Effect of Different Types of Oral Iron Therapy Used for the Treatment of Iron Deficiency Anemia and Their Effects on Some Hormones and Minerals in Anemic Rats

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Abstract: Iron deficiency anemia is the most common type of anemia related to malnutrition world wide. It represents a major problem in developing countries, especially in Egypt. The aim of this study was carried out to elucidate the effect of different types of oral iron therapy (used for the treatment of iron deficiency anemia) on some hormones and minerals in anemic rats. Forty weanling male Sprague-Dawley rats divided into 4 groups (10 rats each), G1; control group as negative control G2; anemic rats as positive control., G3; anemic rats receiving iron chelating amino acids (IDA+ICAA, 40 mg Fe/kg), G4; anemic rats receiving ferrous sulphate (IDA+$FeSO_4$, 40 mg Fe/kg). Anemia was induced through feeding iron deficient diet (3-5 mg Fe/kg). At the end of the experiment, plasma, kidney and liver were used for determination of blood indices, tT3, tT4, Cu, Ca, Fe and MDA. Induction of iron in the diet improves body weight but still significantly lower than control group. Rats fed iron deficient diet had a significant lower Hb level, Hct value, RBCs count than normal controls. tT3 and tT4 levels of anemic rats were significantly lower than normal control (-15.16 & -30.59 % respectively). Treatment with ICAA gives better result than inorganic $FeSO_4$. tT3/tT4 ratio was significantly higher in all treated groups than normal control group. A significant inverse correlation was found between tT3/tT4 ratio and liver Fe in anemic rats. Treatment of IDA rats with ICAA improves lipid peroxidation. Cu level of IDA group was significantly higher than normal control group, treatment with ICAA or $FeSO_4$ returning Cu level to near normal. The plasma Ca level of ICAA treated groups was significantly higher than IDA groups. Plasma level of Fe or Fe/Cu ratio of IDA is significantly lower than normal control group, it reach less than half (58.3% decrease, $P < 0.0001$). A significant direct correlation was found between Ca level and kidney Fe in iron deficient anemia rats treated with iron chelating amino acids therapy. In Conclusion, the high bioavailability, easily tolerated doses of ferrous iron amino acid chelate allow lower doses to be used in IDA treatment than inorganic iron salts. [Journal of American Science 2010;6(6):109-118]. (ISSN: 1545-1003).

Keywords: Iron deficient anemia, iron chelating amino acids, inorganic iron

1. Introduction

Iron deficiency anemia is the most prevalent nutritional deficiency worldwide and it is often associated with some trace elements change (iron, zinc, copper). It is a major public health problem with adverse consequences, especially for women and children. Globally, iron deficiency affects over 2 billion people, mainly young women and children are iron-deficient (WHO/UNICEF/UNU, 2001). Anemia affects one-quarter of the world's population and is concentrated in preschool-aged children and women, making it a global public health problem (McLean et al., 2009). An estimated 39% of preschool children are anemic. Over 90% of affected individuals live in developing countries. The main cause of iron deficiency is the low iron bioavailability of the diet. The consequences of iron deficiency are many and serious, affecting not only individuals health but also the development of societies and countries. The distinction between "iron deficiency" and "anemia" is important. They often go hand in hand, but people can be iron deficient without being anemic. Iron deficiency is a depletion of iron stores while anemia refers to the depletion of iron in the red blood cells. Only 50% of anemia is caused by iron deficiency, the remainder is caused by vitamin A, B12, folate deficiencies, malaria, HIV, other infectious diseases, sickle cell disease and other inherited anemia (Yip, 1994). Tradition treatment of iron deficiency anemia include treatment with ferrous iron as ferrous sulfate ($FeSO_4$) which is much better absorbed than ferric iron e.g. ferric citrate (Davidsson et al., 2000), but it is known to produce intestinal side effects such as constipation, nausea, and bloating. Other forms of iron supplements as iron amino acid chelate are readily absorbed and less likely to cause intestinal side effects. Amino acids are ideal, good chelators or

readily absorbed and less likely to cause intestinal side effects. Amino acids are ideal, good chelators or ligands from both chemical and nutritional points of view. The body is efficient at absorbing amino acids and dipeptide. Iron amino acid chelate has been shown to have an increased bioavailability and reduced irritability over inorganic sources of iron (Pineda et al., 1994).

Studies in animals and humans have shown that iron deficiency anemia (IDA) impairs thyroid metabolism that associated with lower plasma thyroid hormone concentrations in rodents and, in some studies, in humans. Oxidant stress has been shown to play an important role in the pathogenesis of IDA and there are association between lymphocyte DNA damage, total antioxidant capacity and the degree of anemia in patients with IDA (Aslan et al., 2006). Amino acid–Fe(II)–chelator complexes exhibit strong antioxidant activity. Iron amino acid chelate is said to have more rapid effect with less GIT side effects so we tried to see if ICAA has more beneficial effects than the traditional iron therapy. The aim of this study was carried out to elucidate the effect of different types of oral iron therapy (used for the treatment of iron deficiency anemia) on some hormones and minerals in anemic rats.

2. Materials and Methods

Approval of the experimental protocol had been taken from the research ethics committee of General Organization of Teaching Hospitals and Institutes (GOTHI), Cairo, Egypt.

Four types of diet were prepared:

- Standard diet (iron sufficient diet, ISD, 40 mg/kg diet): according to the AIN-93G formulation (Reeves et al. 1993 and National Research Council Committee on Animal Nutrition, 1978).
- Iron deficient diet (IDD): standard diet without Fe in the mineral mixture (Fe 3-5 mg/kg diet as assessed by atomic absorption Unicam).
- Standard diet supplemented with Fe chelating amino acids (ICAA, 40 mg/kg diet) supplied by Nerhadou international.
- Standard diet supplemented with Fe in the form of dietary $FeSO_4$ (40 mg/ kg).

Some modifications were done as: menadione was used instead of phylloquinone; corn starch instead of sucrose; corn starch instead of cellulose (cellulose was omitted as a fiber source because of its variable iron content) as recommended by the American Institute of Nutrition, 1980. The iron content of all diets at baseline was confirmed by atomic absorption analysis. Rats were given free access to food and deionized water.

Forty weanling male Sprague-Dawley rats weighing 68 - 86 g were purchased from the breeding unit of the Egyptian organization for biological products and vaccines (Helwan, Egypt). They were obtained at 21 day of age and housed individually in stainless steel cages. They were fed on standard diet for 10 days before experiments began (adaptation period). Thirty rats of them will be anemic by introducing basal diet without Fe source (iron deficient, ID, 3-5 mg Fe/kg) for 21 days. The animals were divided into 4 groups consisting of 10 rats each and were maintained as follows: Group 1 (control group); rats fed on basal diets. Group 2 (IDA; iron deficient anemia); anemic rats, it will be sacrificed acting as positive control. Group 3 (IDA+ICAA; iron deficient anemia treated with iron chelating amino acids therapy); rats fed basal diet containing ICAA as the only source of Fe for treatment. Group 4 (IDA+$FeSO_4$, iron deficient anemia treated with dietary $FeSO_4$); anemic rats treated with conventional iron therapy (rats fed basal diet containing dietary Fe as the only source of Fe for treatment).

At the end of the experimental period (6 weeks), rats were fasted over night before sacrificing, blood was collected, centrifuged; serum was stored at - 20°C until analysis. Part of the blood is collected on tubes coated with EDTA for hemoglobin (Hb) and hematocrite (Hct) determination. Some hormones as tT3 and tT4, some minerals as Fe, Cu and Ca were determined. After sacrificing, liver and kidney were removed, washed with saline. Parts of liver and kidney each alone were homogenized in 1.15% KCl and used for malonedialdehyde (MDA) determination. Another part of each liver and kidney were kept for Fe determination.

Biochemical Analysis:

Hemoglobin was measured using the cyanomethaemoglobin method using Randox kits, Randox: Laboratories, USA (Dacie and Lewis, 1975). Hematocrite was measured by centrifugation of blood collected into heparinized microcapillary tubes no. 563 supplied by Bio Merieux (Mciniory, 1954). Hematocrite was calculated using the equation: Hct = length of red cell column (mm) / length of total column (mm). Red blood cells count (RBCs) was counted manually (Monica, 2004). Mean cell hemoglobin concentration (MCHC) was calculated using the equation: MCHC = [(Hb*100) / Hct]. Mean cell hemoglobin (MCH) was calculated using the equation: MCHC = [(Hb*10) / RBC]. Mean red cell volume (MCV) was calculated using the equation: MCV = [(Hct*10) / RBC].

Plasma, liver and kidney MDA were used as marker of (*in vivo*) lipid peroxidation and measured according to the method of Yoshioka et al., 1979. Plasma iron and total iron binding capacity, (TIBC) were determined colorimetrically using SGM kits according to the methods of Ruutu, 1975, Ceriotti and Ceriotti, 1980 respectively. Nearly all the binding capacity is due to transferrin. For standards, iron, copper and calcium reference solutions (1g Fe/L, Ca/L, 1g Cu/L; Merck KGaA, Darmstadt, Germany) were used. A control serum sample (Iron, copper and calcium /Seriscann ® normal) Control, QCA, Amposta, Spain), was included in the analysis of the plasma iron, TIBC, copper and calcium determinations to verify accuracy of measurements. Liver and kidney non-heme iron was measured colorimetrically after acid digestion of tissues (Torrance and Bothwell, 1980). Calcium was measured according to the method of Moorehead and Biggs 1974 using QCA kits (Quimica Clinica Aplicada S. A., Amposta/Spain). Copper was measured using Greiner kits (Greiner Diagnostic G mbH-Unter Gereuth 10- Bahlingen-Germany) according to the method of Abe et al., 1989. Total triiodothyronine (tT3) and total thyroxine (tT4) were determined according to the method of Chopra (1977) and Chopra et al. (1971) respectively. using monobind, INC, Costa Mesa, CA 92627 (USA), AccuBind ELISA microwells, 125-300 and 225-300.

Statistical analysis:

All results were expressed as the mean ±SD. Statistical analysis was performed with statistical package for the social science for windows (SPSS, version 13.0, Chicago, IL, USA). The data were analyzed by one-way analysis of variance (ANOVA). To compare the difference among the groups, post hoc testing was performed by the Bonferroni test. Pearson's correlation analysis was used to determine the correlation among the parameters assessed. The p- value < 0.05 was considered statistically significant (Dawson and Trapp, 2001).

3. Results

The body weight of the experimental rats at the beginning showed no significant differences. Anemic rats showed significant decrease in body weight compared with normal control group. The mean body weight of anemic rats at the end of the study was 62% of normal rats. Induction of iron in the diet improves body weight but still significantly lower than control group (table 1) being more pronounced in groups receiving iron chelating amino acids. Mean body weight of ICAA treated rats was 93, 96 % of that of normal control rats. Daily food intake were significantly lower in anemic rats than normal control or treated groups.

Rats fed the iron deficient diet had a significant lower blood Hb concentration, Hct value, RBC count than normal controls (table 2). Red blood indices showed that MCHC of ICAA treated group was significantly higher than normal control or IDA groups. MCH and MCV of IDA group were significantly higher than normal control. The reticulocyte count of normal control (expressed as a percentage of the total number of RBC) was about twice as high as in IDA rats as in controls (P < 0.0001). It is believed that most of the Fe in plasma comes from continuous recycling of heme from senescent RBC through the reticuloendothelial system (Knutson and Wessling-Resnick 2003). In general treatment with ICAA improves level of IDA to normal or near normal better, faster than traditional treatment.

Table 1. Initial and final body weight, body weight gain and daily food intake in the different experimental studied groups (Means ± SD)

Parameters	Experimental groups			
	1	2	3	4
IBW (gm)	74.3 ± 4.75	75.6 ± 5.52	75.1 ± 5.64	74.7 ± 5.93
IBW2 (gm)	127.3 ± 8.05	-----	128 ± 7.04 ^{a}	126.2 ± 8.49 ^{a}
FBW (gm)	195.4 ± 12.27	121.3 ± 10.08 ^{a}	191.2 ± 12.04 ^{a,b,d}	187.80 ± 13.97 ^{a,b,c}
BWG (gm)	68.30 ± 9.97	45.7 ± 6.00 ^{a,c,d}	63.2±7.27 ^{a,b,d}	61.60 ± 8.34 ^{a,b,c}
Food intake(gm/day)	17.19 ± 2.09	10.82 ± 1.01 ^{a,c,d}	15.6 ± 0.79 ^{a,b,d}	13.97 ± 0.80 ^{a,b,c}

IBW: Initial Body weight; IBW2: Initial Body weight after adaptation period, FBW: Final Body weight;; BWG: Body Weight Gain; $^{a}p < 0.0001$ *Vs. Group (1),* $^{b}p < 0.0001$ *Vs. Group (2),* $^{c}p < 0.0001$ *Vs. Group (3),* $^{d}p < 0.0001$ *Vs .Group (4),*

Table 3 revealed that levels of tT3 and tT4 in anemic rats were significantly lower than normal control (-15.16% and -30.59% respectively). Treatment with ICAA gives better result then inorganic $FeSO_4$. tT3/tT4 ratio was significantly higher in all treated groups than control group. An inverse correlation was found between tT3/tT4 ratio and liver Fe in anemic rats ($r = -0.663$, $P = 0.037$) (Fig. 1). Also, there was a positive correlation between tT3/tT4 ratio and plasma Ca in ICAA treated group ($r = 0.662$, $P = 0.037$) (Fig. 2).

Table 4 showed the level of MDA in plasma, liver and kidney. They showed significant decrease than normal control group. Treatment of IDA rats with ICAA improves lipid peroxidation even exceed level of the normal control group

Table 5 showed the levels of Cu, Ca, and Fe in normal, IDA and treated groups. The level of Cu in IDA group was significantly higher than normal control group, treatment with ICAA or $FeSO_4$ returning Cu level to near normal. Both types of treatment gives the same effects with no significant difference. No statistically significant changes of plasma Ca level between iron deficient rats and normal control animals; on the other hand, the level of Ca in ICAA treated groups were significantly higher than IDA groups ($P < 0.05$). Both treatments for IDA showed no statistically significant changes when compared with normal control group. The plasma levels of Fe and Fe/Cu ratio of IDA were significantly lower than normal control group, it reach less than half (58.3% decrease, $P < 0.0001$). A positive correlation was found between plasma Ca and kidney Fe in ICAA treated group ($r = 0.743$, $P = 0.014$) (Fig. 3).

Table 6 showed the levels of non-heme iron in liver and kidney, TIBC and transferrin saturation in the studied groups. Plasma, liver and kidney Fe and transferrin were significantly lower. than control group. Treatment with ICAA or $FeSO_4$ improves these parameters, being more pronounced in groups treated with ICAA.

Table 2. Biological profile in the different experimental studied groups

Parameters	**Experimental groups**			
	1	**2**	**3**	**4**
Hb (g/dl)	13.49 ± 0.68	9.29 ± 0.69 $^{a, c,d}$	12.56 ± 0.94 ^{b,d}	11.08 ± 0.79 ^{a,b,c}
Hct (%)	42.02 ± 2.08	29.06 ± 2.46 ^{a,c,d}	38.17 ± 3.15 ^{a,b,d}	34.18 ± 2.73 ^{a,b,c}
MCHC (g/dl)	32.11± 0.85	32.01 ± 0.68 ^{c}	32.94 ± 0.80 ^{a,b}	32.43 ± 0.55
MCH (Pg)	19.39 ± 1.01	23.57 ± 1.12 ^{a,c,d}	19.68 ± 0.93 ^{b,d}	18.36 ± 1.62 ^{b,c}
MCV (fl)	60.47 ± 4.31	73.62 ± 2.46 ^{a,c,d}	59.76 ± 2.72 ^{b,d}	56.65 ± 5.11 ^{b}
RBC ($x10^6/\mu l$)	6.98 ± 0.60	3.95 ± 0.34 ^{a,c,d}	6.40 ± 0.61 ^{a,b,d}	6.06 ± 0.53 ^{a,b,c}

$^a p < 0.0001$ Vs. Group (1), $^b p < 0.0001$ Vs. Group (2), $^c p < 0.0001$ Vs. Group (3), $^d p < 0.0001$ Vs. Group (4),

Table 3. Plasma total triiodothyronine (tT3); total thyroxine (tT4) and tT3/tT4 ratio in the studied groups

Parameters	**Experimental groups**			
	1	**2**	**3**	**4**
tT3 (ng/L)	4.40 ± 0.44	3.74 ± 0.32 ^{a,c,d}	5.02 ± 0.44 ^{a,b}	4.61 ± 0.24 ^{d}
tT4 (µg/L)	0.44 ± 0.085	0.31 ± 0.04 ^{a,c,d}	0.37 ± 0.04 ^{a,b,d}	0.34 ± 0.03 ^{a,b}
tT3/tT4 ratio	10.27 ± 0.71	12.34 ± 1.73 ^{a}	13.74 ± 1.63 ^{a}	13.87 ± 1.68 ^{a}

$^a p < 0.0001$ Vs. Group (1), $^b p < 0.0001$ Vs. Group (2), $^c p < 0.0001$ Vs. Group (3), $^d p < 0.0001$ Vs. Group (4).

Table 4. Plasma, liver and kidney MDA in experimental studied groups

Parameters	**Experimental groups**			
	1	**2**	**3**	**4**
Plasma MDA (nmol/L)	61.27 ± 4.10	55.97 ± 4.29 ^{a,c}	69.85 ± 3.46 ^{a,b,d}	58.17 ± 2.90 ^{c}
Liver MDA (nmol/g)	60.44 ± 3.95	40.78 ± 3.74 ^{a,c,d}	67.56 ± 4.28 ^{a,b,d}	58.67 ± 2.70 ^{b,c}
Kidney MDA (nmol/g)	184.27 ± 18.95	157.81 ± 14.08 ^{a,c,d}	236.45 ± 11.59 ^{a,b}	219.99 ± 23.66 ^{a,b}

$^a p < 0.0001$ Vs. Group (1), $^b p < 0.0001$ Vs. Group (2), $^c p < 0.0001$ Vs. Group (3), $^d p < 0.0001$ Vs. Group (4).

Table 5. Plasma levels of Cu, Ca, Fe and Fe/Cu ratio in the studied groups.

Parameters	Experimental groups			
	1	2	3	4
Cu (μg/dl)	36.28 ± 4.17	55.57 ± 6.37 ^{a,c,d}	39.47 ± 2.19 ^{b}	40.99 ± 2.45 ^{b}
Ca (mg/dl)	8.72 ± 0.57	8.51± 0.46 ^{c}	8.90 ± 0.35 ^{b}	8.61± 0.43
Fe (μg/dl)	158.12 ± 19.03	65.27 ± 7.88 ^{a,c,,d}	150.26 ± 19.10 ^{b,d}	135.08 ± 8.66 ^{b}
Fe/Cu ratio	4.40 ± 0.66	1.19 ± 0.22 ^{a,c,d}	3.78 ± 0.53 ^{a,,b}	3.45 ± 0.26 ^{a,b}

$^{a}p < 0.0001$ *Vs. Group (1),* $^{b}p < 0.0001$ *Vs. Group (2),* $^{c}p < 0.0001$ *Vs. Group (3),* $^{d}p < 0.0001$ *Vs. Group (4).*

Table 6. Iron status in plasma, liver and kidney in the studied groups

Parameters	Experimental groups			
	1	2	3	4
Plasma Fe (μg/dl)	158.12 ± 19.03	65.27 ± 7.88 ^{a,c,,d}	150.26 ± 19.10 ^{b,d}	135.08 ± 8.66 ^{b}
Liver Fe (μg/g)	104.27 ± 12.56	79.11 ± 10.29 ^{a,c,d}	97.04 ± 7.48 ^{b,d}	91.84 ± 5.17 ^{a,b}
Kidney Fe (μg/g)	139.79 ± 11.16	85.76 ± 8.42 ^{a,c,d}	114.25 ± 8.38 ^{a,b,d}	106.19 ± 4.48 ^{a,b}
TIBC (μg/dl)	309.03 ± 27.07	442.09 ± 16.72 ^{a,c,d}	292.21 ± 45.06 ^{b,d}	300.91± 27.03 ^{b}
Transferin Sat (%)	51.14 ± 4.02	14.76 ± 1.60 ^{a,c,d}	51.66 ± 2.70 ^{b,d}	45.07 ± 3.18 ^{a,b,c}

$^{a}p < 0.0001$ *Vs. Group (1),* $^{b}p < 0.0001$ *Vs. Group (2),* $^{c}p < 0.0001$ *Vs. Group (3),* $^{d}p < 0.0001$ *Vs. Group (4).*

4. Discussion

All iron supplements are not the same. Ferrous iron (e.g. ferrous sulfate) is much better absorbed than ferric iron (e.g. ferric citrate) (Davidsson et al., 2000). The most common form of iron supplement is ferrous sulfate, but it is known to produce intestinal side effects such as constipation, nausea, and bloating in many users (Hansen, 1994). Some forms of ferrous sulfate are enteric-coated to delay tablet dissolving and prevent some side effects, but enteric-coated iron may not absorb as well as iron from standard supplements (Rickettes, 1993). Other forms of iron supplements, such as ferrous fumarate, ferrous gluconate, and iron glycine amino acid chelate (Fox et al., 1998) are readily absorbed and less likely to cause intestinal side effects. In order to overcome the problems (side effects) created from using tradition treatment of anemia, we tried to use iron chelated amino acids. Iron amino acid chelate with the iron in the ferrous state to develop an organic mineral delivery system that is negatively charged which is believed to overcome the problems. The ferrous form of iron amino acid chelate belongs to the class of bicyclic chelates where each respective amino acid bonds the same iron atom through its carboxyl oxygen and -amino groups. This iron amino acid chelate has been shown to have an increased bioavailability and reduced irritability over inorganic sources of iron (Pineda et al., 1994).

Ferrous iron is the form that is mostly used for correction of iron deficiency. About 3–5% of the iron present in alimentary canal in ferrous form is absorbed. Acidic milieu facilitates the absorption by keeping iron in the ferrous form. Ferrous iron is a central pro-oxidant that propagates free radical reactions through Fenton chemistry both locally in the gastrointestinal tract and systemically. An excess of pro-oxidants over antioxidants results in oxidative stress (Kurtoglu et al., 2003).

In this study, the primary cause of anemia was feeding iron deficient diet for a long period (3 weeks). Anemia is considered as one of the most common index of nutritional deficiency worldwide and is caused by iron deficiency store or iron deficiency erythropoiesis (Lin et al., 2003). Several authors have reported that IDA is mainly caused by some food constituents that may contribute to inhibition of iron absorption, hence contribute to the high prevalence of ID, and IDA (Lin et al., 2003).

The highly significant differences in blood indices response (increase of Hb level, Hct, MCHC, MCH, RBC count, returning to near normal level especially when using ICAA) could be explained by the increased assimilation by chelation process as indicated by Burns (2002). Forbes and Erdman (1983) found that increased assimilation by as much as 300%. It seems that the effect of the ingested doses of ICAA were enough to stimulate iron absorption in

experimental rat groups with highly significant increase. Insufficient body stores of iron lead to a

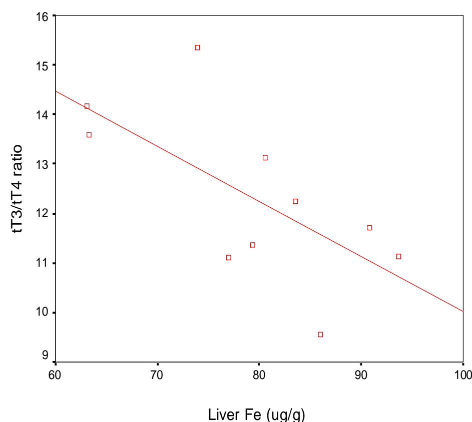

Fig. 1: Correlation between tT3/tT4 ratio and liver iron in Iron Deficient Anemia (IDA) rats (r = – 0.663, P = 0.037)

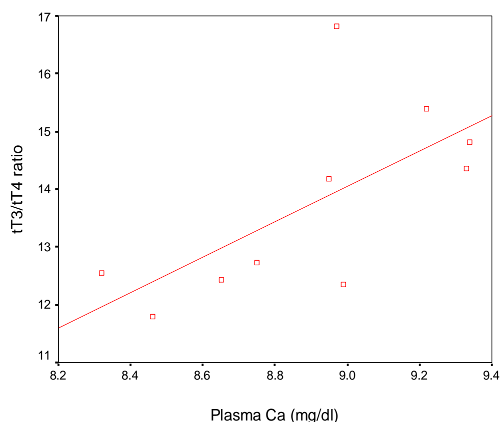

Fig. 2: Correlation between tT3/tT4 ratio and plasma Ca in Iron Deficient Anemia rats treated with Iron Chelating Amino Acids therapy) (IDA+ICAA) (r = 0.662, P = 0.037)

depleted RBC mass which, in turn, leads to a decreased hemoglobin concentration (hypochromia) and decreased oxygen-carrying capacity of the blood which agree with Campos et al. (1998). Also this decrease seems to indicate that the organism is unable to maintain haemoglobin levels, as it cannot obtain iron from its reserves without endangering the activity of the iron-dependent enzymatic mechanisms as Campos et al.(1998) stated. Such mechanisms are essential to the organism, although, as reported by Beutler (1988) some of these enzymes would already be depleted.

In our study, plasma tT4 and tT3 concentrations were significantly lower in Fe-deficient rats than controls which are in agreement with Chen et al. (1983), Martinez-Torres et al. (1984), Beard et al. (1990), Zimmermann and KÖhrle (2002). The decrease in tT3 and tT4 concentration of IDA reach 15.16%, 30.59% respectively when compared with normal control group. The two initial steps of thyroid hormone synthesis are catalyzed by thyroperoxidases (heme–containing thyroid peroxidase) and are dependent on iron. Studies in human and animals have shown that iron deficiency impairs thyroid metabolism, lower thyroperoxidase activity and interfere with the synthesis of thyroid hormones. Hess et al. (2002) have shown that thyroid peroxidase activity is significantly reduced in iron deficiency anemia. In rats, iron deficiency decreases plasma thyroid hormone concentration, impairs peripheral

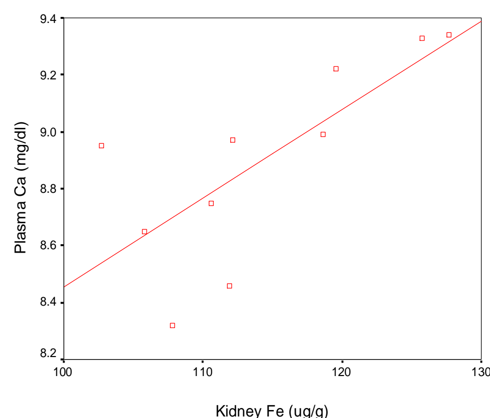

Fig. 3: Correlation between plasma Ca and kidney Fe in Iron Deficient Anemia rats treated with Iron Chelating Amino Acids therapy) (IDA+ICAA) (r = 0.743, P = 0.014)

conversion of T4 to T3 (Dillman et al., 1980), reduces the activity of hepatic thyroxine 5'-deiodinase, which catalyzes the conversion of T4 to T3, and blunts the thyrotropin (TSH) response to thyrotropin releasing hormone, TRH, (Beard et al.,1998 and Hess et al., 2002). These reasons might be the reason for the significantly decreased level of T3, T4 found in the present study. These results are in agreement with the previous studies. The T3 levels in IDA rats were only 85% of those in the control group.

In biological systems, the steady-state level of lipid peroxidation is often assessed by the measurement of lipid peroxidation breakdown products such as malondialdehyde, MDA (Draper and Hadley 1990 and Janero, 1990). Increased lipid peroxidation in IDA rats identifies adverse effect of

iron deficiency and further emphasizes the need for preventing and correcting it. Lipid peroxidation is initiated by reactive oxygen species, and also stimulated by iron ions (Braughler et al., 1986), which can catalyze the formation of te hydroxyl radical and accelerate the decomposition of lipid hydroperoxides (Davies and Slater 1987). Despite extensive literature on iron and lipid peroxidation, few studies have investigated the effects of oral iron supplements on lipid peroxidation. In our study, results showed that iron deficiency anemia is associated with decrease in lipid peroxidation which is indicated by significant decrease in liver and kidney MDA and slight decrease in plasma MDA which are in agreement with Rao and Jagadeesan (1996). Our study disagree with Sundaram et al. (2007) who found an elevated level of MDA in anemic patients and a significant decrease in MDA after treatment. Kurtoglu et al. (2003) observed a significant decrease of oxidative stress in IDA patients. Also Aslan et al. (2006) and D az-Castro et al. (2008) has reported that thiobarbytric acid reactive substances (TBARs) production was similar in liver cytosols from iron deficiency anemia and control rats (IDA did not affect lipid peroxidation in rats), suggesting that hepatic antioxidant capacity is normal or there is enough compensatory capacity to keep antioxidant defenses high, probably due to the adequate antioxidant enzyme activities as glutathione peroxidase, Catalase and glutathione reductase.

The source of iron is either from food supply (heme) or free iron from other sources. Both kinds of iron are processed by the gut (stomach and intestine) where they are converted to a form of iron readily used by the body. Finally, the iron winds up in the intestinal epithelial cells, ready for export to red blood cells, muscle tissue and organs. It has to get out of the gut into the blood stream, but this is particularly difficult because the so-called "hydrophobic" intestinal membrane wants to reject the charged iron molecule. Fe is absorbed from the intestinal lumen into the circulation through a series of steps beginning with uptake into the enterocyte by the apical membrane transporter DMT1 "divalent metal transporter 1" (Gunshin et al., 1997). This step requires Fe in the reduced state which is carried out by a reductase, duodenal cytochrome b, located in the enterocyte apical membrane (McKie et al., 2001). The Fe taken into the cell likely equilibrates with various Fe pools. Fe is exported from the enterocyte to the circulation by the basolateral membrane transporter, ferroportin (McKie et al., 2000) which apparently can transport Fe only in the oxidized state. To accomplish this, a Cu-dependent ferroxidase protein, hephaestin (Hp) (Vulpe et al., 1999), in the intestinal enterocytes, is thought to oxidize Fe_2 to Fe_3 and may act as a helper molecule forming a complex with a yet unknown transport protein where it allows iron to make its way through the membrane (Vulpe et al., 1999). Fe_3 is then transported into the circulation and is bound to apotransferrin .

Many factors affect absorption of minerals and one of the most important ones is the interaction of one mineral with another to the extent that absorption and utilization are reduced or enhanced. There are nutrients which need to be present for iron absorption as B12, folic acid, vitamin C, vitamin A, copper, calcium, manganese, molybdenum and other of the B complex vitamins. According to Ebihara and Okano (1995), sufficient iron is absorbed via the large intestine for recovery from IDA. In this study IDA rats showed significant decrease in plasma Fe concentration when compared with the control group and after iron therapy, mean Fe level increase which agree with Sundaram et al. (2007). Results of this study shows that there is a significant decrease in the concentration of iron in the liver and serum; and this confirms the findings of Milne et al. (1990) who stated that in nutritional IDA all the reserves of iron in the organism were first depleted before hemoglobin levels were drastically affected, and this differ from hemolytic anemia which does not deplete the organism's reserves of iron in such a generalized manner (Flanagan et al., 1980 and Kalpalathika et al., 1991).

The study showed increased level of Cu in IDA rats when compared with the control normal group which agree with GÃrgÃze et al. (2006) and Yokoi et al. (1991) and disagree with Van et al. (2006).

Our results demonstrated a non significant change in Ca level of IDA rats when compared with the control normal group which disagree with Yokoi et al. (1991). Little information is available on the influence of the development of IDA on the metabolism of calcium. Campos et al., 1998 found that the digestive utilization of calcium was higher in IDA than in the control animals. The greater absorption of calcium might also be due to the phenomenon described by Hill and Matrone (1970) who showed that the deficiency of a divalent cation, such as iron, in the intestinal region produces an increase in the absorption of other divalent cations such as calcium. These interactions, which have been studied by Cook et al. (1992). Hallberg et al. (1991) showed that when dietary calcium content increases, the absorption of iron falls; in our study, the administration of an iron-deficient diet led to an

increase in calcium absorption, accompanied by a parallel increase in the absorption of phosphorus and magnesium. In this study our results for IDA rats also agree with Campos et al. (1998). Hallberg et al. (1991) speculated that calcium and iron might competitively bind to one or more substances that are important in the transcellular absorptive pathway, resulting in the inhibitory effect of calcium on iron absorption. Actually in rats some duodenal and intestinal proteins such as mobilferrin and calreticulin have affinity for both calcium and iron (Conrad et al., 1993).

This research describes an effective dietary intervention for the treatment of iron deficiency anemia through specific ferrous iron amino acid chelate. Due to its high bioavailability, relatively small, easily tolerated doses may be administered. The high bioavailability of iron as the ferrous amino acid chelate allows lower doses of iron on a daily basis than would be expected to allow repletion of iron stores if supplied as an inorganic iron salt.

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References

1. Abe A, Yamashita S, and Noma A. Sensitive direct colorimetric assay for copper in serum. Clin Chem. 1989; 35(4): 552-554.
2. American Institute of Nutrition. Second report of the ad hoc committee on standards for nutritional studies. J. Nutr., 1980; 110: 1726-1732.
3. Aslan M, Horoz M, Kocyigit A, Ozgonul S, Celik H and Celik M. Lymphocyte DNA Damage and oxidative stress in patients with iron deficiency anemia. Mutat. Res., 2006; 601: 144-149.
4. Beard JL, Borel MJ and Derr J. Impaired thermoregulation and thyroid function in iron-deficiency anemia. Am. J. Clin. Nutr., 1990; 52: 813–819.
5. Beard JL, Brigham DE and Kelley SK, et al., Plasma thyroid hormone kinetics are altered in iron-deficient rats. J. Nutr., 1998; 128: 1401–1408.
6. Beutler E. In: Modern nutrition in health and disease, 6th edn, ed. Ruckenbush, Y. & Thivend, P., 1988 pp. 298-326. Salvat, Barcelona.
7. Braughler, JM, Duncan, LA and Chase RL. The involvement of iron in lipid peroxidation. Importance of ferric to ferrous ratios in initiation. J. Biol. Chem., 1986; 261: 10282–10289.
8. Burns D. Enhanced transport of amino acid chelated minerals. Research and Development Department, Seroyal Brands, Inc., Concord, California 2002.
9. Campos MS, Barrionuevo M, Alferez MJM, Gomez-Ayala AE, Rodriguez-Matas MC, Lopez Aliaga I and Lisbona F. Interactions among iron, calcium, phosphorus and magnesium in the nutritionally iron-deficient rat. Experimental Physiology, 1998; 83: 771-781.
10. Ceriotti F and Ceriotti G. Improved direct specific determination of serum iron and total iron-binding capacity. Clin. Chem., 1980; 26: 327–331.
11. Chen SCH, Shirazi, MRS and Orr RA. Triiodothyronine and thyroxine levels in iron-deficient, hypertriglyceridemic rats. Nutr. Res. 1983: 3: 91–106.
12. Chopra IJ. Radioimmunoassay of iodothyronine-handbook of Radioimmunoassay. GE Abraham. Ed. New York. Marcel Dekker, Inc., 1977.
13. Chopra IJ, Solomon DH and Ho RS. A radioimmunoassay of thyroxine. J. Clin. Endocrinol. Metab., 1971; 33 (5): 865-868.
14. Conrad ME, Umbreit JN and Moore EG. Rat duodenal iron-binding protein mobilferrin is a homologue of calreticulin. Gastroenterology, 1993; 104: 1700-1704.
15. Cook JD, Baynes RD and Skikne BS. Iron deficiency and the measurement of iron status. Nutr. Res. Rev., 1992; 5: 189 –202.
16. Dacie JU and Lewis SM. Basic hematology techniques. In: Dacie JU, Lewis SM (Eds). Practical hematology. Churchill Livingston: London. 1975; pp 21–96.
17. Davidsson L, Kastenmayer P and Szajewska H. et al., Iron bioavailability in infants from an infant cereal fortified with ferric pyrophosphate or ferrous fumarate. Am. J. Clin. Nutr., 2000; 71:1597–602.
18. Davies MJ and Slater TF. Studies on the metal-ion and lipoxygenase catalysed breakdown of hydroperoxides using electron-spin-resonance spectroscopy. Biochem. J., 1987; 245: 167–173.
19. Dawson B and Trapp RG. Basic and clinical biostatistics, third edition , pbl. Lange Medical Books/McGraw-Hill .U.S.A. 2001.
20. D az-Castro J, Alf rez MJM, L pez-Aliaga I, Nestares T, Granados S, Barriomuevo M and Compos MS. Influence of nutritional iron deficiency anemia on DNA stability and lipid peroxidation in rats. J. Nutr., 2008; 24: 1167-1173.
21. Dillman E, Gale C and Green W. et al., Hypothermia in iron deficiency due to altered

triiodothyronine metabolism. Am. J. Physiol., 1980; 239: R377–381.
22. Draper HH and Hadley M. Malondialdehyde determination as index of lipid peroxidation. Methods Enzymol., 1990: 186: 421-431. 29.
23. Ebihara K and Okano J. Comparison of bioavailability and hemoglobin repletion of ferric and ferrous ion infused into the cecum in anemic rats. Nut. Res., 1995; 15: 889-897.
24. Flanagan PR, Haist J and Valberg, LS. Comparative effects of iron deficiency induced by bleeding and a low-iron diet on the intestinal absorptive interactions of iron, cobalt, manganese, zinc, lead and cadmium. Journal of Nutrition, 1980; 110: 1754-1763.
25. Forbes RM and Erdman JW Jr. Bioavailability of the trace mineral elements. Ann. Rev. Nutr., 1983; 3: 213-231.
26. Fox TE, Eagles J and Fairweather-Tait SJ. Bioavailability of iron glycine as a fortificant in infant foods. Am. J. Clin. Nutr., 1998; 67: 664–668.
27. GÃrgÃze MK, OÃÃÃ A, AygÃn AD, Taskin E, and KiliÃ M. Serum and hair levels of zinc, selenium, iron, and copper in children with iron-deficiency anemia. Biol. Trace Elem Res., 2006; 111 (1-3): 23-29.
28. Gunshin H, Mackenzie B, Berger UV, Gunshin, Y, Romero MF, Boron WF, Nussberger S, Golan JL and Hediger MA. Cloning and characterization of a mammalian proton-coupled metal ion transporter. Nature (Lond.) 1997; 388: 482–488.
29. Hallberg L, Brune M, Erlandsson M, Sandberg AS and Hulten, LR. Calcium effect of different amount on non-heme and heme iron absorption in humans. Am. J. Clin. Nutr., 1991; 53: 112-119.
30. Hansen CM. Oral iron supplements. Am Pharm 1994; 34: 66 –71.
31. Hess SY, Zimmermann MB, Arnold M, Langhans W and Hurrell RF. Iron deficiency anemia reduces thyroid peroxidase activity in rats. J. Nutr., 2002; 132: 1951-1955.
32. Hill CH and Matrone G. Chemical parameters in the study of in vivo and in vitro interactions of transition elements. Fed. Proc., 1970; 29(4): 1474-1481.
33. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radic. Biol. Med., 1990; 9: 515–540.
34. Kalpalathika M, Mahoney A, whirraker P and Hendricks D. Incorporation of absorbed iron from different dietary sources into hemoglobin. Nutrition Research, 1991; 11: 185-195.
35. Kurtoglu E, Ugur A, Baltaci AK., and U Dar L. Effect of iron supplementation on oxidative stress and antioxidant status in iron-deficiency anemia. Biol. Trace Elem. Res., 2003; 96 (1-3): 117-123.
36. Knutson M and Wessling-Resnick M. Iron metabolism in the reticuloendothelial system. Crit. Rev. Biochem. Mol. Biol., 2003; 38: 61–88.
37. Lin XM, Wang Z, Shen XY, Long Z, Liu WJ, Guo YM, and Tang Y. Iron status and the effect of early iron supplementation on sub-clinical iron deficiency in rural school-age children from mountainous areas of Beijing (China). Zhonghua Yu Fang Yi Xue Za Zhi., 2003; 37 (2): 231-246.
38. Martinez-Torres C, Cubeddu L and Dillmann E, et al., Effect of exposure to low temperature on normal and iron-deficient subjects. Am. J. Physiol., 1984; 246: R380 – 383.
39. Mciniory RA. A microhematocrite for determining Packed Cell and hemoglobin concentration on capillary blood. J. Clinc. Pathol., 1954; 7: 32-36.
40. McKie AT, Barrow D, Latunde-Dada GO, Rolfs A and Sager G. et al. An iron-regulated ferric reductase associated with the absorption of dietary iron. Science (Washington, DC). 2001; 291: 1755–1759.
41. McKie, AT, Marciani P, Rolfs A, Brennan, K., Wehr K, Barrow D, Miret S, Bomford A, Peters T.J, Farzaneh F, Hediger MA, Hentze MW and Simpson RJ. A novel duodenal iron-regulated transporter, IREG1, implicated in the basolateral transfer of iron to the circulation. Mol. Cell., 2000; 5: 299–309.
42. McLean E, Cogswell M, Egli I, Wojdyla D, and de Benoist B. Worldwide prevalence of anaemia, WHO Vitamin and Mineral Nutrition Information System, 1993-2005. Public Health Nutr., 2009; 12 (4): 444 - 454.
43. Milne, DB, Gallacher SK and Nielsen FH. Response of various indices of iron status to acute iron depletion produced in menstruating women by low intake and phlebotomy. Clinical Chemistry, 1990; 36: 487-491.
44. Monica Cheesbrough. District laboratories practice in tropical countries. Part 2. Cambridge Low-Price Eddition, 2004; PP: 308-318.
45. Moorehead WR, and Biggs HG. 2-Amino-2-methyl-1-propanol as the alkalizing agent in an improved continuous-flow cresolphthalein complexone procedure for calcium in serum. Clin. Chem., 1974; 20 (11): 1458 -1460.
46. National Research Council Committee on Animal Nutrition. Nutrient requirement of laboratory animals. No. 10 3rd revised edition.

National academy of science, National Research Council, Washington, DC, 1978.
47. Pineda O, Ashmead HD, Perez JM and Lemus CP: Effectiveness of iron amino acid chelate on the treatment of iron deficiency anemia in adolescents. J .Appl. Nutr., 1994; 46: 2 – 13.
48. Rao J and Jagadeesan V. Lipid peroxidation and activities of antioxidant enzymes in iron deficiency and effect of carcinogen feeding. Free Radic. Biol. Med., 1996; 21: 103–108.
49. Reeves PG, Nielson FH, and Fahey GC Jr. Ain 93 Purified diets for laboratory rodents: Final report of the American Institute of Nutrition and HOC Wriling Committee on the Reformation of the Ain 76 A rodent diet. J. Nutr., 1993; 123: 1939-1952.
50. Rickettes CD. Iron bioavailability from controlled-release and conventional iron supplements. J . Appl. Nutr ., 1993; 45:13–19.
51. Ruutu R. Determination of iron and unsaturated iron-binding capacity in serum with ferrozine. Clin. Chem. Acta, 1975; 61: 229 –232.
52. Sundaram RC, Selvaraj N, Vijavav G, Bobby Z, Hamide A., and Rattina Dasse N. Increased plasma malondialdehyde and fructosamine in iron deficiency anemia: effect of treatment. Biomed Pharmacother., 2007; 61(10): 682 - 685.
53. Torrance JD and Bothwell TH. Tissue iron stores. In: Methods in Hematology: Iron (Cook, J. D., ed.), pp. 90 –115, Churchill Livingstone, New York, NY. 1980.
54. Van NN, Khan NC, Yabutani T, Ninh NX, Kassu A, Huong BT, Do TT, Motonaka J and Ota F. Serum levels of trace elements and iron-deficiency anemia in adult Vietnamese. Biol Trace Elem. Res., 2006; 111 (1-3):1- 9.
55. Vulpe CD, Kuo YM, Murphy TL, Cowley L, Askwith C, Libina N, Gitschier J. and Anderson G J. Hephaestin, a ceruloplasmin homologue implicated in intestinal iron transport, is defective in the sla mouse. Nat. Genet. 1999; 21: 195 –199.
56. WHO/UNICEF/UNU: Iron deficiency anemia: Assessment, prevention and control. A guide for program managers (2001).
57. Yip R. Iron deficiency: contemporary scientific issues and international programmatic approaches. J. Nutr., 1994; 124: 1479S –1490S.
58. Yokoi K, Kimura M and Itokawa Y. Effect of dietary iron deficiency on mineral levels in tissues of rats. Biol. Trace Elem. Res., 1991; 29 (3): 257-265.
59. Yoshioka T, Kawada K, Shimada T and Mori M. Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood. Am J Obstet Gynecol., 1979; 135 (3): 372 - 376.
60. Zimmermann MB and Köhrle J. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. Thyroid, 2002; 12 (10): 867-878.

4/1/2010