IRON DEFICIENCY ANEMIA AND CATECHOLAMINE METABOLISM

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ABSTRACT

To assess the effects of iron therapy on platelet monoamine oxidase (MAO) activity and urinary excretion of total metanephrines (MN) in infants and young children with iron deficiency anemia, 24 subjects were tested before and after one month of oral iron treatment. Thirteen healthy children comprised the control group. In the control group, platelet MAO level was 0.21±0.02 U/mg protein (mean±SE), urinary total metanephrine was 2.51±0.47 μg/mg creatinine. In cases with iron deficiency, mean platelet MAO level was 47.6% lower ($p < 0.005$) whereas mean urinary metanephrine plus normetanephrine (MN-NMN) was only 20.7% lower ($p > 0.05$) than the control values. After one month, the anemic patients receiving oral iron therapy showed a significant increase in hemoglobin concentration, per cent transferrin saturation and platelet MAO activity ($p < 0.05$). However, urinary metanephrine excretion was found to be lower in this group when compared to the metanephrine levels in iron deficiency before the medication ($p < 0.05$).

Although hemoglobin and transferrin saturation did not return to normal levels, these findings suggested that platelet MAO activity increased and urinary excretion of metanephrines decreased after iron medication.

Key words: *Platelet MAO, Urinary metanephrines, Iron deficiency, Iron therapy.*

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Iron deficiency anemia is defined as a hypochromic microcytic anemia apparent when the body iron stores are severely depleted. Although the determination of prevalence of iron deficiency anemia is complicated by disagreement over the hemoglobin concentration, it has been proposed that a hemoglobin(Hb) of less than 11 g/dl or a transferrin saturation capacity less than 15% may be used to identify anemia.

Previous studies about the effects of iron deficiency anemia on human behaviour have focused on alterations, in mental and psychomotor development indexes. Work in animals, adults, older children and young adolescents showed that iron deficiency anemia may adversely affect the cognitive development performance and behaviour(1,2). It has been reported that the patients with iron deficiency anemia complain of fatigue, weakness and lack of ability to concentrate and clinical reports on anemic children indicate that they are irritable and anorexic(1,2). It is suggested that the correlation between anemia and developmental changes can be related to disordered cerebral oxidative metabolism secondary to suboptimal levels of various heme-containing and iron-dependent enzymes. In this concept, it is suggested that the enzyme apparently sensitive to the state of body iron stores is mitochondrial monoamine oxidase (MAO)(3). Platelet monoamine oxidase (MAO-β) is the possible peripheral marker of the activity of the enzymes.

It was concluded that in iron deficiency anemia, MAO activity was decreased but when serum iron level rose to its normal value MAO activity was restored to normal limits(4). However, Oski and Hanig reported that iron deficiency in infants produced developmental alterations and these changes are rapidly reversible following

iron therapy(5).

This study was, therefore, designated to determine first, correlations between urinary catecholamines and platelet MAO activity in healthy controls and patients with iron deficiency anemia and second, the effects of iron therapy on platelet MAO activity and urinary MN-NMN excretion in iron deficient children.

Material and Methods

Twenty-four cases (ranging in age from 1.4 to 14 years: 11 males and 13 females) admitted to the Pediatric Department of the Erciyes University Hospital, Kayseri were selected for the present study. All subjects were iron deficient and anemic as judged by the following criteria: hemoglobin of less than 11.0 g/dl; mean corpuscular volume of 75 μ^3 or less; and serum transferring saturation of 15% or less. All were free of infection during the study, and none had a chronic disease. All anemic cases had some problems such as anxiety, anorexia, developmental deficits, alterations in attention and lack of interest in the surroundings. Thirteen healthy controls comprised our control group. Their age range was 1.4-14 years. There were 7 males and 6 females in that group.

Hemoglobin (Hb), hematocrit (HTC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were determined by a "coulter counter". Serum iron and total iron binding capacity (TIBC) were performed by a commercial kit obtained from SIGMA Chemical Company, and transferrin saturation were calculated as percentage. Platelet MAO activity was estimated by method of McEwen Mc, Jr(6).

Twenty-four hours urine samples were collected in acid containing bottles for metanephrine determinations.

Since there would be some errors in collection of urine in infants, the creatinine values of urine samples were also determined and values of MN-NMN were defined as mg MN-NMN/mg creatinine(7). All measurements were carried out in duplicate. Protein was determined by biuret method(8).

Iron therapy (6 mg/kg/day) was begun for all patients for a period of four weeks. Patients were announced for control after this five weeks period. Only, 15 patients completed the therapy. At the end of which time full hematologic evaluation, serum iron and TIBC, platelet MAO activity and urine MN-NMN analysis were repeated.

Statistical significances between the control and pre-treatment group were determined by Student's 't' test. Results from the 15 iron deficient children whose urine and blood samples were analyzed by the Wilcoxon two sample test(9).

Results

The mean platelet MAO, transferrin saturation, Hb, urinary MN+NMN values in controls as well as in cases with iron deficiency are shown in *Table I*. In control group the mean Hb level was 12.40 ± 0.14 (mean $\pm$ SE), with a range of 11.5-13.0 and the mean platelet MAO level was 0.21 ± 0.02 with a range of 0.16-0.56. The urinary MN+NMN level was 2.51 ± 0.47 with a range of 1.08-6.72. In iron deficiency, the mean platelet MAO level was 0.11 ± 0.02 with a range of 0.06-0.56, the mean Hb level was 8.3 ± 0.5 with a range of 4.6-11.0. The urinary MN+NMN level was 1.99 ± 0.28 with a range of 0.35-6.77.

After oral iron medication, the differences before and after treatment were

TABLE I—*Relationship Between the Mean Values of the Anemic and Control Groups*

Parameter	Anemic groups (n=24) Mean±SE	Control groups (n=13) Mean±SE	t	p value
Hb (g/dl)	8.34±0.53	12.40±0.14	5.8	$p < 0.05$
Transferrin saturation (%)	4.73±0.39	25.63±2.19	9.5	$p < 0.05$
Mao Activity (U/mg protein)	0.11±0.02	0.21±0.02	3.6	$p < 0.005$
Urinary MN-NMN (μg/mg creatinine)	1.99±0.28	2.51±0.47	0.9	$p > 0.05$

calculated for the difference observed for both available readings. There was a significant increase in Hb concentration and percent transferrin saturation ($p < 0.05$). Even one patient had 11 g Hb/dl and other patient had a transferrin saturation higher than 15% after medication. Platelet MAO activity was significantly increased after the therapy ($p < 0.05$), whereas there was a significant decrease in urinary metanephrine levels in iron treated group ($p < 0.05$) (*Table II*).

Discussion

It has been demonstrated by the Bayley Mental Development Index that iron deficiency, even in the absence of anemia, results in biochemical alterations that impair behaviour in infants(10,11). The biochemical link between iron deficiency and physiological function is related to abnormal catecholamine metabolism. MAO that plays a central role in the degradation of catecholamines has been the possible candidate for the explanation of alterations in behaviour in iron deficiency. MAO is naturally present in the central nervous systems, platelets and in the other tissues of mammals(3,12,13). Platelet MAO activity has been used as a possible "peripheral marker" of the enzyme in the brain. It has been stated that iron might be necessary for the synthesis or the function of MAO. A lowering of activity in platelets has been demonstrated in iron deficient subjects and rats(4).

In conformation with this, in the present study significantly decreased activity of MAO was observed in iron deficient group ($p < 0.005$) as compared to controls. It is well known that MAO activity increases with age(14). Since our study group is in childhood, we observed lower levels of MAO activity in our control group than healthy adults reported by Balon *et al.*(14).

A neurotransmittor epinephrine is metabolized to metanephrine by the action of COMT, then MAO catabolizes metanephrine to 4-hydroxy-3-methoxy mandelic aldehyde and VMA. The mean control values of urinary total metanephrines in the present series are in accordance with those of previous studies(15). It has been reported that urinary total metanephrine levels changes between 0.001 and 5.38 μg/mg creatinine in healthy children aged 1 to 15 years. In our control group we observed mean total metanephrine levels as 2.51-0.47 μg/mg creatinine. One of the controls had total metanephrine value (6.72 μg/mg creatinine) higher than 5.38.

One would expect to find increased amounts of metanephrines in the urine of iron-deficient children if MAO activity is reduced. Woorhess *et al.* found no significant difference between pre- and post-iron dextran therapy, MN-NMN excretion in

TABLE II—*Biochemical and Hematological Determinations in Anemic Subjects, Before and After Iron Therapy*

No.	Age (yr)	Hb (g/dl)		Transferrin saturation (%)		MAO activity (U/mg protein)		MN-NMN (μg/mg creatinine)	
		Before	After	Before	After	Before	After	Before	After
1.	1.5	8.5	10.6	5.0	10.5	0.08	0.427	0.808	0.401
2.	1.5	9.6	9.8	9.7	11.3	0.10	0.134	0.412	0.408
3.	1.5	9.5	10.4	4.2	8.6	0.07	0.181	1.371	1.187
4.	1.5	7.6	8.3	7.0	9.7	0.07	0.108	1.733	1.265
5.	1.5	8.8	–	2.9	–	0.07	–	3.228	–
6.	2.	8.8	9.8	2.9	4.6	0.10	0.128	3.531	3.187
7.	2	7.5	9.1	3.0	7.8	0.11	0.139	0.802	0.682
8.	3	8.5	–	3.0	–	0.11	–	2.714	–
9.	3	9.6	–	4.0	–	0.08	–	1.474	–
10.	3	8.5	–	3.2	–	0.11	–	1.259	–
11.	3	4.6	–	5.5	–	0.06	–	1.674	–
12.	4	8.8	10.2	3.8	9.1	0.17	0.191	2.295	1.348
13.	4	8.5	10.4	3.1	10.1	0.16	0.204	1.466	2.052
14.	5	6.7	7.3	6.1	5.9	0.10	0.122	2.714	2.522
15.	6	7.5	–	4.6	–	0.14	–	0.350	–
16.	6	8.8	9.8	8.1	15.4	0.08	0.174	2.834	1.920
17.	7	6.9	–	8.6	–	0.08	–	1.681	–
18.	7	11.0	11.5	6.1	13.7	0.21	0.315	6.770	4.228
19.	10	9.6	10.4	2.7	6.5	0.09	0.124	3.328	2.834
20.	11	8.8	10.4	3.2	3.3	0.05	0.086	3.122	2.841
21.	11	4.6	6.9	3.8	7.8	0.12	0.153	1.409	1.210
22.	12	9.3	–	5.1	–	0.56	–	1.371	–
23.	14	9.3	–	4.6	–	0.08	–	0.559	–
24.	14	9.5	10.8	3.5	10.3	0.07	0.182	0.879	0.753
		$p^* < 0.05$ $t = 6.4$		$p^* < 0.05$ $t = 7.2$		$p^* < 0.05$ $t = 3.0$		$p^* < 0.05$ $t = 2.75$	

*Statistical evaluation : Wilcoxon two-sample test.

the group of eight anemic children(16). In our study, although the levels of urinary MN-NMN excretion were lower than control, no statistical difference was determined ($p > 0.05$). On the other hand, we observed significant difference between pre- and post therapy MN-NMN excretion in our iron deficient group ($p < 0.05$). The variation in the observed values could be due to the small number of cases in Woorhess *et al.*'s series(16).

In another study, Woods *et al.* showed that the increase in platelet MAO activity following an oral iron therapy for a month period and the difference in MAO activity before and after therapy was statistically significant(4). As it is expected, we also observed increasing levels of MAO activity after therapy ($p < 0.05$) and the changes in platelet MAO activity were correlated well with the changes in transferrin saturation values ($r = 0.051$, $p < 0.05$) (*Fig. 1*). We could not come across any similar reference in literature for comparing our results on the correlation.

Oski and Honig demonstrated that improvement in the developmental alterations has been obtained within several days after the institution of iron therapy long before any significant rise in Hb, and these symptoms are a manifestation of lack of iron rather than anemia(5). In another study it has been reported that short term oral iron therapy did not reverse these deficits(17). Although we did not do any developmental test, our clinical observations are in good agreement with those of Oski and Honig, and indicate that deficits in behavior are altered by iron therapy in iron deficient children(5).

We conclude that treatment of iron deficiency in young children produces a quantifiable improvement in measures of Hb and transferring saturation within a short

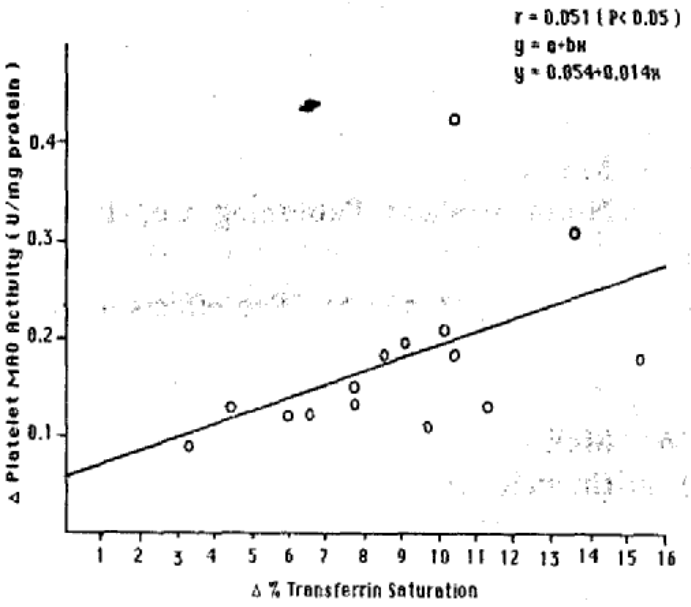

Fig. 1. The relationship between changes in platelet MAO activity and changes in per cent transferrin saturation after oral iron treatment.

Δ Platelet MAO activity (units)/mg protein = Platelet MAO activity (units)/mg protein after iron therapy-before iron therapy.

Δ Per cent Transferrin saturation = Per

period of time and the changes in transferrin saturation values were correlated with changes in platelet MAO activity. This study also demonstrates improvement in behaviour, decrease in anorexia, anxiety and attention before any important rise in Hb concentration after seven days of ferrous sulfate (Ferrosanal) therapy.

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