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Long lasting effects of infancy iron deficiency – Preliminary results

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Summary The long-term effects of rehabilitated infancy (1 year old) iron deficiency (ID) were examined at age 10. The children were examined for the following variables: auditory system function, the level of morning cortisol, I.Q. score (WISC-R), and behavioral profile. The results indicate that while the former ID children's hearing system appears to function well, there was a delay in brain stem processing of the auditory signals. In addition, the level of morning cortisol was reduced, the general I.Q. scores were lower than the normal group (mainly in the performed subtest), and more sleep disturbances and fatigue during day were reported. These outcomes are consistent with established reports on the effect of iron deficiency on the rate of myelination in selected brain areas during critical period of 1 year olds. The findings of increased sleep disturbances and lower I.Q. tests require further study.

Introduction

Despite some successful intervention programs, iron deficiency (ID) and anemia remain the most common nutritional disorders in the world. Numerous behavioral manifestations can be observed among ID children and adults, such as lethargy, irritability, apathy, restlessness, fatigue, lack of concentration, paropghia (pathological craving for ice) and pica (pervert craving for substances unfit for food), inattention, hypoactivity (or hyperactivity) and sleep disturbances. However, the most disturbing functional hallmark of ID is a decrease in mental performances (Youdim and Yehuda, 2000).

While the frequency of ID is greater among children in the Third World, many cases of ID can be found at all ages, even among people in wealthy countries (Scrimshaw, 1991, 1998). Recently the population of healthy elderly people, living in Old Age Homes was identified as a high ID risk group (Buzina et al., 1998). In addition, pregnant women and children were identified as two other high-risk groups.

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Many ID effects are mediated via brain biochemistry, with prominent modification in the level and the activity of brain neurotransmitters (mainly the dopaminegic system and DA D2 receptors). In addition, iron plays a major role in fatty acid and lipid metabolism via the family of cytochrome P-450. There is an important link between iron and lipids that affects myelin and all its processes, especially myelin formation and myelin disintegration. An adequate supply of iron and essential fatty acids during the developing period is a requirement for the normal rate of myelination (Youdim and Yehuda, 2000). Using Luxol fast blue stain, Yu et al. (1986) confirmed earlier findings by Youdim and Yehuda, that the degree of myelination is decreased in pups of pregnant ID rats.

It seems that the ID also affects the sensory system. For example, ID rats are much more sensitive to strong noise. Hearing loss is induced in low amplitude noise in ID rats compared to normal rats (Sun et al., 1991). This finding was explained as due to ID induced changes in the inner ear. Similarly, a delay in maturation of the auditory brain stem responses was found in ID infants (Roncagliolo et al., 1998). The explanation was based on a delay in brain and auditory system myelination. In addition, some endocrinological changes were found in ID rats. Lower levels of thyroid hormone concentration were found in ID humans and rats (Beard et al., 1997), along with lower levels of cortisol in ID humans and rats (Weinberg et al., 1981; Saad et al., 1991).

In general the infancy period is a ''critical period'' for proper development of several sensory systems. Animal studies have shown that cognitive deficits induced by iron deficiency are very difficult to rehabilitate (Ben-Shachar et al., 1986; Youdim et al., 1989; Lozoff et al., 1996).

The aim of this study was to examine the long lasting effects of infancy ID. While many studies have investigated

the effect of an iron deficient diet, this study took advantage of a group of children in the age of 8–10 years old, who were ID at age 1 year and now are considered rehabilitated with respect to normal values of iron and hemoglobin. We tested their I.Q., auditory system, and basic cortisol levels, in order to find which parameters were not fully rehabilitated after so many years.

Methods

Subjects

The participants of this study were rehabilitating non-anemic children who were healthy full-term infants (birth weights $\geq$3.0 kg, with no perinatal complications, nor acute or chronic illnesses. As infants they were identified as having (Iron deficiency anemia) IDA at 12 mo. Anemia was defined as venous Hb $<$110 g/L at 12 mo. Iron deficiency was defined as two of three iron measures in the iron-deficient range [mean cell volume $<$70 fL, erythrocyte protoporphyrin $>$100 μg/dL red blood cells (1.77 μM), serum ferritin $<$12 μg/L) and/or an increase in Hb $\geq$10 g/L after 6 mo of iron therapy. Very strict exclusion criteria were used (e.g., chronic disease, head injury, jaundice, syphilis, toxoplasma, rubella, frequent ear infections, ototoxic medication, etc). A matched group of children, who were non-anemic during infancy served as a control group. 17 children, (13 boys and 4 girls, age 5.8 $\pm$ 1 year) tested in the audiometric test, 27 children (20 boys and 7 girls, age 10.2 $\pm$ 1 year) were tested in all other studies.

The ethics committees of Kaplan Hospital and Bar Ilan University approved the study.

Audiometric tests

All audiometric measures were obtained and processed without knowledge of whether a given child had been diagnosed as IDA or control. The children were studied while awake during the daytime. Audiometric tests included air and bone thresholds, in each ear, in frequencies of 250, 500, 1000, 2000, 4000, and 8000 Hertz, and SRT (Speech Reception Threshold). ABR (Auditory Brainstem Response) recordings were carried out in a quiet, dimly lit, and electrically shielded room using an integrated instrument (Bio Logic). ABR recorded using silver-silver chloride disk electrodes placed according to the 10–20 International. Interelectrode impedance was kept below 5 kilo ohms.

Using the same procedures as for infants, ABR were performed with the child in the supine position, elicited monaurally, stimulating the ipsilateral ear with a series of square wave rarefaction clicks (0.1 ms) through TDH-39 headphones at 80 dB nHL. The ABR were recorded twice to insure reproducibility. Results were stored for off-line analyses. The following parameters were determined for every response: absolute latency and amplitude for waves I, III, and V, and inter-peak latencies I–III, III–V, and I–V.

Cortisol

Morning salivary cortisol samples were collected at 08:00 PM, while the subjects were still at home. No food intake was allowed 30 min. before taking the sample. Samples were collected using cotton swabs chewed for 2 min. and inserted into a plastic test tube and then to a commercially available double-chamber device Salivette® (Sarstedt, Nümbrecht, Germany). After centrifuging and cooling to 4°C and storage at −20°C, the cortisol level was measured by radioimmunoassay.

WISC

Intelligence was tested using the Wechsler Intelligence Scale for Children-Revised (WISC) that included Verbal, Performance, and Full-Scale IQ derived from 12 subtests of different facets of cognitive functioning.

Behavior

After completion of the WISC, all subjects were asked to complete a 5-point rating scale for each of the following six dimensions (with 5 meaning "very good" and 1 meaning "very bad"):

1. *Appetite*.
2. Overall mood state.
3. *Concentration* during the school day.
4. *Fatigue* experienced during the day.
5. Ability to *organize* materials for home work.
6. *Quality* of sleep.

Results

Hemoglobin

The mean Hb level of the experimental group at the time of diagnosis (12.4 months) was 9.68 gr/DL $\pm$ 0.70, and 11.80 $\pm$ 0.70 at the time of the test (9.6 $\pm$ 1.1 years old). The Hb level of control group was 12.01 $\pm$ 0.90.

Audiometric test

Seventeen subjects completed the auditory test. All subjects exhibited normal hearing of pure tunes (250–8000 Hz) threshold, and normal SRT threshold. Both were less than 20 dB in both ears.

In the ABR test, (Tables 1 and 2) there was a slight (0.09–0.20 ms), yet statistically significant, prolongation of waves I, III, and V, and I–III and III–V latencies were

Table 1. *Left ear ABR (milliseconds)*

	ABR wave I	ABR III	ABR wave V	ABR IPL I–III	ABR IPL III–V	ABR IPL I–V
Population mean	1.63	3.86	5.74	2.15	1.93	4.08
Population SD	0.13	0.32	0.19	0.15	0.13	0.19
Normal value	1.54	3.69	5.54	2.14	1.86	4
Normal value SD	0.12	0.1	0.19	0.23	0.14	0.2
T-test *p*-value	$p = 0.013^*$	$p = 0.044^*$	$p = 0.006^*$	$p = 0.79$	$p = 0.046^*$	$p = 0.11$

* Statistical significant difference, $p = 0.05$

Table 2. *Right ear ABR (milliseconds)*

	ABR wave I	ABR III	ABR wave V	ABR IPL I–III	ABR IPL III–V	ABR IPL I–V
Population mean	1.64	3.75	5.68	2.13	1.92	3.93
Population SD	0.15	0.18	0.22	0.18	0.19	0.22
Normal value	1.54	3.67	5.52	2.13	1.85	3.98
Normal value SD	0.11	0.12	0.22	0.14	0.17	0.19
T-test P-value	$p = 0.015^*$	$p = 0.08$	$p = 0.001^*$	$p = 1$	$p = 0.15$	$p = 0.37$

* Statistical significant difference, $p = 0.05$

similar to the normal group. Increased I–V latency was found only in the left ear.

Cortisol

The mean level of saliva cortisol of the 27 experimental group children was 2.8 ± 0.4 nmol/L, while the level of 27-matched control group was 2.5 ± 0.7 nmol/L ($p = 0.001$).

WISC

The I.Q. test revealed some cognitive deficiencies in the experimental group. While all subjects were in the normal range of I.Q., some statistical differences between the experimental and control group were found. The experimental group scored lower mainly in the performance subtests and the serial learning subtest (Table 3).

Behavior

The analysis of the results showed that in general the members of the experimental group self-rated themselves lower than the members of the control groups in all 6 tested variables. In two variables, the differences were statistically significant viz. more fatigue experienced during day and more reported sleep disturbances (Table 4).

Table 3. *Results of WISC*

	Experimental group ($n = 27$)	Control group ($n = 27$)
Full Scale I.Q.	$96.7 \pm 2.1^{**}$	105.1 ± 1.3
Verbal I.Q.	$99.1 \pm 2.4^*$	104.5 ± 1.8
Performance I.Q.	$95.9 \pm 2.2^{**}$	104.9 ± 2.4

Statistical significant, * $p = 0.05$; ** $p = 0.001$

Table 4. *Behavioral variables*

	Former ID	Control
Appetite	3.5 ± 0.6	4.9 ± 0.7
Good mood	3.8 ± 0.8	4.8 ± 0.8
Ability to concentrate	3.5 ± 0.6	4.7 ± 0.5
Fatigue during day	$3.0 \pm 0.5^*$	4.5 ± 0.8
Organizing academic materials	$4.0 \pm 0.0.6$	4.8 ± 4.6
Quality of sleep	$3.1 \pm 1.3^*$	4.8 ± 1.0

*Statistical significant difference, $p = 0.05$

Discussion

The results of this study showed that despite rehabilitation of the hematological profile of infant ID as a group, those children still carried some deficiencies at age 10. Those deficiencies are not pathological, but they are at the lower range of the normal values. All subjects had normal hearing, however they had a delay in processing auditory signals. They exhibited lower morning cortisol values, lower scores in the WISC test, mainly in the performance subtests, and they reported more sleep disturbances and fatigue during daytime. They also rated themselves low on other behavioral variables.

Some of the results of this study confirm earlier results obtained by Lozoff's group. They found that former ID infants exhibited modifications in the auditory and visual systems at age 5 (Algarin et al., 2003), despite normal values of blood iron and hemoglobin. When similar groups were tested at age 10, changes in behavioral and cognitive variables were similar to those found in our study (Lozoff et al., 2000). They explained their results by delay in the myelination process in the brain stem attributable to an insult during a critical developmental period.

Reduced cortisol secretion in ID patients has been reported earlier (Saad et al., 1991), similar to the decreased level of cortisol that we found in former ID children. The significance of this finding is still not clear, however, many confirmed Attention-Deficit Hyperactivity Disorder (ADHD) children were found to be ID. It is not clear to us if ID is a part of the ADHD syndrome, or whether the ID is due to very poor eating habits. It is noteworthy that a lower level of cortisol, and a modified response to stress was found among ID children (King et al., 1998; Kariyawasam et al., 2002).

In terms of behavioral variables, the experimental group rated itself much lower (but not statistically significant) with respect to appetite, general mood, ability to concen-

trate and organization of school materials. The somewhat surprising finding was their report (statistically significant) about their sleep disturbances (mainly insomnia) and fatigue during day. It was reported that sleep disturbances are correlated with decreased morning awakening salivary cortisol (Backhaus et al., 2004).

The WSIC scores showed that the experimental group archived lower scores than the control group. While the scores are within the normal range, the scores in the performance subtests were even lower. This interesting finding (which had been found also by Lozoff's group) might indicate that ID has preferential effects on spatial learning and memory, more than on serial learning and memory. Animal studies showed similar effects in that the spatial learning and memory of ID rats (as measured by Morris Water Maze) were substantially poorer than serial learning (Youdim et al., 2000).

We would suggest that all deficits found in this study share the same basic cause i.e., the long term effects of infant ID.

This study also demonstrates the significance of critical periods during development. A delay in myelanation of several brain areas in the critical period modifies physiological, endocrinological and cognitive variables that persist for a number of years.

The main conclusion from this study is that despite rehabilitation of the hematological profile of infant ID, there are some physiological and cognitive functions that do not rehabilitate even after 10 years. The new findings of this study, e.g., reduced morning cortisol and sleep disturbances, indicate that this group of former ID infants, should be under close observation, and may need special care and intervention. More studies are needed to establish those effects and to devise methods to overcome those deficits.

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