2. MATERIALS AND METHOD

2.1 Study Population

Twenty seven children were enrolled in this cross sectional case-control study. They presented to the Outpatient Clinic in the Children's Hospital, Ain Shams University Cairo, Egypt with symptoms and signs suggestive of iron deficiency anemia (IDA). IDA was confirmed by laboratory investigations (Hemoglobin<11gm/dl [10] and serum ferritin level less than 12ng/ml [11]). The enrolled children were 3 to 12 years old, having negative family history of hemolytic anemia and free from any neurologic or chronic disease or infection. Fifty clinically healthy age and sex matched children were enrolled from healthy sibs who were accompanying patients as controls. All patients and controls were inhabitants of Al-Abbasia district, Cairo, Egypt who usually seek medical care in Ain Shams University Hospitals.

After the approval of the pediatric board of the Children's Hospital, Ain Shams University; a clear explanation about the nature and procedure of the study and the role of the participated children was given to enrolled children and their parents or caregivers and an informed written consent was obtained (an informed verbal consent was obtained if parents were illiterate).

All enrolled children were subjected to:

- Full history taking with special emphasis on the education and occupation of parents in order to assess the social status according to Park and Park score [12] and symptoms of IDA.
- Thorough clinical examination laying stress on anthropometric measurements [weight and height]. Body mass index (BMI) was estimated, followed by calculation of z-score values of weight for age, height for age and BMI for age according to CDC Growth Charts,2000 [13] and signs of IDA.

2.2 Laboratory Investigations

Venous blood samples were taken under complete aseptic conditions using sterile dry disposable syringes and were put in two clean tubes. One tube contained EDTA for complete blood picture assessment using Coulter AC-T10 Hematology Analyzer, and the other was left to clot then centrifugation was performed at 3000g for 15 minutes at room temperature to assess of serum ferritin [14] as well as BDNF. The latter was measured by ELISA technique [15] using the Quantikine Human BDNF Immunoassay supplied by R&D Systems, Inc. Minneapolis, USA.

2.3 IQ Assessment

All children were evaluated for their verbal and performance (non-verbal) IQ using Wechsler Intelligence Scale for Children (WISC*)* [16]. The child's verbal IQ score was derived from scores on several subtests which are; information, digit span, arithmetic, comprehension, vocabulary and similarities. On the other hand, the child's performance (non-verbal) IQ was derived from scores on other subtests which are; picture completion, picture arrangement, block design, object assembly and coding search. Scores on the performance subtests were based on both the speed of response and the number of correct answers. Parents or caregivers were allowed to attend the test without participation as they had no role in conducting the test. Each test item was first explained to the child, his response modified repeatedly to make the child familial to the test before starting. Time of whole test ranged between 25-40 minutes.

2.4 Statistical Analysis

Standard computer program SPSS for Windows, release 13.0 (SPSS Inc, USA), was used for data entry and analysis. Since some variables were not normally distributed, all variables are presented as median (interquartile range), as well as mean±standard deviation (SD). Comparison of different variables in various groups was done using student t and Mann Whitney tests for normal and nonparametric variables respectively. Pearson's and Spearman's correlation test were used for correlating normal and non-parametric variables respectively. Chi-square test was used to compare frequency of qualitative variables among different groups. Multiple regression analysis was also performed to determine effect of various factors on a dependent variable. For all tests a probability (p) less than 0.05 was considered significant [17].

3. RESULTS AND DISCUSSION

3.1 Results

In the current study, there were no significant differences between IDA patients and controls regarding age, sex and social status Table 1.

Pallor and anorexia were the commonest manifestations among IDA children (100%), followed by easy fatigability (88.8%) and tachycardia (77.7%) then pagophagia (51.8%).

Regarding anthropometric measurements, both IDA patients and controls were within the normal range of weight, height and BMI according to their age and sex with no significant differences between patients and controls Table 1.

Regarding laboratory parameters, IDA patients had significantly lower values of hemoglobin (Hb %), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) and significantly higher red cell width % (RDW %) values, compared to controls. Further, significant lower serum ferritin was found in IDA patients compared to control subjects Table 2.

There was no statistical difference between IDA patients and controls regarding BDNF level Fig. 1.

The results of Wechsler intelligence test showed significantly lower values of verbal, performance and total IQ scores among IDA patients compared to the controls Table 3.

Correlation studies revealed that total IQ score had a positive significant correlation with Hb % (r=0.93, P<.001) and a negative significant correlation with RDW % (r=0.99, P<.001). Further, BDNF level showed a positive significant correlation with object assembly Fig. 2.

Multiple regression analysis using total IQ as a dependent variable showed that RDW was the most determinant factor affecting IQ scores (t=4.61, P< .001).

Table 1. Comparison between IDA patients and controls regarding demographic and clinical data

Variable	IDA patients N= 27 (mean±SD)	Controls N= 50 (mean±SD)	Test	P-value
Age (years)	7.96±3.06	7.50±3.05	1.136*	0.259
Sex				
Males	15 (55.6%)	30 (60.0%)	0.018•	0.892
Females	12 (44.4%)	20 (40.0%)		
Social status				
Low	10 (37.04%)	12 (24.0%)		
Middle	7 (25.92%)	20 (40.0%)	2.036•	0.360
High	10 (37.04%)	18 (36%)		
Anthropometry				
Wt Z-score	1.09±0.35	1.12±0.26	0.427*	0.670
Ht Z-score	0.96±0.81	0.98±0.74	0.109*	0.913
BMI Z-score	0.87±0.52	0.71±0.33	1.650*	0.103

*Wt: weight, Ht: height, BMI: body mass index, IDA: iron deficiency anemia *: Independent t-test; •: Chi-square test*

Table 2. Comparison between IDA patients and controls regarding blood picture and serum ferritin

Variable	IDA patients N= 27 (mean±SD) (median [IQR])	Controls N= 50 (mean±SD) (median [IQR])	t/z*	P value
HB (g/dl)	8.62±0.83 8.90 [1.00]	12.36±0.69 12.10 [1.00]	5.21*	<.001
MCV (fl)	74.33±4.92 76.00 [5.00]	81.68±4.20 83.00 [7.00]	-4.75	<.001
MCH (pg/ml)	23.61±1.70 23.00 [2.00]	28.75±2.18 28.00 [3.00]	5.23*	<.001
RDW (%)	16.84±1.20 16.00 [2.00]	13.20±1.25 13.0 [2.00]	5.05*	<.001
Serum ferritin (ng/ml)	1.89 ±1.47 1.98 [2.90]	28.23±12.63 30.15 [21.00]	11.09	<.001

*HB: Hemoglobin concentration, MCV: Mean corpuscular volume, fL: Femtoliter, MCH: Mean corpuscular hemoglobin, pg/ml: Pictogram per milliter, RDW: Red cell distribution width, ng/ml: nanogram per milliter, IDA: iron deficiency anemia, IQR: Interquartile range, SD: Standard deviation. t: Independent t-test, * z: Mann-Whitney test for non parametric data*

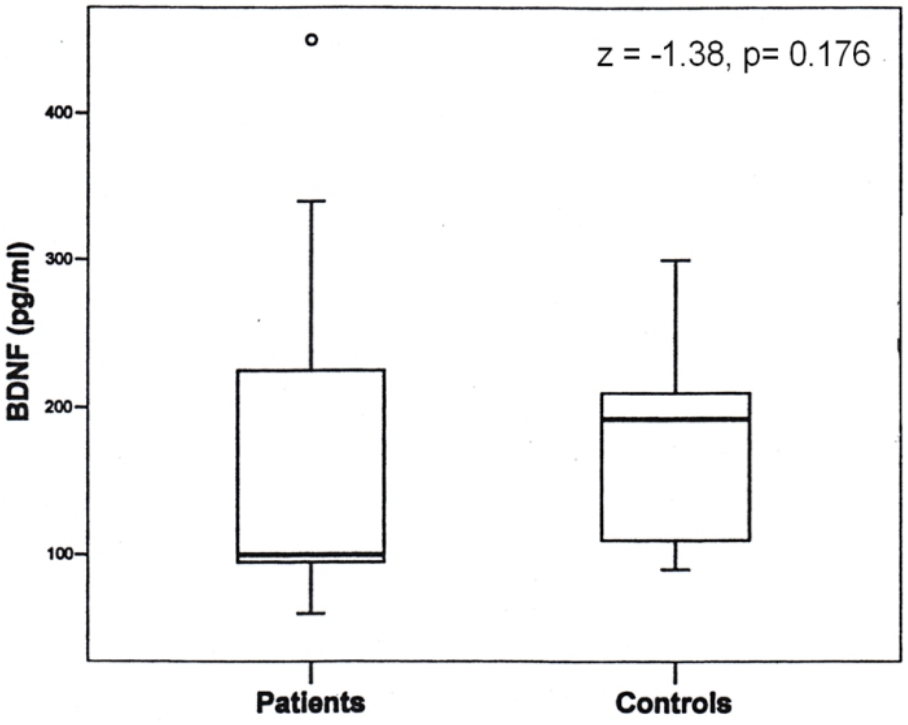

Fig. 1. Comparison between IDA patients and controls regarding BDNF serum levels

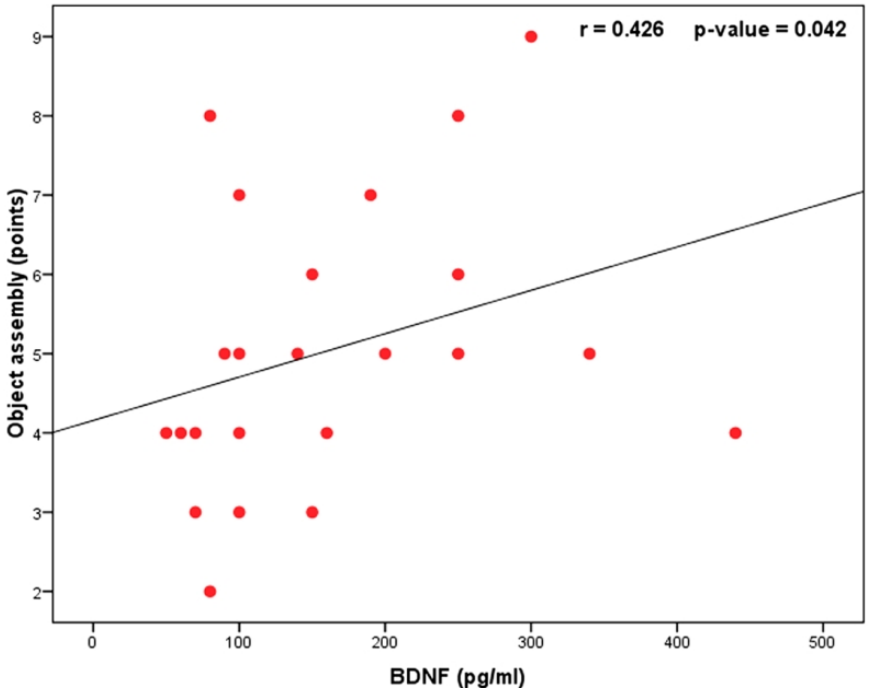

Fig. 2. Correlation between BDNF serum levels and object assembly test

Table 3. Comparison between IDA patients and controls regarding Wechsler IQ

Variable	IDA patients N= 27 (mean±SD (median [IQR])	Controls N= 50 (mean±SD) (median [IQR])	t/z*	P value
Information	7.70±2.26 8.00 [3.00]	11.14±2.93 10.50 [5.00]	-4.16	<.001
Comprehension	6.70±1.83 6.00 [3.00]	8.14 ±3.15 8.00 [5.00]	1.52*	.135
Arithmetic	6.67±2.21 7.00[4.00]	9.86±4.38 12.00[8.00]	1.46*	.151
Similarities	10.07±2.48 10.00[3.00]	12.07±3.02 12.50[5.00]	-2.26	.029
Vocabulary	5.33±1.61 5.00[2.00]	8.86±2.72 9.00[4.00]	-4.15*	<.001
Digit span	5.22±2.13 5.00[4.00]	3.93±1.07 4.00[1.00]	-1.84*	.071
Verbal IQ	42.81±5.89 41.00[7.00]	53.93±12.60 55.50[22.00]	2.93*	.003
Picture completion	8.59±3.63 41.00[7.00]	9.29±2.48 9.00[5.00]	-1.17*	.246
Picture arrangement	7.26±1.25 7.00[2.00]	9.07±2.01 10.00[3.00]	-2.91-	.004
Block design	9.26±2.30 9.00[3.00]	8.71±2.30 10.00[4.00]	-0.57*	.577
Object assembly	5.04±1.65 5.00[2.00]	8.14±2.07 8.50[4.00]	-3.92*	<.001
Performance IQ	30.±5.65 30.00[8.00]	35.07±7.64 33.50[13.00]	-2.30	.027
Total IQ	80.19±6.18 79.00[8.00]	92.07±10.43 91.00[20.00]	-3.47*	<.001

*IQ: Intelligence equation, IQR: Interquartile range, SD: Standard deviation. t: Independent t-test , * z: Mann-Whitney test for non parametric data*

3.2 Discussion

In the current study, no significant difference was found regarding socioeconomic status between IDA patients and controls; although low social class has been claimed in the past years to be one of the causes of IDA in developing countries due to poor diet quality and low bioavailability of dietary iron [18,19]. Likewise, in developed countries the widespread availability of dietary energy is not always matched with proportional availability of micronutrients due to high proportion of empty calories in the modern urban diet [20]. This might obviate the impact of social status on prevalence of IDA.

Also, patients and controls were comparable regarding age and sex adjusted anthropometric measures. Similar to these findings, a study conducted in Brazil on 240 preschool children showed no significant association between vitamin A, iron and zinc deficiencies and the anthropometric indices studied [21]. Moreover, meta-analysis of randomized controlled trials found no significant effect of iron intervention on gestational, infant or child physical growth. [22].

Significantly lower scores in verbal, performance and total IQ were obvious in our series of IDA patients compared to controls. Furthermore, significant correlations were detected between total IQ and CBC parameters namely Hb % and RDW %. The later was the most determinant factor that affected IQ scores, by regression analysis. IDA has been found to affect brain metabolism, neurotransmitter function, and myelination [4]. A recent study by Muñoz and Humeres [5] on humans and rodents found that iron plays an important physiological role in neuronal processes such as myelination, synaptogenesis, behavior and synaptic plasticity (SP), that may produce lasting neurological consequences even after correction of ID. They demonstrated changes in the hippocampus, striatum, amygdale or prefrontal cortex, in addition to interaction among these systems. They found that cognitive alterations were correlated with changes in neural plasticity which is the possible cellular substrate of memory and learning. Nevertheless, there was no convincing evidence that iron treatment of young children with IDA has an effect on psychomotor development or cognitive function within 30 days after commencement of therapy, yet the effect of longer-term treatment remains unclear [6].

BDNF level was not statistically different between IDA patients and controls. Nevertheless, BDNF level showed a significant positive correlation with object assembly in those patients. A recent study reported that iron deficiency may induce cognitive deficits by decreasing the expression and function of specific growth factors IGF-I/II and BDNF in specific areas of the brain [7]. Another study found that attenuation of activity-dependent BDNF expression significantly impairs spatial memory reversal and contextual memory extinction, two executive functions that require intact hippocampal-prefrontal cortex (PFC) circuitry. Thus, activity-dependent BDNF expression in the hippocampus and PFC may contribute to cognitive and behavioral flexibility [23].

Recent studies highlighted the role of BDNF on cognitive functions. Parkhurst et al. [24] reported that microglia serves important physiological functions in learning and memory by promoting learning-related synapse formation through BDNF signaling. Moreover, Yuan et al. [25] explored that transplantation of BDNF gene-modified umbilical cord mesenchymal stem cell (UCMSC) improved neurological function in rats after brain trauma.

4. CONCLUSION

Wechsler IQ test results were adversely affected in IDA patients. BDNF level was not significantly lower in IDA children but it showed a significant positive correlation with one of Wechsler IQ test items in such patients. Larger scale studies are recommended to further investigate BDNF as a possible mediator that disturb cognitive functions in IDA and explore other mediators.

5. STUDY LIMITATIONS

The study is limited by the small number of enrolled patients. Additionally, other possible mediators for impaired cognition could have been done simultaneously to strengthen the work.

CONSENT

Written informed consents were obtained from the parents or care givers of the enrolled children.

ETHICAL APPROVAL

Authors obtained the necessary ethical approval from Institutional ethical Committee, Pediatric department, Ain Shams University on March 21st 2009. The parents of the enrolled cases were briefed with the results of the tests and their implications.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, et al. A systematic analysis of global anemia burden from 1990 to 2010. Blood. 2014;30;123(5):615-24.
2. WHO (2013). Micronutrient deficiencies - World Health Organization. Available: www.who.int/nutrition/topics/ida/en. Last accessed on 12/3/2014.
3. Austin AM, Fawzi W, Hill AG. Anaemia among Egyptian children between 2000 and 2005: Trends and predictors. Matern Child Nutr. 2012;8(4):522-32.
4. Madan N, Rusia U, Sikka M, Sharma S, Shankar N. Developmental and neurophysiologic deficits in iron deficiency in children. Indian J Pediatr. 2011;78(1):58-64.
5. Muñoz P, Humeres A. Iron deficiency on neuronal function. Biometals. 2012;25(4):825-35.
6. Wang B, Zhan S, Gong T, Lee L. Iron therapy for improving psychomotor development and cognitive function in children under the age of three with iron deficiency anaemia. Cochrane Database Syst Rev. 2013;6;6:CD001444.
7. Estrada JA, Contreras I, Pliego-Rivero FB, Otero GA. Molecular mechanisms of cognitive impairment in iron deficiency: Alterations in brain-derived neurotrophic factor and Insulin-like growth factor expression and function in the central nervous system. Nutr Neurosci; 2013. [Epub ahead of print]
8. Baj G, Carlino D, Gardossi L, Tongiorgi E. Toward a unified biological hypothesis for the BDNF Val66Met-associated memory deficits in humans: A model of impaired dendritic mRNA trafficking. Front Neurosci. 2013;30;7:188.
9. Tran PV, Carlson ES, Fretham SJB, Georgieff MK. Early-life iron deficiency anemia alters neurotrophic factor expression and hippocampal neuron differentiation in male rats. J Nutr. 2008;138(12):2495–501.
10. US Preventive Services Task Force: Screening for iron deficiency anemia including iron prophylaxis. In: Guide to Clinical Preventive Services, 2nd edition, Baltimore MD, Williams& Wilkins. 1996:231-46.
11. Dallman PR. Influence of age on laboratory criteria for the diagnosis of iron deficiency anemia and iron deficiency in infants and children. In: Iron Nutrition in Health and Disease. Looker AC, Johnson CL and Carrol M (eds), John Libbey Company, London. 1996;65-74.
12. Park JE, Park K. Textbook of preventive social medicine, 7th edition, Messers Banarsidas, Bhanot Publisher, 1268, Napier Town. 1979:81.
13. Centers for Disease Control and prevention: CDC growth charts. Available: www.cdc.gov/growth charts/zscore.htm. Last updated: August 4,2009. Last accessed on 12/3/2014.

14. Dawson DW, Hoffbrand AV, Worwood M. Assay of serum ferritin. In: Practical Hematology. Dacie JV, Lewis SM (eds), 7th edition, Churchill living stone, London. 1991;414-30.
15. Rosenfeld RD, Zeni L, Haniu M, Talvenheimo J, Radka SF, Bennett L, et al. Purification and identification of brain-derived neurotrophic factor from human serum. Protein Expr Purif. 1995;6(4):465-71.
16. Groth-Martnat, G. Wechsler intelligence scales. In: G. Groth-Martnat (ed), Handbook of psychological assessment, 4th edition. New York: John Wiley and Sons. 2003;129-195.
17. Daniel WW. Biostatistics: A foundation for analysis in the health sciences. 6th edition. John Wiley and sons, Inc, New York; 1995.
18. Tatala S, Svanberg U, Mduma B. Low dietary iron availability is a major cause of anemia: A nutrition survey in the Lindi district of Tanzania. Am J Clin Nutr. 1998;68:171-8.
19. Wu AC, Lesperance L, Bernstein H. Screening for iron deficiency. Pediatric Rev. 2002;23(5):171-8.
20. Poti JM, Slining MM, Popkin BM. Where Are Kids Getting Their Empty Calories? Stores, Schools, and Fast-Food Restaurants Each Played an Important Role in Empty Calorie Intake among US Children During 2009-2010. J Acad Nutr Diet; 2013. [Epub ahead of print].
21. Pedraza DF, Rocha AC, Sousa CP. Growth and micronutrient deficiencies: profile of children attended at the day care center for the government of Paraiba, Brazil]. Cien Saude Colet. 2013;18(11):3379-90. [English abstract]
22. Vucic V, Berti C, Vollhardt C, Fekete K, Cetin I, Koletzko B, et al. Effect of iron intervention on growth during gestation, infancy, childhood, and adolescence: A systematic review with meta-analysis. Nutr Rev. 2013;71(6):386-401.
23. Sakata K, Martinowich K, Woo NH, Schloesser RJ, Jimenez DV, Ji Y, et al. Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. Proc Natl Acad Sci USA. 2013;10;110(37):15103-8.
24. Parkhurst CN, Yang G, Ninan I, Savas JN, Yates JR , Lafaille JJ, et al. Microglia promote learning-dependent synapse formation through brain-derived neurotrophic factor. Cell. 2013;19;155(7):1596-609.
25. Yuan Y, Pan S, Sun Z, Dan Q, Liu J. Brain-derived neurotrophic factor-modified umbilical cord mesenchymal stem cell transplantation improves neurological deficits in rats with traumatic brain injury. Int J Neurosci; 2013. [Epub ahead of print].

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