Comparison of the efficacy of iron amino acid chelate and ferrous sulfate in the treatment of iron deficiency anemia among pregnant women seen at the out-patient department of a tertiary medical center on 2016-2017*

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ABSTRACT

Background: Anemia is a major global problem that affects women and prevalent during pregnancy. Effective management is needed to prevent adverse maternal and pregnancy outcomes. Ferrous iron salts are the preparation of choice and recommended for both prevention and treatment of iron deficiency anemia (IDA). However, most commonly available iron supplement are poorly absorbed, with gastrointestinal disturbances as side effect.

Objective: To compare the efficacy of iron amino acid chelate and ferrous sulfate in the treatment of IDA among pregnant women seen at the out-patient department of a tertiary medical center.

Methodology: This study is a single blind randomized clinical trial which included women 18 to 40 years old, with singleton pregnancies diagnosed with IDA without any co-existing fetal and maternal complications seen at the Out-Patient Department. Forty eight eligible participants were randomized, with 24 women allocated on each treatment arm who took their assigned treatment twice a day for 90 days. Hemoglobin, hematocrit, MCHC, MCV, RDW & serum ferritin levels were taken at baseline and monitored on days 30, 60 and 90 from initiation of treatment. Mean blood parameters between two treatment arms were compared on days 30, 60 and 90 post-treatment as well as the mean difference of blood parameters on days post-treatment from the baseline using T-test. Chi-square was used to compare adverse effects between two treatment arms.

Results: No statistically significant differences in the mean blood parameters on days 30 and 60 of treatment between Iron amino acid chelate and Ferrous sulfate. It was only on day 90 from initiation of treatment when there were a significantly higher hematocrit and MCHC and lower RDW in Iron amino acid chelate compared to Ferrous sulfate group. All of the CBC parameters on days 30, 60, and 90 post-treatment when compared to baseline level were significantly increased for both treatment arms. However, day 90 level of serum ferritin in the Iron amino acid chelate group significantly increased unlike those in ferrous sulfate group.

Conclusion: Iron amino acid chelate is comparable to Ferrous sulfate in the treatment of IDA among pregnant women. Iron amino acid chelate was found to be superior to Ferrous sulfate in achieving optimum treatment response even at a lower dose with lesser adverse effects. Hence, better oral iron treatment tolerability, thereby, compliance to long-term therapy can be expected resulting to successful treatment outcome.

Keywords: iron deficiency anemia in pregnancy, iron amino acid chelate, ferrous sulfate, hemoglobin, hematocrit, MCV, MCHC, RDW, serum ferritin

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INTRODUCTION

The World Health Organization (WHO) estimates that global prevalence of anemia is 25%, with pregnant woman comprising 41.8%. Southeast Asia has the highest burden of anemia in pregnant women (48.2%). In the Philippines, according to the Food and Nutrition Research Institute (FNRI) in year 2012, the prevalence of maternal anemia is 43.9% which proves that anemia is a severe public health significant factor in our country.[1]

There are various causes of anemia during pregnancy such as hemolysis, and nutritional deficiencies due to inadequate intake. Other diseases that can cause significant anemia are those that decrease absorption, increase demand and increase loss of iron. Hence, the most common type of anemia is due to iron deficiency.[1]

In a typical singleton gestation, the maternal iron averages close to 1000 mg. Of this, 300 mg is for the fetus and placenta; 500 mg for maternal hemoglobin mass expansion; and 200 mg that is shed normally through the gut, urine, and skin. The total amount of 1000 mg considerably exceeds the iron stores of most women and results in iron deficiency anemia unless iron supplementation is given.[2,5,7,18,19]

In an iron-deficient individual, about 50 to 100 mg of iron can be incorporated into hemoglobin daily. However, about 25% of oral iron given as ferrous salt can be absorbed. This justifies the administration of 200 to 400 mg of elemental iron daily to correct iron deficiency. Trying therefore to reach the therapeutic doses of iron supplementation promotes acute side effects. Not to mention that the amount of iron absorbed decreases with increasing doses. Patients unable to tolerate such large doses of iron can be given lower daily doses of iron, which results in slower but still complete correction of iron deficiency. Treatment with oral iron should be continued for 3 to 6 months to correct the anemia and replenish iron stores.

Most commonly available iron supplements are poorly absorbed from the intestine due to their low solubility and with gastrointestinal disturbances such as nausea and constipation as a frequent side effect. There are preparations wherein additional minerals are added to enhance their bioavailability. “Chelated” minerals are among the mineral supplements used for their improved absorption.

There has been no local study comparing the efficacy of iron amino acid chelate and ferrous sulfate in the treatment of iron deficiency anemia among pregnant women. Hence, in this study, we would like to determine if there is a difference between the efficacy of Iron Amino Acid Chelate and Ferrous Sulfate in the treatment of iron deficiency anemia among pregnant women.

BACKGROUND OF THE STUDY AND REVIEW OF RELATED LITERATURE

Iron requirements increase during pregnancy, to accommodate fetal and placental needs, expansion of the maternal RBC mass, and blood loss during delivery.[33] Recall that each 1 mL of erythrocytes contains 1.1 mg of iron. Because most iron is used during the latter half of pregnancy, the iron requirement becomes large after mid-pregnancy and averages 6 to 7 mg/day. In most women, this amount is usually not available from iron stores. Although the serum ferritin concentration initially rises during pregnancy, this is followed by a progressive fall by 32 weeks to about 50% pre-pregnancy levels. This is due to hemodilution and mobilization of iron. A concentration below 15 µg/L indicates iron depletion in all stages of pregnancy. Without supplemental iron, the optimal increase in maternal erythrocyte volume will not develop, and the hemoglobin concentration and hematocrit will fall appreciably as plasma volume increases. At the same time, fetal red cell production is not impaired because the placenta transfers iron even if the mother has severe iron deficiency anemia.[34]

It follows that the amount of dietary iron, together with that mobilized from stores, will be insufficient to meet the average demands imposed by pregnancy. If the non-anemic pregnant woman is not given supplemental iron, then serum iron and ferritin concentrations decline after midpregnancy.[34]

The high physiological requirement for iron in pregnancy is difficult to meet with most diets. Therefore, pregnant women should routinely receive iron supplements in almost all contexts. The prevalence of anemia in pregnant women is high (40% or more), supplementation should continue into the postpartum period to enable women to acquire adequate iron stores.

According to experts, there are several measures to correct IDA. First, an increased intake of iron-rich foods such as liver, meat, fish and eggs. These are good sources of heme-iron, the form that is readily absorbed in the body. Other sources such as grains, legumes and vegetables are also sources of non-heme iron, the form that is less absorbed unless taken together with meat, vitamin C-rich foods or processed in a way to enhance iron absorption. Another method that is preventive as well as therapeutic measure is iron supplementation using iron tablets or capsules. The cost of taking iron supplements is very minimal when compared to the benefits of preventing IDA and its devastating effect on both the mother and the child.

Iron supplementation has long been advocated for anemia control. A wide variety of oral iron preparations are available. Since ferrous iron is most efficiently absorbed, only ferrous salts should be used. The salt preparations

include ferrous sulfate, gluconate, and fumarate. They are all effective and inexpensive and are recommended for the treatment of iron deficiency anemia. Different iron salts provide different amounts of elemental iron. Fumarate is highest (106 mg elemental iron/tablet), followed by sulfate (65 mg elemental iron/tablet) then gluconate (28-36 mg elemental iron/tablet). However, these ferrous salts show only marginal differences in efficacy of iron absorption. In the same manner that dietary iron is only partially absorbed, this is also true with iron salts.

In pregnant women, iron therapy should be tried initially. Current guidelines recommend empiric treatment in pregnant women with iron deficiency anemia. However, if the hemoglobin level does not increase by 1 gram per deciliter (10 gram per liter) after one month of therapy in pregnant women, further evaluation may be indicated.[4,15,16] In pregnant patients, poor compliance or intolerance should be considered, and parenteral iron may produce a better response.[15]

A dietary supplement is unavailable to the majority of the population. Hence, many governments have mandated iron fortification of basic foods.[22,23] Frequently, however, in order to retain palatability and retard oxidation of certain food components, the iron salts selected for fortification have had low solubility. Low solubility generally equates to low bioavailability.[24,25] For example, ferrous fumarate, ferrous glycine sulfate, ferrous sulfate, ferrous citrate, ferrous tartrate, ferrous pyrophosphate, ferric coline citrate, ferric sulfate, ferric citrate, and ferric ethylenediaminetetraacetate showed decreasing degrees of iron absorption, respectively, with the ferric compounds being absorbed at less than half of the first three ferrous sources[26]. Increasing iron levels in a meal does not proportionately increase iron absorption. The mean absorption of non-heme iron has been reported to decrease from 18% to 6.4% as the non-heme iron content of the meals was increased from 1.52 mg to 5.72 mg[27]. Although 6.4% of 5.72 mg is higher than 18% of 1.52 mg (0.37 mg versus 0.27 mg), the amount of increased absorption is not proportional to the increased dose. It is, in fact, greatly suppressed. Increasing doses of unprotected iron is, thus, far more likely to elicit toxicity, than significantly increasing absorbed iron.

In order to enhance iron bioavailability and still avoid interactions with food ingredients, chelating iron with amino acids has been employed. A nutritionally viable iron amino acid chelate must have a stability constant that is higher than the potential formation constants which would result if the iron were chelated or complexed to the food ligands found in the stomach and intestines[30]. This is necessary for the original chelate to remain intact in the gastrointestinal tract prior to absorption. If the chelate dissociates in the gut, it has no more value than ionized iron from a soluble salt. The stability constant should also be high enough to allow the chelate to cross the intestinal cell membrane into the cytoplasm, and yet be low enough that the cytoplasmic ligands are capable of removing the iron from the absorbed amino acid chelate by complexing with the absorbed iron. In this fashion, the rate of delivery to the target tissue or enzyme from the mucosal cell is controlled.[31]

An iron-amino acid chelate has relatively high bioavailability. Theoretically, the conjugation of ferrous iron with amino acid prevents the iron from forming insoluble ferric hydroxide in the small intestine hence making it less likely to cause gastrointestinal intolerance than ferrous sulfate, ferrous gluconate, or ferrous fumerate.[35] Theoretically, chelated irons are better tolerated and absorbed compared to other forms of iron.

To be able to gather the evidence regarding the use of iron in management of IDA, a computerized literature search was done. The following search terms were used: anemia, pregnancy, ferrous sulfate and iron amino acid chelate. The union and intersection of the free text and MeSH terms of these search terms yielded 73 articles. Among these articles, only 27 studies were relevant to our study. These are 4 individual studies and the other 23 were included in a meta-analysis.

These studies were conducted by Olivares et al. in Chile,[21] Bovell-Benjamin et al. in the United States,[23] and Layrisse et al. in Venezuela.[24] In all of these studies iron absorption was evaluated by labeling the iron amino acid chelate with radioactive or stable iron isotopes and measuring the amount incorporated into red blood cells approximately two weeks later.

In the research by Olivares et al.[21] the absorption of iron from ferrous bisglycinate in water given to 14 adult women was compared with its absorption from milk in a different group of 14 similar individuals. Only two subjects were iron deficient. Because different subjects were used in each study, and the amount of iron absorbed by each individual is affected by their iron status, all subjects also consumed a reference dose of ferrous ascorbate to correct for interindividual differences in iron status. Ferrochel iron was less well absorbed from the milk (11%) than from the water (46%). Also, adding ascorbic acid increased the absorption of Ferrochel iron from milk by 38%, to 15%. These results suggest that inhibitors and enhancers can affect the absorption of the bisglycinate iron. The authors commented that there was a much larger (approximately 250%) increase in iron absorption, however, when ascorbic acid was added to ferrous sulfate in a previous experiment. Another limitation of this study was that there was no assessment of the absorption of ferrous sulfate iron from milk; the authors reported that in a previous study they found this to be only 4%, which would mean

approximately three times more ferrous bisglycinate iron than ferrous sulfate iron is absorbed from milk.

In the study by Bovell-Benjamin et al.[23] in the United States, the investigators[23] assessed whether the absorption of the iron from Ferrochel is down-regulated normally by higher iron stores. Iron absorption from ferrous ascorbate in water and Ferrochel in water was compared in 21 healthy adult women with a range of iron status (serum ferritin from 2 to 63 g/L). When given in water, iron absorption from the bisglycinate was 31% compared with 72% from the ascorbate. Absorption of iron from both compounds was inversely and similarly correlated with iron status (r 0.61 between ferrous ascorbate and serum ferritin, and r 0.78 between ferrous bisglycinate and serum ferritin). Finally, the absorption of ferric trisglycinate iron in water (39%) was similar to that of the ferrous bisglycinate iron, but the trisglycinate iron was quite poorly absorbed (2.3%) from the maize porridge meal. This study shows the superior absorption of iron from the ferrous bisglycinate in the presence of high phytate and suggest that the bisglycinate iron is taken into the intestinal cells in the chelated form. It is probable, however, that the chelate is degraded within the intestinal cell because the absorption of its iron is regulated normally by iron status.

Layrisse et al. studied iron bioavailability from breakfasts enriched with ferrous bisglycinate to which phytates and polyphenols (iron absorption inhibitors) were added.[24] Five different experiments were conducted in a total of 74 subjects. When ferrous bisglycinate and ferrous sulfate were given together or in different meals (in breads made from corn flour or white wheat flour, with cheese and margarine) the iron absorption from the bisglycinate was twice that from the sulfate although it was slightly less than iron absorption from iron EDTA. The efficiency of iron absorption from the fortified corn flour was 5.1% from ferrous sulfate and 10.1% (signicantly higher) from the ferrous bisglycinate; these data support the study by Bovell-Benjamin et al.[23] that found higher absorption of the bisglycinate iron from maize. However, the addition of phytase to the bisglycinate fortifed corn bread did increase iron absorption by approximately 30%, indicating that there was some inhibition of absorption of the bisglycinate iron by phytate. The polyphenols in espresso coffee and tea reduced iron absorption from the ferrous bisglycinate by 50% but there was no ferrous sulfate control for comparison.

Based on the 3 studies described above, it shows that the advantage of using ferrous bisglycinate as an iron fortificant is its much higher absorption from foods that are high in iron absorption inhibitors. The investigators concluded that ferrous bisglycinate is a suitable compound for food fortification.

There has been many iron supplementation trials involving pregnant women over the past 30 years. Sloan, et al., conducted a meta-analysis on the effects of iron supplementation on maternal hematologic status in pregnancy. Data from randomized controlled trials published between 1966 and 1998 were pooled. There are 70 studies of iron supplementation in pregnant women identified and reviewed; two thirds of these studies were from developing countries.

The criteria used to determine whether to include a study in this meta-analyses were the study: 1) must have been a randomized controlled trial, 2) must have reported the initial sample size, baseline and follow-up or change in hemoglobin concentrations or hematocrit and their associated variance, 3) the daily dose of supplemental elemental iron, duration of therapy, proportion of women with whom follow-up was conducted, 4) general reasons for loss to follow-up, and 5) additional nutritional supplementation or medical therapy provided. Only 23 studies—15 of which were conducted in developing countries—met these criteria. Almost all studies drew their samples from women attending prenatal clinics; 2 drew their samples from rural antenatal programs.[11,12]

According to this meta-analysis, pooled data indicated that the average baseline hemoglobin level of women in 13 of the 15 randomized controlled trials conducted in developing countries reviewed in this article was less than 11 g/dL. Relative to no supplementation (by any nutrient or medication), iron supplementation alone increased hemoglobin change by 1.00 ± 0.013 g/dL (P<.001, n=1118, df=13). The average daily dose of iron in women receiving supplementation was 114 ± 65 mg. In combination with other nutrients iron supplementation increased hemoglobin change by 1.20 ± 0.023 g/dL (P<.001, n=1077) relative to no supplementation, and the average daily dose of iron was higher: 132 ± 76 mg (P<.001). In studies reporting serum ferritin change and its variance, iron supplementation alone increased serum ferritin levels by 9.48 ± 0.0174 μ/L (the mean effect on hemoglobin was 0.87 ± 0.017 g/dL with an average daily dose of 132 ± 77 mg iron, n=578, P<.001) relative to no supplementation.

In a few studies reporting iron deficiency, iron supplementation alone reduced the percentage of women with hemoglobin levels of less than 11 g/dL by 38% ± 0.101% (the mean effect on hemoglobin was 1.20 ± 0.039 g/dL with an average daily dose of 167 ± 82 mg, n=431, P<.001). All but 1 study, with 30 subjects, of the effect of iron supplementation alone used placebos. The average effect of iron supplementation in the subsample of studies using placebos was 0.99 ± 0.013 g/dL (n=1088) at an average daily dose of iron supplementation of 112 ± 64 mg.

The effect of adherence to iron supplementation and the reason of non-adherence are also reviewed in this meta-analysis. Adherence to the regimen affects the effectiveness of the supplementation.[2,25,36] Side effects of iron supplementation include constipation, diarrhea, vomiting, or epigastric pain. These effects are reported to increase with dose[21,26] and may have caused some women to abandon therapy or take less than the recommended dose. Although many of the studies reviewed report that adherence was a problem, few measured compliance. Adherence to iron supplementation was found to be poor (approximately 42%) in a study that carefully measured this factor in Tanzania[27]; it was better (61%) with a slow-release gastric delivery system in another study.[21]

In this meta-analysis, they also reviewed studies on the effects of iron supplementation on hemoglobin between developed and developing countries. They stratified the relative change in maternal hemoglobin by initial hemoglobin levels, duration of supplementation, and daily gestational supplement dose and supplementation with other nutrients.

In relation to the initial hemoglobin level, the effects of iron supplementation was greater in women from developing countries with initial levels of less than 11 g/dL relative to unsupplemented women (P<.001). The effect of iron supplementation in women with initial hemoglobin values of less than 10 g/dL[18] and 10 to less than 11 g/dL[11,13,17] was 1.13 ± 0.120 g/dL (P < .05) and 1.10 ± 0.045 g/dL, respectively (P < .001). The effect was smaller—0.85 ± 0.018 g/dL (P < .001)—in women from developing countries with initial levels of 11 to less than 12 g/dL.[17,23] No data were available on iron supplementation alone for women from developing countries with initial levels of 12 g/dL or higher. There were no studies of women in developed countries with low initial hemoglobin levels. The effect of iron supplementation alone in women in developed countries with initial mean hemoglobin levels of more than 11 g/dL to less than 12 g/dL[28,29] and at least 12 g/dL[24,25,30] averaged 1.17 ± 0.022 g/dL and 1.16 ± 0.045 g/dL above unsupplemented comparisons (both P<.001), respectively.

Second aspect they looked into was studies from developed and developing countries. They compared the effect of daily dose iron supplements between these countries. The studies showed a positive dose–response relationship exists between iron dose and change in studies with unsupplemented comparison groups (P < .001). Women receiving no more than 60 mg daily iron supplementation alone, with an average daily dose of 42 ± 23 mg iron,[11,28,39] had a 0.41 ± 0.027 g/dL increase in change compared with unsupplemented women (P < .01). Women receiving between 61 and 90 mg daily iron supplementation with an average dose of 76 ± 4 mg iron[20,32] showed an average effect of 0.86 ± 0.018 g/dL (P < .01). Women receiving between 91 and 120 mg daily iron supplementation with an average dose of 117 ± 6 mg iron[15,16,18,29,30] had an average effect of 1.87 ± 0.027 g/dL (P < .001), and those receiving more than 120 mg daily iron supplementation with an average dose of 223±20 mg iron[16,17,23,24] had an average increase of 1.78 ± 0.042 g/dL (P < .001), compared with women not receiving supplementation. Only 2 studies compared hematologic change associated with iron alone in women receiving higher (167 ± 26 mg) versus lower (45 ± 22 mg) average daily supplementation.[29,31] These 2 studies showed a 0.33 ± 0.012 g/dL improvement with higher iron supplementation (P<.05).

The third aspect in their analysis is the effect of duration of treatment on hemoglobin. Studies from developed and developing countries providing up to 10 weeks,[18,22] 11 to 13 weeks,[17] and 14 to 19 weeks[15,16,29] of iron supplementation alone found benefits of 0.84 ± 0.017 g/dL (P < .001), 1.37 ± 0.062 g/dL (P < .05), and 1.16 ± 0.022 g/dL (P < .001), respectively, relative to no supplementation. The effect of 20 weeks or more of iron supplementation in women in developed countries[23,24,28,30] (1.00 ± 0.039 g/dL, P < .001) was smaller than that in other women receiving more than 10 weeks but less than 20 weeks of supplementation. The effect of duration of supplementation is attributable to differences in iron supplementation dose.

Finally, they analyze studies on the effect of iron supplementation with other nutrients. Women receiving combined average daily iron (176 ± 65 mg) and folate (5 ± 0 mg) supplementation had 1.37 ± 0.093 g/dL (df = 8, P < .001) better change than did women not receiving supplementation,[15,16,28] but there was no additional effect (–0.07 ± 0.009 g/dL, df = 19, P < .001) from folate (5 ± 0 mg with 170 ± 64 mg iron) compared with iron supplementation alone (158 ± 74 mg in those without folate).[15,16,28,31] The combined effect of folate and iron supplementation (average daily doses: 66 ± 18 mg iron and 4.6 ± 0.5 mg folate) on maternal hematologic change above folate supplementation alone (average daily dose: 4.7 ± 0.4 mg folate) was 1.22 ± 0.047 g/dL (df = 3, P < .001).[12,15,21,28]

Another evidence is a random, prospective, open study with individual benefit was performed by Bayoumeu F, et al., in a Maternity Hospital at Nancy, France. The aim of this study was to compare intravenous iron sucrose versus oral iron sulfate in anemia at 6 months of pregnancy. This study involved 50 patients with hemoglobin levels between 8 and 10 g/dL and a ferritin value of <50 microgram/L. In the intravenous group (IV group), the iron dose was calculated from the following formula: Weight before pregnancy (kg) x (120 g/L - Actual

hemoglobin [g/L]) x 0.24 + 500 mg. The oral group (PO group) received 240 mg of iron sulfate per day for 4 weeks. Treatment efficacy was assessed by measurement of hemoglobin and reticulocytes on days 8, 15, 21, and 30 and at delivery and of ferritin on day 30 and at delivery. The baby's birth weight and iron stores were noted. Results were expressed as median +/- interquartile range. Mann-Whitney and Wilcoxon tests were used for the analysis, with P <.05 considered significant. In the result, an increase in hemoglobin was observed, rising from 9.6 +/- 0.79 g/dL to 11.11 +/- 1.3 g/dL on day 30 in the IV group and from 9.7 +/- 0.5 g/dL to 11 +/- 1.25 g/dL on day 30 in the PO group (not significant). On day 30 (P <.0001) and at delivery (P =.01) ferritin was higher in the IV group. A mean higher birth weight of 250 g was noted in the IV group (not significant). It therefore concluded that both oral and parenteral iron appears to be both efficacious in the treatment or correction of pregnancy anemia and iron stores depletion.

In monitoring the outcome of oral iron therapy for iron deficient individuals or populations, one established approach involves monitoring changes in hemoglobin or hematocrit after oral iron supplementation. An increase in hemoglobin of 1 g per dL after one month of treatment shows an adequate response to treatment and confirms the diagnosis.[36] In adults, therapy should be continued for three months after the anemia is corrected to allow iron stores to become replenished[37].

In pregnant woman with IDA, once hemoglobin is in the normal range supplementation should continue for 3 months and at least until 6 weeks postpartum to replenish iron stores. The hemoglobin concentration should rise by approximately 20 g/L over 3 to 4 weeks. However, the degree of increase in hemoglobin that can be achieved with iron supplements will depend on the hemoglobin and iron status at the start of supplementation, ongoing losses, iron absorption and other factors contributing to anemia hence monitoring after 2 to 3 months is necessary.

Optimally, iron therapy should be of sufficient duration to allow for complete restoration of the patient's ideal hemoglobin level as well as to provide some storage iron. This will generally involve treatment for three to six months.[38]

In summary, the literatures have demonstrated that iron supplementation during pregnancy increases hemoglobin and serum ferritin levels. The effect of iron supplementation is directly related to dose. The effect also is slightly more pronounced in women with lower initial hemoglobin levels. The effect of duration of therapy was mediated by dose. Inferences about the effects of other nutrients in addition to iron are limited because data from multiple studies are available only for folate and vitamin C. Inferences about the effect of iron supplementation on maternal anemia also are limited by the small number of studies and subjects from which these data were available.

Iron supplementation is an effective strategy to prevent anemia. However, evidence to treat anemia of pregnant women from the developing world with the use of Iron amino acid chelate is scarce. To date, based on literature search, there are still no available studies done, particularly in the Philippines, comparing the efficacy of Iron Amino Acid Chelate and Ferrous Sulfate in the treatment of IDA in pregnant women.

SIGNIFICANCE OF THE STUDY

Continuing rapid advances in understanding the molecular mechanisms of iron absorption and metabolism might enable the development of new strategies to combat iron deficiency. Mineral absorption from supplements has been a contested issue in the supplement industry. Companies naturally desire to provide the best products and gain market share by promoting them as the best. This has led many companies to claim superior absorption from particular formulations of mineral supplements. Understanding and validating some of these claims has often been difficult, due to insufficient or conflicting research. This paper is initiated to address the controversy and establish the state of scientific knowledge about mineral absorption from supplements.

This will compare the efficacy of ferrous sulfate with respect to iron amino acid chelate as a supplement in pregnant women with depleted levels of iron. Most commonly available iron supplements such as ferrous sulfate are poorly absorbed in many people, with gastrointestinal (GI) disturbances such as nausea and constipation a frequent side effect. Iron amino acid chelate is an extremely well tolerated form of iron that been shown in numerous clinical studies to exhibit superior absorption compared to other forms. The superior absorption of iron amino acid chelate compared to ferrous sulfate also means lower doses are required to produce comparable increases in iron levels hence lesser side effects. In addition, its superior tolerability will enable more women to complete the trial until the end. These advantages of iron amino acid chelate will lead pregnant women to be more compliant in taking oral iron therapy hence circumvent hospital stay for possibility of parenteral iron therapy or blood transfusion, which can also improve perinatal outcomes.

It is hoped that the results may contribute, albeit indirectly, to improve the health status of pregnant women with depleted levels of iron who consume iron amino acid chelate.

OBJECTIVES

General Objective: To compare the efficacy of iron amino acid chelate and ferrous sulfate in the treatment of IDA among pregnant women seen at the out-patient department of a Tertiary Medical Center on 2016-2017

Specific Objectives:

1. To compare the mean of the following parameters at days 30, 60 and 90 from treatment initiation between Iron Amino Acid Chelate and Ferrous Sulfate
   a. hemoglobin level
   b. hematocrit level
   c. mean cell volume (MCV)
   d. mean cell hemoglobin concentration (MCHC)
   e. red cell distribution width (RDW)
   f. serum ferritin level
2. To compare the mean difference in the levels of the following parameters taken at days 30, 60 and 90 post-treatment from the baseline level between Iron Amino Acid Chelate and Ferrous Sulfate
   a. hemoglobin level
   b. hematocrit level
   c. MCV
   d. MCHC
   e. RDW
   f. serum ferritin level
3. To compare the adverse effects between Iron Amino Acid Chelate and Ferrous Sulfate

DEFINITIONS OF VARIABLES

A. Independent Variable: This variable refers to the treatment being compared in this study. This will be encoded and entered as:
1 - Ferrous Sulfate
2 - Iron amino acid chelate

B. Dependent Variable: This variable refers to the parameters being assessed in this study.
   a. The actual and mean values of these parameters will be recorded.
      1. hemoglobin level
      2. hematocrit level
      3. MCV
      4. MCHC
      5. RDW
      6. serum ferritin level
   b. Adverse effects – this data was extracted from the participants who experienced the adverse effects during intake of oral iron supplement in this study. The actual side effects will be recorded.
      1. nausea
      2. vomiting
      3. constipation
      4. black stool
      5. epigastric pain

These will be further categorized, encoded and entered based on:

a) Presence/Absence:
   1 – with presence of any of the above adverse effects
   2 – absence of the above adverse effects
b) Number of Side Adverse Effects:
   1 – Single adverse effect
   2 – Combination of adverse effects

METHODOLOGY

A. Design: This is a single-blind randomized controlled study.

B. Setting and Population:

1. Setting: Out-Patient Section
Tertiary Medical Center

2. Population: All pregnant women ages 18 to 40 years old, with singleton pregnancies seen in the out-patient clinic diagnosed with iron deficiency anemia will be included in this trial.

Inclusion Criteria: Included were pregnant women diagnosed with anemia based on the following parameters:

- hgb level of 10 to 11 g/dL during the 1st (until 14 weeks) and 3rd trimester (period of 28-30 weeks AOG), or
- hgb level of 9.6 to 11 g/dL during the 2nd trimester (14 weeks 1 day-27 weeks 6 days AOG)

Exclusion Criteria: Those pregnant women with any of the following will be excluded:

1. hemoglobinopathies and other blood dyscrasias
2. iron metabolism problems (eg, hemosiderosis, hemochromatosis) or high levels of iron in your blood
3. co-existing fetal complications like IUGR or congenital anomalies
4. multifetal pregnancy
5. preexisting medical conditions such as hypertension, DM and thyroid disorders
6. placental abnormalities
7. those residing in high altitude places/ smoker
8. low body mass index (<18 kg/m2) / malnourished

METHODOLOGY PROPER

Pregnant women seen at the out-patient clinic with complete blood count (CBC) showing anemia were evaluated for eligibility for inclusion in the study. Those who were eligible to participate were asked to sign an informed consent prior to the inclusion. (Please see Appendix 1 for details). Once the participant has signed the informed consent, baseline serum ferritin assay was added to the baseline parameters.

The participants were randomized as to their respective treatment group using computer generated random numbers. The subjects assigned to Group 1 were given Iron Amino Acid Chelate while those assigned in Group 2 were given Ferrous sulfate. The elemental iron content of amino acid chelate is 30 mg per tablet while ferrous sulfate contains 65 mg elemental iron per tablet. For both groups, the participants were asked to take the iron preparation 2x a day for 3 months on an empty stomach. The participants were advised to take iron supplements 30 minutes to 1 hour before meals. If tea, coffee, milk, calcium supplements or antacids were to be taken, they were advised to take these 2 to 4 hours apart from intake of iron supplements.

The iron supplement was provided by the primary investigator to the participants. The number of supplement dispensed to the participants was enough to cover for daily intake until their next follow-up. The supplements were refilled during each follow-up. Compliance was monitored by counting the empty foil shells of the treatment as these were returned to the residents assigned at the OPD. Furthermore, the participants were instructed to avoid taking foods fortified with iron which is usually indicated on the label of such foods. They were also given a diary on which they will write their daily food intake.

The participants were also informed regarding the adverse effects such as nausea, constipation, stomach upset, and/or vomiting after taking oral iron as well as darkening of the stool. They were advised to contact the investigator if the adverse effects cannot be tolerated.

The participants were instructed that the storage of ferrous sulfate or iron amino acid chelate should be at room temperature, be kept away from heat, moisture, and light and should not be stored in the bathroom.

The data were recorded in a standard data collection tool provided for each patient. The data included in the standard data collection form were age, gravidity & parity, age of gestation, hemoglobin, hematocrit, MCV, MCHC, RDW, & serum ferritin. The prenatal check-up schedule followed the required interval visits recommended at the OPD. The schedules were as follows: once a month up to 28 weeks, twice a month up to 36 weeks and weekly thereafter.

The blood sample for Complete Blood Count & serum Ferritin were extracted and processed in the Hematology Section of the Clinical Laboratory of the tertiary medical center. CBC was processed using the Sysmex automated machine while the serum ferritin assay was measured spectrophotometrically on Beckman Coulter AU Chemistry Analyzers.

Based on the plan stated in the proposal, if after 30 days from initiation of iron therapy, despite good compliance of the participants and still no improvement noted, further evaluation regarding the underlying cause should be conducted with referral to the Hematology Service. For those participants who can not tolerate the required iron supplementation, an appropriate alternative would be offered. However, none of these were noted in this study, all of the participants tolerated and completed the treatment.

SAMPLE SIZE CALCULATION

For sample calculation in this study, the formula on comparing means from the two independent samples was used. Sample size calculations were based in the study of Stoltzfus et. Al. In this study, the estimated group means of the hemoglobin among pregnant women who took iron supplementation alone is 11.0 g/dL. The other mean hemoglobin 12.17 g/dL was based from the study of Mahomed et al which is the mean hemoglobin among prresegnant women taking iron supplement with folic acid. The test of equality of means was carried out at the 0.05 level of significance. A sample size of 19 per group gives a probability of 80% of rejecting the null hypothesis of equal means if the alternative holds.

DATA MANAGEMENT ANALYSIS

The data entry and encoding was done using Microsoft Excel. Analysis was done using intercooled strata version 9.1. Univariate analysis such as mean, median, mode and range were used to describe the age, gravidity and parity, age of gestation, hemoglobin, hematocrit, MCV, MCHC, RDW, and serum ferritin. Frequency distribution was used to describe the proportion of patients with adverse effects. Student's t-test was used to compare the difference in the mean hemoglobin, hematocrit, MCV, MCHC, RDW and serum ferritin concentration between groups as well as the mean difference before and after 30, 60, and 90 days from the initiation of treatment. Chi-square was used to compare the adverse effects between the two treatment arms. There were no drop outs in this study. All participants completed the treatment assigned to them.

ETHICAL CONSIDERATION

An informed consent was obtained from the eligible participants. This will provide them the information on the nature and purpose of the study, and the possible adverse effects that would arise from the study. Participants were given the assurance on the confidentiality of the results by protecting their identity through the use of number codes on the data collection tool. The participants were given the prerogative to withdraw from the study without

incurring penalty. All participants were informed of the summary of the outcome of the study.

The participant was informed that participation in the study has no involvement of any monetary compensation or other forms of material goods. The blood extraction for complete blood count and serum ferritin tests were shouldered by the primary investigator and the pharmaceutical company provided by the Iron amino acid chelate (Aminofer FA) preparations that will be given to the participants. The pharmaceutical company did not have, in any way have a role in the design, conduct of the study as well as the collection, management, analysis and interpretation of data. They were not involved in the preparation, review and approval of the study. The primary investigator as well as the co-investigators conducted the research study but did not have any incentives nor monetary compensations from the pharmaceutical company. Hence no conflict of interest exists in this study.

When the scheduled laboratory examinations did not fall on the expected prenatal check-ups, transportation fee were provided. Hence no expenses will be shouldered by the participants.

The contact number of the primary investigator was given to all the participants. In the event that any side effects occurred, persisted or become bothersome, they were instructed to contact and seek medical attention with the principal investigator.

For those participants in this study whose complete blood count parameters did not improve or if there is progression of anemia, referral to Hematology Service was contemplated. This is done to ensure that appropriate management was afforded to these participants. However, in this study, none of the participants had progression of the anemia.

Those pregnant women who participated in the study were considered as vulnerable groups. In this regard, the principal investigator guaranteed the participants were well-informed regarding the effects of the medications, necessary interventions as well as the importance of regular prenatal check-ups and intake of vitamins. This was done in order for the participants to fully understand the primary objectives of prenatal care.

This study was reviewed and approved by the Institutional Ethics Review Committee (IERC).

RESULTS

There were 48 pregnant participants in this study who met the inclusion criteria and were included in the trial. For each treatment arm, there were 24 participants. The age range of the participants was 18 to 36 years old with mean age of 26.7 years old. The participants had a gravidity range of 1 to 4 with a mean of 1.9 and parity of 0 to 3 with a mean of 0.78. The age of gestation of the participants included ranged from 9 weeks to 30 weeks with a mean of 20.5 weeks. The participants between the two treatment groups had no statistically significant differences in their baseline characteristics. (Table 1)

Table 1. Baseline patient's characteristics according to treatment arm

Parameter	Iron Amino Acid Chelate Group Mean (SD)	Iron Salt Group Mean (SD)	P value
Maternal age	26.21 (4.19)	27.13 (5.07)	0.49
Gravidity	1.92 (0.83)	1.92 (0.88)	1.00
Parity	0.83 (0.87)	0.75 (0.85)	0.74
Age of Gestation	19.71 (6.77)	21.33 (5.89)	0.38

Statistical test done: t-test Statistically significant p-value <0.05

The CBC parameters used were hemoglobin, hematocrit, MCV, MCHC and RDW. Baseline levels were measured and the results showed hemoglobin ranged from 9.1 to 11.2 g/dL with a mean of 10.4 g/dL; hematocrit ranged from 27 to 35.8% with a mean of 30.87%; MCV ranged from 80.57 to 94.12 um^3 with a mean of 86.64 um^3; MCHC ranged from 27.0 to 38.0 g/dL with a mean of 34.0 g/dL; RDW ranged from 13.7 to 16.1% with a mean of 14.86%. The baseline serum ferritin ranged from 8.68 to 56.89 ng/mL with a mean of 22.86 ng/mL. With regards to the comparison of the baseline blood parameters, there were no significant differences noted between the two treatment arms. (Table 2)

Table 2. Comparison of the Mean of Baseline Blood Parameters according to treatment arm

Blood Parameters (Baseline)	Iron Amino Acid Chelate Group *Mean* (SD)	Iron Salt Group *Mean* (SD)	P value
Hemoglobin	10.12 (0.68)	9.97 (0.60)	0.41
Hematocrit	31.13 (2.43)	30.62 (2.43)	0.47
MCV	86.96 (3.03)	86.32 (2.97)	0.46
MCHC	34.0 (2.00)	32.0 (2.00)	0.99
RDW	14.90 (0.57)	14.82 (0.52)	0.65
Serum Ferritin	21.44 (13.03)	24.27 (13.60)	0.46

Statistical test done: t-test Statistically significant p-value <0.05

The efficacy of iron amino acid chelate and ferrous sulfate in treating IDA based on blood parameters were compared with hemoglobin as the main outcome variable. On day 30 from initiation of treatment, there was a significant increase on all blood parameters from the baseline level

except for serum ferritin. This was noted among the participants for both treatment arms. (Table 3)

Table 3. Comparison of the mean in the levels of the Blood Parameters at day 30 post-treatment from the baseline level

Blood Parameters	IRON AMINO ACID CHELATE			IRON SALT		
	Baseline Mean (SD)	Day 30 Mean (SD)	P value	Baseline Mean (SD)	Day 30 Mean (SD)	P value
Hemoglobin	10.12 (0.68)	11.59 (0.56)	0.0001	9.97 (0.61)	11.45 (1.03)	0.0001
Hematocrit	31.13 (2.43)	34.58 (2.09)	0.0001	30.62 (2.42)	33.83 (3.33)	0.0001
MCV	89.96 (3.03)	90.6 (3.69)	0.0005	86.32 (2.97)	88.69 (3.54)	0.02
MCHC	34 (2.00)	36 (3.00)	0.0009	33 (2.10)	34 (2.00)	0.0002
RDW	14.96 (0.58)	14.32 (0.81)	0.0061	14.82 (0.52)	14.54 (0.58)	0.04
Serum Ferritin	21.45 (13.03)	22.78 (13.22)	0.72	24.27 (13.60)	27.02 (16.86)	0.54

Statistical test done: t-test Statistically significant p-value <0.05

When the blood parameter levels of the participants on day 30 from the initiation of treatment were compared between the 2 treatment arms, no significant differences were noted. (Table 4)

Table 4. Comparison of the Mean of Blood Parameters on Day 30 Post-treatment according to treatment arm

Blood Parameters (After 30 Days)	Iron Amino Acid Chelate Group *Mean* (SD)	Iron Salt Group *Mean* (SD)	P value
Hemoglobin	11.59 (0.55)	11.45 (1.03)	0.72
Hematocrit	34.58 (2.06)	33.82 (3.32)	0.82
MCV	90.62 (3.69)	88.69 (3.54)	0.96
MCHC	0.36 (0.02)	0.34 (0.01)	0.99
RDW	14.31 (0.81)	14.54 (0.57)	0.13
Serum Ferritin	22.78 (13.22)	27.01 (16.86)	0.17

Statistical test done: t-test Statistically significant p-value <0.05

Repeat evaluation after 60 days of treatment for both treatment arms showed that the mean blood parameters of the participants were noted to have significantly higher levels when compared to the baseline. Similar to the day 30 evaluation, only the serum ferritin levels did not show a significant increased from the baseline. This was noted on both treatment arms. (Table 5)

Table 5. Comparison of the mean in the levels of the Blood Parameters at day 60 post-treatment from the baseline level

Blood Parameters	IRON AMINO ACID CHELATE			IRON SALT		
	Baseline Mean (SD)	Day 60 Mean (SD)	P value	Baseline Mean (SD)	Day 60 Mean (SD)	P value
Hemoglobin	10.12 (0.68)	11.83 (0.62)	0.0001	9.97 (0.61)	11.89 (0.89)	0.0001
Hematocrit	31.13 (2.43)	35.34 (2.09)	0.0001	30.62 (2.42)	34.49 (2.87)	0.0001
MCV	89.96 (3.03)	90.24 (3.84)	0.0020	86.32 (2.97)	88.62 (3.68)	0.02
MCHC	34 (2.00)	37 (0.003)	0.0001	33 (2.10)	35 (2.00)	0.0003
RDW	14.96 (0.58)	14.11 (0.77)	0.0002	14.82 (0.52)	14.43 (0.51)	0.0098
Serum Ferritin	21.45 (13.03)	22.90 (13.23)	0.70	24.27 (13.60)	27.11 (16.79)	0.52

Statistical test done: t-test Statistically significant p-value <0.05

However when the blood parameters of the participants between iron amino acid chelate and iron salt groups compared, no significant differences were noted. (Table 6). This was true for both the CBC parameters and serum ferritin assay.

Table 6. Comparison of the Mean of Blood Parameters on Day 60 Post-treatment according to treatment arm

Blood Parameters (After 60 Days)	Iron Amino Acid Chelate Group *Mean* (SD)	Iron Salt Group *Mean* (SD)	P value
Hemoglobin	11.82 (0.62)	11.89 (0.89)	0.38
Hematocrit	35.34 (2.09)	34.49 (2.87)	0.87
MCV	90.24 (3.85)	88.62 (3.68)	0.93
MCHC	0.37 (0.03)	0.35 (0.02)	0.99
RDW	14.11 (0.77)	14.43 (0.51)	0.09
Serum Ferritin	22.90 (13.23)	27.11 (16.79)	0.17

Statistical test done: t-test Statistically significant p-value <0.05

Final evaluation was done after 90 days from the initiation of treatment. All of the participants had blood parameter levels falling within the normal range (Table 7). On this day, only the hemoglobin and serum ferritin levels were not significantly different among the participants between the 2 treatment arms. The hematocrit and MCHC were significantly higher and RDW was significantly lower in the participants who took iron amino acid chelate. On the other hand, the MCV was significantly increased among participants who have taken iron salt.

Table 7. Comparison of the Mean of Blood Parameters on Day 90 Post-treatment according to treatment arm

Blood Parameters (After 90 Days)	Iron Amino Acid Chelate Group *Mean* (SD)	Iron Salt Group *Mean* (SD)	P value
Hemoglobin	12.39 (0.69)	12.26 (0.87)	0.58
Hematocrit	37.90 (1.03)	35.91 (2.43)	<0.001
MCV	90.45 (3.91)	91.19 (4.87)	0.56
MCHC	0.38 (0.01)	0.36 (0.02)	0.02
RDW	11.36 (1.10)	12.35 (1.50)	0.01
Serum Ferritin	30.21 (11.95)	30.77 (16.90)	0.54

Statistical test done: t-test Statistically significant p-value <0.05

With regards to the comparison of the CBC parameters of the participants between day 90 post-treatment and the baseline, similar to day 30 and 60 evaluations, all of their mean levels were significantly increased on day 90. However, for the serum ferritin levels, only the participants taking iron amino acid chelate had a significantly higher mean level at day 90 when compared to the baseline. (Table 8)

Table 8. Comparison of the mean in the levels of the Blood Parameters at days 90 post-treatment from the baseline level

Blood Parameters	IRON AMINO ACID CHELATE			IRON SALT		
	Baseline Mean (SD)	Day 90 Mean (SD)	P value	Baseline Mean (SD)	Day 90 Mean (SD)	P value
Hemoglobin	10.12 (0.68)	12.38 (0.68)	0.0001	9.97 (0.61)	12.26 (0.18)	0.0001
Hematocrit	31.13 (2.43)	37.90 (1.03)	0.0001	30.62 (2.42)	35.91 (2.43)	0.0001
MCV	89.96 (3.03)	90.45 (3.92)	0.0012	86.32 (2.97)	91.19 (4.87)	0.0001
MCHC	34 (2.00)	38 (1.50)	0.0001	33 (2.10)	37 (2.00)	0.0001
RDW	14.96 (0.58)	11.36 (1.10)	0.0001	14.82 (0.52)	12.36 (1.50)	0.0001
Serum Ferritin	21.45 (13.03)	31.21 (11.95)	0.0096	24.27 (13.60)	30.77 (16.91)	0.15

Statistical test done: t-test Statistically significant p-value <0.05

Adverse effects reported by the participants in the study were gastrointestinal in nature such as nausea, vomiting, constipation, black stool and epigastric pain. The most frequently reported adverse effect was black stool (33/48, 68.75%), followed by nausea (29/48, 60.42%) with the least complaint of epigastric pain (5/48, 10.42%). Between the two treatment arms, there were significantly higher proportion of participants in the iron salts with nausea (p value 0.04) and vomiting (p value 0.02). There were no significant differences in the occurrence of other adverse effects such as constipation, black stool and epigastric pain between the two treatment arms. (Table 9)

Table 9. Comparison of Adverse Effects Observed between Treatment arms

Adverse Effects	Total Number of Participants N=48 *n (%)*	Iron Amino Acid Chelate Group *n (%)*	Iron Salt Group *n (%)*	P value
Nausea	29 (60.42)	11 (45.83)	18 (75.00)	0.04
Vomiting	26 (54.16)	9 (37.50)	17 (70.83)	0.02
Constipation	18 (37.50)	7 (29.17)	11 (45.83)	0.23
Black stool	33 (68.75)	16 (66.67)	17 (70.83)	0.75
Epigastric pain	5 (10.42)	2 (8.33)	3 (12.5)	0.63

Statistical test done: chi-square statistically significant p-value <0.05

Majority of the participants experienced combination of adverse effects (30/48, 62.5%) compared to single adverse effect (18/48, 37.5%). The most common adverse effect combination that was reported by the participants was nausea, vomiting and constipation (8/30, 26.67%). Most of the participants with combined side effects were from Ferrous Sulfate group (19/24, 79.17%) while most of the participants with single side effect came from the Iron Amino Acid Chelate group (18/24, 37.5%). This difference between the two groups was statistically significant (p value 0.016). (Table 10)

Table 10. Comparison of the Numbers of Adverse Effects Observed between Treatment arms

Number of Adverse Effects	Total Number of Participants N=48 *n (%)*	Iron Amino Acid Chelate Group *n (%)*	Iron Salt Group *n (%)*	P value
Single	18 (37.50)	13 (54.17)	5 (20.83)	0.016
Combination	30 (62.50)	11 (45.83)	19 (79.17)	

Statistical test done: chi-square statistically significant p-value <0.05

DISCUSSION

Iron deficiency anemia has been considered as a financial burden. The WHO/World Bank-supported analysis of the Global Burden of Disease has ranked iron deficiency anemia as the third leading cause of loss of disability-adjusted life years (DALYs) for females aged 15 to 44 across the globe. Hence, the prevention of IDA can

translate to high cost efficiency.[34]

Iron deficiency is more common in developing countries and countries on the tropical zones commonly due to inadequacy of nutrition.[10] In our institution, based on our department's statistics, anemia belongs to the top 15 leading causes of morbidity in the past 5 years (2013 to 2017) which accounts for the 2.1 to 3.8% of cases during pregnancy. According to the National Nutrition survey of 2003 by the Food and Nutrition Research Institute (FNRI) of the Philippines, women in the reproductive age group, 15 to 45 years old are the ones mostly affected with Iron deficiency anemia. Approximately 43.94% of those women with IDA are pregnant[12]. In this study, the age range of the pregnant with IDA was 18 to 36 years old.

Pregnancy with anemia has a significant impact on the health of fetus as well as that of the mother. For this reason, all pregnant women should be screened for IDA as recommended by both international and local clinical practice guidelines. Diagnosis of IDA requires laboratory-confirmed evidence of anemia and low iron stores.[1] Laboratory studies such as CBC and serum ferritin are helpful in the diagnosis of IDA. The CBC parameters provide guidance on interpreting the red cell portion of hematologic report while serum ferritin confirms the diagnosis of iron deficiency.

Measurement of hemoglobin and hematocrit are the most efficient and commonly used method to screen for anemia. Hemoglobin and hematocrit concentration indicate the erythroid mass. Between the two, hemoglobin – an iron-containing protein in red blood cells, is a more sensitive and direct indicator of anemia. It reflects the composite effects of the mechanisms that control the size of the red cell mass – that was being maintained by the stimulation of red cell production by the humoral factor erythropoietin. Hematocrit is simply the ratio of red cells to the volume of whole blood; however it does not supply any information about anemia that cannot be obtained from hemoglobin concentration. Hence it has no advantage compared to hemoglobin measurement.[39]

The measurement of hemoglobin concentration and hematocrit cannot be used to determine the cause of anemia. To further identify the etiology such as iron-deficiency anemia, use of the CBC parameters such as MCV, MCHC and RDW should be evaluated. The RBC parameters indicate the morphology (size and degree of hemoglobinization) of the RBC. The MCV defines the size of RBCs because it measures the average red blood cell volume. This index has been most widely used for the evaluation of nutritional iron deficiency. MCHC indicates the amount of hemoglobin relative to the size of the cell or hemoglobin concentration per red blood cell. It is more useful since it provides information regarding the appearance of a cell by measuring the concentration of hemoglobin in each erythrocyte. Lastly, RDW quantifies the variation in red cell size and is a good indicator of the degree of anisocytosis.[40] Findings on CBC that is highly suggestive of IDA are low levels of hemoglobin, hematocrit, MCV, MCHC and high level of RDW from their respective cut-off values.[39] Serum ferritin is still considered as the gold standard to diagnose IDA since it is an indicator of total body iron stores. In cases of IDA, serum ferritin is decreased.

Once IDA is diagnosed, treatment involves iron supplementation. Based on the Philippine Obstetrical and Gynecological Society clinical practice guidelines on anemia, it is recommended to give 100-200 mg/day in pregnant women with IDA. To monitor optimal response to treatment, repeat CBC and serum ferritin determination is warranted.[33] Kuizon et al.[18] stated the positive role of iron supplementations to improve hematological status. An increased in the hemoglobin and hematocrit reflect short-term alterations concerning the quality of erythropoiesis. However, during pregnancy, it is difficult to assess whether an increase in hemoglobin concentration and reticulocytes is due to increased activity of erythropoiesis after supplementation, or if this is due to a less rapid increase in plasma volume in late pregnancy. Other several investigators reported a 6 g/dL increase in hemoglobin during late pregnancy even without iron supplementation.[4,5,7] But after 1 month of compliance to iron therapy the hemoglobin is expected to increase by 1 g per dL.[39]

In study of Kuizon et al, anemic pregnant ladies were treated with 195 mg crude iron per day and found a significant increase in hemoglobin by 1 + 0.7 g/dL and hematocrit level by 3% after 4 weeks of treatment. In our study, after iron supplementation, an increase in hemoglobin of approximately 2 to 3 g/dL was observed both in iron amino acid chelate and ferrous sulfate group. With regards to RBC indices, low MCV and MCHC with subsequent increased RDW values as shown in the baseline CBC of the participants was indicative of enhanced erythropoiesis or an increased in the degree of heterogeneity in the size of RBCs (anisocytosis) which was reflective of anemia. In this study, hematocrit and MCHC were significantly higher while RDW was significantly lower after 90 days of treatment favoring iron amino acid chelate group.

The results of our study signify that daily iron supplementation in pregnant with iron deficiency anemia increases different hematological parameters after 30, 60 and 90 days post-treatment while serum ferritin only significantly increased on 90 days post-treatment. This proved that oral iron therapy is recommended to give with a minimum of 3 months and maximum of 6 months to replenish iron stores.

In trying to reach the therapeutic doses of iron supplementation promote acute side effects. Most adverse effects related to oral Iron therapy were gastrointestinal (nausea, vomiting, constipation, black stool, epigastric pain). Not to mention that the amount of iron absorbed decreases with increasing doses. The Institute of Medicine through the National Institute of Health published the Tolerable Upper Intake Level for Iron. The UL is the maximum daily intake unlikely to result in adverse health effects and for iron, its only 45 mg/day.[36] There is also a phenomenon known as Mucosal Block whereby the intestine becomes refractory to iron absorption following the ingestion of a large dose of iron. The mechanism is unclear but in this phenomenon, the mucosal receptors are somehow intimidated by the large dose of iron ingested, they close, and the iron is only wasted. According to CDC, the amount of iron absorbed decreases with increasing doses. For this reason, if higher amount of elemental iron is needed, the iron must be taken daily in several equally spaced small doses.[41]

To overcome these challenges in iron supplementation, an innovative product was made whereby the iron is complexed or chelated with amino acids. This iron amino acid chelate has a stability constant higher than the potential formation constant of iron with other food particles. Thus, the iron passes undisturbed through the acidic medium of the stomach overcoming the challenge of absorption inhibitors. Once the chelate is at the absorption window of the intestinal mucosa, the amino acid ligands fall off allowing now the iron to enter the intestinal cell. Therefore, the advantages of this iron chelate can be stated in the following manner: Because the iron is protected by the amino acids, other food products do not form insoluble complex with the iron. This ensures greater bioavailability of iron resulting to greater mucosal absorption which can relate to more iron easily available when the need arises.

In the study of Pineda et. Al in Cairo proved the greater bioavailability or iron amino acid chelate wherein 10 anemic students were identified. Each student was given 1 cookie daily for 30 days. The cookie contains a mixture of iron absorption inhibitors and 30 mg of iron amino acid chelate. The result revealed an increase from 8 to 10.1 g/dL and the result was significant. This proved that even though the iron amino acid chelate was combined with a multitude of dietary iron absorption inhibitors, the actual absorption of iron is still not affected.[41]

Another study by Ashemead proved the greater mucosal absorption of iron amino acid chelate. In this in vitro study, rat jejunal segments were washed, randomized and exposed for 120 seconds to identical gastric solutions containing either iron oxide, iron sulfate, iron carbonate and iron amino acid chelate. After exposure, assay of the jejunal segments revealed 4.7 – 7.2 x greater iron absorption in the iron amino acid chelate than the other organic salts.[42]

In 2004, Coplin et al. compared therapeutic efficacy and tolerability of ferrous sulfate and iron bis-glycinate chelate in among pregnant women with IDA. They found that both ferrous sulfate and iron bis-glycinate chelate had equivalent therapeutic efficiency at a similar dose (50 mg) but the side effects were greater (37% vs. 21%) in the group that received ferrous sulfate.[20] The frequency and intensity of these complaints are related to the amount of elemental iron released in the stomach.[1,39] Likewise in this study, participants in both arms complained of combinations of gastrointestinal side effects but the proportion of the participants with single versus combined adverse effects were significantly lesser for the Iron Amino Acid Chelate group. This is true due to the fact that because of the amino acid bonding, there is less direct exposure of iron to the gastrointestinal mucosa cells; this can reduce local toxicity and side effects attributable to iron compounds present in the intestinal lumen.[18,19,33,37,38] Overall, both oral iron preparation were generally well tolerated by all of the participants hence none withdrawn from the study.

Our study demonstrated that Iron Amino Acid Chelate is comparable to Ferrous Sulfate in the treatment of IDA of pregnant women despite a 2.2-fold lower levels of elemental iron. Iron Amino Acid Chelate preparation only contains 30 mg elemental iron compared to a Ferrous Sulfate preparation with 65 mg of elemental iron content. This can be explained by the improved bioavailability of iron amino acid chelate. Both treatment arms improved the levels of the RBC parameters evaluated up to 90 days post-treatment. On the other hand, after 90 days from initiation of treatment, the mean hematocrit and MCHC were found to be higher and RDW was found to be lower among pregnant women taking Iron amino acid chelate.

The study was only limited to pregnant women who were diagnosed to have Iron Deficiency Anemia. This study described the prevalence of iron deficiency anemia among pregnant women consulting at the out-patient clinic in one private tertiary medical center. Hence this may not reflect the findings in women from other institutions. Moreover, compliance to ferrous sulfate and iron amino acid chelate intake was only based on monitoring through out-patient follow-up and collection of empty foil medicine shells. Therefore, compliance is not directly observed by the investigator. The study duration was 90 days hence, occurrence of relapse or sustaining the normal iron levels beyond the study period was not determined. ■

CONCLUSIONS

Iron deficiency can be adequately treated with oral iron supplements after 3 months of compliance to the prescribed dose. In terms of improvement in CBC parameters and serum ferritin levels, both iron amino acid chelate and ferrous sulfate are efficacious. But in this study, iron amino acid chelate was found to be superior than ferrous sulfate in achieving optimum treatment response with lesser adverse effects. This may be brought by its unique formulation that improved bioavailability and absorption of iron with less irritating properties to the gastrointestinal tract. Hence, with lesser adverse effects this can further make oral iron treatment tolerable. Thereby, compliance to long-term (3 to 6 months) therapy can be expected resulting to successful treatment outcome.

RECOMMENDATION

Further studies on the effectiveness of Iron amino acid chelate on patients with other forms of anemia and those patients with co-morbid conditions predisposing to anemia and those who are at higher risk to develop iron deficiency anemia should be included.

REFERENCES

1. Philippine Obstetrical & Gynecological Society (Foundation), Inc. *Clinical Practice Guidelines on Iron Deficiency Anemia*. November 2009, p.2.
2. Mehler CC. History of Medicine. NY, McGraw-HiII, 1947, p.177.
3. Bryan CP. The Papyrun Ebers. NY, D. Appleton and Co., 1931, p.156.
4. Monard M. Joyful news out of the new found world. 1500 (Trans.) NY, A. Knopf, 1925, p.135.
5. Herchberg S. La Carence en Fer en Nutrition Humaine. París, Editions Medicals Intemationales, 1988, p.3.
6. Combs, GF. Iron in Poultry Nutrition. En: Iron in Animal and Poultry Nutrition. HR Conrad, DR Zimerman, & GF Combs. West Des Moines, IA, NFIA, 1980.
7. Guthrie HA. Introductory Nutrition. St. Louis, MO, Times Mirror, 1989, p.290-293.
8. Ponka P, HM Schulman & RC Woodworth. (Eds.) Iron Transport and Storage. Boca Raton, FL, CRC Press, 1990, Preface.
9. Beutler E. Iron. En: Modem Nutrition in Health and Disease. RS Goodhart & ME Shils. (Eds.) Philadelphia, PA, Lea & Febiger, 1980, p.324.
10. Charlton RW and TH Bothwell. Definition, prevalence and prevention of iron deficiency. Clin Haematol 1982;11:309.
11. Demaeyer EM. Epidemiologic, traitement et prevention de la carence ferriprive. Rev Epiden et Sante Publ. 1980;28:235.
12. Herchberg S & C Rousud. La carence en fer. Cab Nutr Dict. 1981;16:219.
13. World Health Organization. Control of nutritional anemia with special reference to iron deficiency. Technical Report No. 580. Geneva, WHO, 1975.
14. Finch CA & H Huebers. Perspectives in iron metabolismo. *N Engl J Med.* 1982; 306:1520-1528.
15. Beutler E. Iron. En: Modem Nutrition in Health and Disease. RS Goodhart & ME Shils. (Eds.) Philadelphia, PA, Lea & Febiger, 1980, p. 339.
16. Layrisse M & C Martinez- Torres. Absorption of iron from foods. *Prog Hematol*. 1971; 7:37.
17. Gillooly M, TH Bothwell, JD Torrance, AP MacPhail, DP Derman, WR Bezwoda, W Mills, RW Charlton & F Mayet. The effects of organic acids, phytates, and polyphenols on the absorption of iron from vegetables. *Br J Nutr*. 1983; 49:331-342.
18. Monsen ER, L Hallberg, M Layrisse, DM Hegsted, JD Cook, M Mertz & CA Finch. Estimation of available dietary iron. *Am J Clin Nutr.* 1978; 31:134-141.
19. Cook JD & ER Monsen. Food iron absorption in human subjects. III Comparison of the effect of animal proteins on nonheme iron absorption. *Am J Clin Nutr.* 1976; 29:859-867.
20. Hallberg L & L Rossander. Improvements of iron nutrition in developing countries: comparison of adding meat, soy protein, ascorbic acid, citric acid, and ferrous sulphate on iron absorption from a simple Latin American-type of meal. *Am J Clin Nutr.* 1984; 39:577.
21. Fairbanks FV. Iron in medicine and nutrition. En: Modern Nutrition in Health and Disease. ME Shils, JA Olson & M Shike. (Eds.) Philadelphia, PA, Lea and Febiger, VI, 1994, p.186.
22. Beisel WR. Magnitude of the host nutritional responses to infection. *Am J Clin Nutr.* 1977; 30:1236-1247.
23. Morton I. How mineral deficient are we? Paper presented at Booker Conference, London, England, 1976.
24. Ashmead HD, EJ Rapp & JJ Name. Uso de amino acidos quelatos na fortificaco de alimentos. Proc O Seminario Brasilero de Alimentos Enriquecidos, 1994, p.32-37.
25. Allen LH. Properties of iron amino acid chelate as iron fortificants for maize. In: Proceedings International Conference on Human Nutrition. Salt Lake City, UT, Albion Laboratories, Inc., 1998, p.96-108.

26. Marchetti M, HD Ashmead, N Tossani, S Marchetti, & SD Ashmead. Comparison of the rates of vitamin degradation when mixed with sulfates or amino acid chelates. J Food Comp Analy. In Press.

27. Brise H & L Hallberg. Iron absorption studies II. *Acta Med Scandinav.* 1962; 171(Supp 376):1-73.

28. Bezwoda WR, et al. The relative dietary importance of heme and nonheme iron. *S Afr Med J.* 1983; 64:552.

29. Werner A. Beitrag Zur Konstitution Anaorganischer Verbindungen. Z, Anorg. U Aligem. *Chem.* 1893; 3:267.

30. Morgan G & H Drew. Research on residual affinity and coordination. II Acetylacetones of selenium and telluriumn. *J Chem Soc.* 1920; 117:1456.

31. Williarns RJP. An introduction to the nature of iron transport and storage. En: Iron Transport and Storage. Ponka, P, HM Schulman & RC Woodworth. (Eds.) Boca Raton, FL, CRC Press, 1990, p.I-15.

32. Kratzer FH & P Vohra. Chelates in Nutrition. Boca Raton, FL, CRC Press, 1986.

33. Achebe MM, Gafter-Gvili A. How I treat anemia in pregnancy: iron, cobalamin, and folate. Pubmed (Internet). 2017 Feb;129 (8):940-949. Available from: https://www.ncbi.nlm.nih.gov/pubmed?term=28034892.

34. Cunningham, F., et.al. (2014). Williams Obstetrics, 24th Edition, Chapter 4, p.55.

35. Comparison of oral iron supplements. *Pharmacist's Letter/ Prescriber's Letter.* 2008; 24(8):240811.

36. Baker RD, Greer FR; Committee on Nutrition, American Academy of Pediatrics. Diagnosis and prevention of iron deficiency and iron-deficiency anemia in infants and young children (0–3 years of age). *Pediatrics.* 2010; 126(5):1040-1050.

37. 37. Goddard AF, James MW, McIntyre AS, Scott BB; British Society of Gastroenterology. Guidelines for the management of iron deficiency anaemia. *Gut.* 2011; 60(10):1309-1316.

38. Peter R. Dallman, Iron Deficiency: Diagnosis and Treatment, San Francisco.

39. Bresani, Cristiane C. et.al, Erythrocyte indices in the second trimester of pregnancy: are reference values well established? *Revista Brasileira De Hematologia E Hemoterapia*. 2009; 31(1):37-40.

40. Lynch, Sean, Indicators of the iron status of populations: red blood cell parameters. Available from: http://www.who.int>9789241596107_annex1.