

# Substitution of hemoglobin levels in pregnant women with iron supplement: A prospective randomized clinical study

A. Daniilidis<sup>1</sup>, G. Dryllis<sup>2,\*</sup>, G. Chorooglou<sup>2</sup>, M. Politou<sup>2</sup>, R. Dampali<sup>1</sup>, K. Dinas<sup>1</sup>

<sup>1</sup>2nd University Clinic in Obstetrics and Gynecology, Hippokratio General Hospital, Aristotle University of Thessaloniki (Greece)

<sup>2</sup>Hematology Laboratory-Blood Bank, ARETAEIO Hospital, National and Kapodistrian University of Athens, Athens (Greece)

## Summary

**Introduction:** Iron deficiency anemia (IDA) is the most common form of anemia. Women who are pregnant or lactating and young children are the most affected. Iron protein acetyl aspartate is a ferroprotein complex that is more efficient versus iron protein succinylate in relatively short periods of time, for example 30 days. **Objective:** The aim of this clinical study was to investigate the efficacy of acetyl aspartate iron protein supplement in the treatment of anemia in pregnant women after the first trimester. This is the first real-time clinical study testing the usage of an iron protein supplement in a specific population, as there are not sufficient data for such studies in the literature. **Study Design:** 28 pregnant women with hemoglobin < 10.5 gr/dL and after completing the 12<sup>th</sup> week of pregnancy, initiated acetyl aspartate protein iron at a daily dose yielding 80g of elemental iron in two uptakes. Similarly, 35 women were studied who received different iron supplements at the same daily dose. After 30 days, hemoglobin values were measured. Inclusion criteria included: age > 18, gestational week > 12, Hb < 10.5 g/dL, Ht < 32%. Exclusion criteria included: age < 18, pregnancy < 12 weeks, many pregnancies, history: allergy to iron preparations, ulcer, cirrhosis, hemodialysis, hemochromatosis, aplastic anemia, chronic disease anemia, pancreatitis, renal disease. **Results:** Treatment with acetyl aspartylated iron lead to higher levels of hemoglobin after four weeks of treatment. Following successive measurements of hemoglobin levels in the 28 women of the study group, Hb levels increased from an initial mean Hb value of 10.04 gr/dL to 10.69 gr/dL. In the control group of 35 pregnant women, an increase in the mean hemoglobin from Hb was observed from 9.99 gr/dL to 10.46 gr/dL. The difference was statistically significant with  $p < 0.0001$ . **Conclusions:** The use of acetyl aspartate iron protein is a very promising option, as there has been an increase in hemoglobin levels to 0.5 gr/dL.

**Key words:** Iron deficiency anemia (IDA); Iron protein acetyl aspartate; Iron protein succinylate; Pregnancy.

## Introduction

Iron deficiency anemia (IDA) is the most common form of anemia, afflicting over two billion people worldwide. It affects mainly infants, children, adolescents, and women of childbearing potential, whether pregnant or not [1-3].

Women who are pregnant or lactating and young children are the most affected, especially in the developing world, but IDA frequently occurs in developed countries as well [1-3]. World Health Organization (WHO) data showed that IDA in pregnant women has a prevalence of 17.4% in industrialized countries, whereas the underdeveloped countries have a 4x higher prevalence [2, 3].

Iron deficiency anemia leads to poor pregnancy outcomes and increases the risk for preterm labor, low-birth-weight babies, and infant mortality [4, 5]. Both iron deficiency (ID) and IDA in pregnant women are associated with increased risks of developing preeclampsia, low birth weight, prematurity, perinatal mortality, delayed fetal maturation, and irreversible compromise to infants' neurocognitive development and motor capacity [6, 7]. The incidence of ID and IDA in infants born to mothers who are anemic is higher [6, 7]. Animal model studies consistently show that inadequate iron intake during pregnancy would lead to permanent changes in the offspring's brains (struc-

ture and function) [7].

The prevention of ID and IDA in pregnancy in many countries is commonly done with routine iron supplementation (IS), once the iron obtained from diet fails to reach the recommended daily intake levels [8, 9].

The Centers for Disease Control and Prevention (CDC) recommends that all pregnant women should begin with a 30 mg/d iron supplement at the first prenatal visit, whereas the WHO suggests a dosage of 60 mg/d for all pregnant women [10-13].

The equivalent of 60 mg of elemental iron is 300 mg ferrous sulfate heptahydrate, 180 mg ferrous fumarate or 500 mg of ferrous gluconate. In the first and third trimesters, the hemoglobin (Hb) threshold for diagnosing anemia is 110 g/L; in the second trimester, the threshold is 105 g/L. If a woman is diagnosed with anemia during pregnancy, her daily elemental iron should be increased to 120 mg until her Hb concentration rises to normal. Later, she can resume the standard daily antenatal iron uptake in order to prevent recurrence of anemia (WHO, iron and folate supplementation) [2, 10, 14].

Iron protein acetyl aspartate, a ferroprotein complex containing 5% trivalent iron bound to sucrose casein, is a product of long-term research and of multiple clinical studies. It is more efficient versus iron protein succinylate in

Table 1. — Mean value, standard deviation, and median value of women participating in the treatment and control groups

	Treatment group (N = 28) (Mean $\pm$ SD, median)	Control group (N = 35) (Mean $\pm$ SD, median)	<i>p</i>
Age (years)	29.89 $\pm$ 5.18, 31	31.09 $\pm$ 4.48, 31	0.4109
Number of pregnancies	1.63 $\pm$ 0.9, 1	1.74 $\pm$ 0.83, 2	0.5353
Gestational age at therapy initiation (weeks)	21.26 $\pm$ 1.91, 21	21.47 $\pm$ 1.69, 21	0.7261
Gestational age at therapy end (weeks)	25.26 $\pm$ 1.91, 25	25.47 $\pm$ 1.69, 25	0.7261
Therapy duration (weeks)	4 $\pm$ 0, 4	4 $\pm$ 0, 4	1
Hb before treatment (gr/dL)	10.04 $\pm$ 0.28, 10	9.99 $\pm$ 0.28, 10	0.5212

Table 2. — Mean value, standard deviation, and median value of women participating in the treatment and control groups, along with statistical significance tests results (bold values indicate significant results)

gr/dL	Treatment group (N = 28) (Mean $\pm$ SD, median)	Control group (N = 35) (Mean $\pm$ SD, median)	<i>p</i>
Hb before treatment	10.04 $\pm$ 0.28, 10	9.99 $\pm$ 0.28, 10	0.5212
Hb after treatment	10.69 $\pm$ 0.26, 10.7	10.46 $\pm$ 0.29, 10.5	0.0074
Hb increment	0.65 $\pm$ 0.17, 0.7	0.47 $\pm$ 0.06, 0.5	< 0.0001
Hb increment percentage	6.47% $\pm$ 1.84%, 6.73%	4.71% $\pm$ 0.64%, 4.93%	< 0.0001

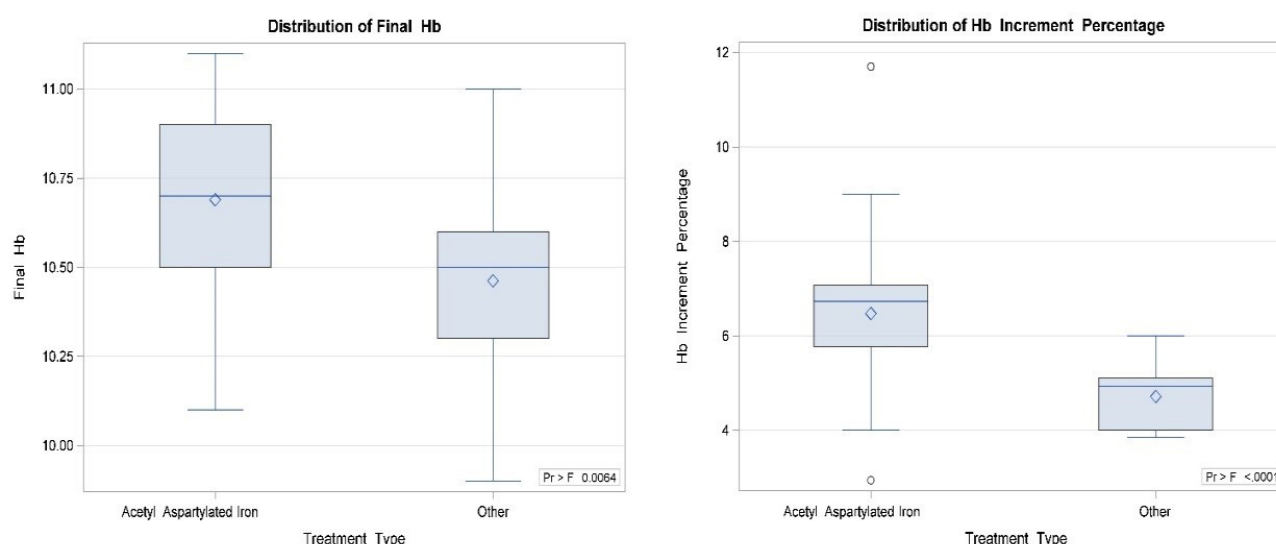


Figure 1. — Box and whisker plots for the treatment and control groups.

relatively short periods of time, for example 30 days. [15] Also, it achieves a higher increase in hemoglobin and iron levels into the serum, compared to iron protein succinylate [15].

The aim of this clinical study was to investigate the efficacy of acetyl aspartate iron protein supplement in the treatment of anemia in pregnant women after the first trimester. This is the first real-time clinical study testing the usage of an iron protein supplement in a specific population, as there are not sufficient data for such studies in the literature. Eligible women attending antenatal clinics as outpatients of the Second Obstetrics Clinic of Hippokrateio Hospital in Thessaloniki, were enrolled according to the protocol approved by the Research Ethics Committee.

## Materials and Methods

Sixty-three pregnant women aged 21 to 39 years (mean  $30.56 \pm 4.78$  years) consecutively presenting in outpatient clinic were enrolled in the study, with hemoglobin <10.5 gr/dL at the completion of 12 weeks of pregnancy. They began a 3-6 month treatment with different iron supplements in a daily dose yielding 80 mg of elemental iron in two daily shots. According to the minimum sample size calculation method, the annual population of pregnant women is estimated to be about 88,533, while the pregnant women presenting with anemia are approximately 18,000 (18% of pregnant women); so the authors used a confidence interval of 12 and a confidence level of 95% in this study. Inclu-

sion criteria were: age  $\geq 18$  years, gestational week  $\geq 12$ , Hb  $\leq 10.5$  gr/dL, and Ht  $\leq 32\%$ . Exclusion criteria were: age  $< 18$  years, absence of registration consent, step of pregnancy less than 12 weeks, coadministration formulations iron oral or parenterally, background of liver cirrhosis, background of hemosiderosis, background acquired or chronic hemochromatosis, aplastic, hemolytic anemia and chronic diseases, chronic pancreatitis, subjective renal and/or liver disease, hypothyroidism or hyperthyroidism. The study took place from January 2015 to December 2017 and was approved by the medical ethics committee of Hippokratio General Hospital, University of Thessaloniki; the study code was NCT02957643.

Twenty-eight pregnant women, were treated with acetyl aspartate protein iron supplement (Omalin trademark) at a twice daily dose yielding 80 mg of elemental iron (study group), while 35 women received different iron supplements (as ferrus sulfate, ferrous fumarate, and ferrus gluconate) at the same daily dose (control group). Upon giving consent to participate in this study, patients were allocated into the two groups randomly and were very strictly enrolled according to all inclusion and exclusion criteria.

According to the study protocol, the treatment duration was four weeks, and Hb values were remeasured with a hematologic analyzer. The treatment effects were tested by comparing between the two groups: a) the Hb levels after treatment b) the increase in Hb level, and finally c) the percentage of Hb increment in relation to the initial measurement.

Consent forms were used, and the study was approved by the hospital's scientific council. Randomization was based on a single or even number, as derived from the sum of the first five digits of patient's social security number.

Statistical analysis was performed by the Kruskal-Wallis test using the SAS for 9.4 software platform. The significance level ( $p$ -value) was set at 0.05. For completeness, the descriptive statistics are reported as mean  $\pm$  SD (standard deviation and median value).

## Results

The descriptive statistics between the treatment and control group are presented in Table 1. There was no statistically significant difference in terms of age, parity, week of gestation, and Hb levels between the two groups ( $p > 0.05$  in all cases).

Treatment with acetyl aspartylated iron led to higher levels of Hb after four weeks of treatment. Following successive measurements of Hb levels in the 28 women of the study group, the levels increased from an initial mean value of 10.04 gr/dL to 10.69 gr/dL. In the control group of 35 pregnant women, an increase in the mean Hb was observed from 9.99 gr/dL to 10.46 gr/dL. The difference was statistically significant with  $p < 0.0001$ . The increment in the treatment group was  $0.65 \pm 0.17$ , while for the control group it was  $0.47 \pm 0.06$  ( $p < 0.0001$ ). While comparing the increment as a percentage in relation to initial Hb lev-

els, it was found that for the treatment group, the increment percentage was  $6.47\% \pm 1.84\%$  while in the control group it was  $4.71\% \pm 0.64\%$  ( $p < 0.0001$ ). Results are presented in Table 2 and Figure 1.

Considering that the increment in Hb level was on a four-week basis, it was calculated that on average, the treatment group had increased by  $0.65/4 = 0.1625$  units per week, while the control group had an increment of  $0.47/4 = 0.1175$  units/week. At this rate, the control group would require 5.5 weeks (1.5 additional weeks) to reach similar Hb levels. This additional time, especially if iron supplements should be provided for longer time periods, may be eventually, important for fetal development and women's health. In both groups, no specific side effects were recorded and treatment was well tolerated.

## Discussion

Iron deficiency accounts for 75% of cases of anemia in pregnancy. The most common clinical manifestations of iron deficiency include epigastric pain, fatigue, tachycardia, constipation, diarrhea, dyspepsia, suboptimal work performance, postpartum depression, impaired lactation, low birth weight, premature delivery, intrauterine growth retardation, and increased fetal and neonatal mortality [16-19]. During pregnancy, the total maternal need for extra iron averages close to 1,190 mg, with a net iron balance during pregnancy of 580 mg, equating to a requirement of 2 mg daily. The fetus acquires about 280 mg of iron and a further 400–500 mg is required for the temporary expansion of maternal red cell mass. Another 200 mg of iron is lost with the placenta and with bleeding at delivery. The placental and fetal requirement is obligatory, though even with an iron-rich diet, this is difficult for the mother to maintain [20-22].

Current recommendations suggest that pregnant women should receive 15-30 mg daily of supplemental elemental iron. Nevertheless, it is not certain that the use of iron supplementation during pregnancy has a clear benefit in pregnancy outcomes. Much of the reportedly poor compliance with oral iron therapy is due to the associated side effects. Moreover, the increase in the number of tablets that are required daily is also prone to invite noncompliance. Therefore, a single daily dose of iron supplementation may be preferred. Ferrous gluconate is better tolerated due to fewer gastrointestinal effects than ferrous sulfate. For women who do not tolerate oral iron, parenteral iron may be used [21-23].

The main characteristic of the new iron protein compared complex with iron protein acetyl-aspartate is that it is well absorbed by the gastric mucosa and does not irritate the gastric mucosa. This makes it a better choice for oral iron supplementation compared to ferrous sulfate, considering that it has an improved compliance in treatment and it well-tolerated by patients, avoiding most of the adverse effects of oral iron supplementations. The efficacy of iron protein acetyl-aspartate compared to iron gluconate

is similar. Both treatments have shown improvements in clinical and hematological parameters including significant increases in Hb and serum iron levels [24].

Dose-response studies demonstrated that 80 mg of iron protein acetyl-aspartilate is able to elicit an extremely rapid increase in serum iron levels. Iron protein acetyl-aspartilate is more effective than iron protein succinylate during short time periods. Also, iron protein acetyl-aspartilate achieves a larger increase in Hb and serum iron levels, compared to iron protein succinylate [24].

The present study showed that there was a statistically significant difference between the 28 women receiving acetyl aspartate protein iron and the 35 pregnant women receiving different iron preparations, in the one-month increase in Hb values compared to the initial mean Hb value ( $p < 0.0001$ ). These findings are in line with other clinical studies, where acetyl aspartate protein iron was good or satisfactory in 97.9% of cases compared with 84.6% of reference drug cases, in pregnant population, and other populations with iron deficiency like pediatric, geriatric, and post-hemorrhagic patients [15, 24-31].

In conclusion, the proposed twice-daily dosage of 40 mg iron protein acetyl-aspartilate seems to significantly improve the hematologic parameters of anemia in a short period of time. This dosing regimen has a favorable risk-benefit relationship and is both statistically and clinically effective. These findings are in accordance with other clinical studies [24]. Nevertheless, larger clinical studies are required to prove that acetyl aspartate protein iron is more tolerable and efficient compared with IPS.

## Conclusions

This is the first real-time clinical study testing the use of an iron protein supplement in a specific population, demonstrating that the use of acetyl aspartate iron protein is a very suitable option, as there has been a significant increase in Hb levels.

## Trial Registration

Clinical Trials.gov, ID: NCT02957643. Registered on 10 December 2016.

## Acknowledgments

The authors appreciate the participation of all the study participants from Greece.

## Conflict of Interest

The authors declare no competing interests.

Submitted: July 04, 2019

Accepted: September 23, 2019

Published: August 15, 2020

## References

- [1] Silla L.M., Zelmanowicz A., Mito I., Michalowski M., Hellwing T., Shilling M.A., et al.: "High prevalence of anemia in children and adult women in an urban population in southern Brazil". *PLoS One*, 2013, 8, e68805.
- [2] Khalafallah A.A., Dennis A.E.: "Iron deficiency anaemia in pregnancy and postpartum: pathophysiology and effect of oral versus intravenous iron therapy". *J. Pregnancy*, 2012, 2012, 630519.
- [3] Roodenburg A.J.: "Iron supplementation during pregnancy". *Eur. J. Obstet. Gynecol. Reprod. Biol.*, 1995, 61, 65.
- [4] Guo S., Frazer D.M., Anderson G.J.: "Iron homeostasis: transport, metabolism, and regulation". *Curr. Opin. Clin. Nutr. Metab. Care*, 19, 276.
- [5] liveira F., Rocha S., Fernandes R.: "Iron metabolism: from health to disease". *J. Clin. Lab. Anal.*, 2014, 28, 210.
- [6] Zhou S.J., Gibson R.A., Makrides M.: "Routine iron supplementation in pregnancy has no effect on iron status of children at six months and four years of age". *J. Pediatr.*, 2007, 151, 438.
- [7] Zhou S.J., Gibson R.A., Crowther C.A., Baghurst P., Makrides M.: "Effect of iron supplementation during pregnancy on the intelligence quotient and behavior of children at 4 y of age: long-term follow-up of a randomized controlled trial". *Am. J. Clin. Nutr.*, 2006, 83, 1112.
- [8] Peña-Rosas J.P., De-Regil L.M., Garcia-Casal M.N., Dowswell T.: "Daily oral iron supplementation during pregnancy". *Cochrane Database Syst. Rev.*, 2015, 7, CD004736.
- [9] Friedrich J.R., Friedrich B.K.: "Prophylactic iron supplementation in pregnancy: a controversial issue". *Biochem. Insights*, 2017, 10, 1178626417737738.
- [10] Achebe M.M., Gafer-Gvili A.: "How I treat anemia in pregnancy: iron, cobalamin and folate". *Blood*, 2017, 129, 940.
- [11] World Health Organization: "Iron Deficiency Anemia. Assessment, Prevention and Control: A Guide for Programme Managers", Geneva: World Health Organization, 2011.
- [12] Stevens G.A., Finucane M.M., De-Regil L.M., Paciorek C.J., Flaxman S.R., Branca F., et al.: "Global regional, national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995- 2011: a systematic analysis of population-representative data". *Lancet Glob. Health*, 2013, 1, e16.
- [13] Pavord S., Myers B., Robinson S., Allard S., Strong J., Oppenheimer C., British Committee for Standards in Haematology: "UK guidelines on the management of iron deficiency in pregnancy". *Br. J. Haematol.*, 2012, 156, 588.
- [14] Yip R., Parvanta I., Cogswell M.E.: "Recommendations to prevent and control iron deficiency in the United States". *MMWR Recomm. Rep.*, 1998, 47, 1.
- [15] Geisser P., Burckhardt S.: "The pharmacokinetics and pharmacodynamics of iron preparations". *Pharmaceutics*, 2011, 3, 12.
- [16] Lee K.A., Zaffke M.E., Baratte-Beebe K.: "Restless legs syndrome and sleep disturbance during pregnancy: the role of folate and iron". *J. Womens Heal. Gend. Based Med.*, 2001, 10, 335.
- [17] LMurray-Kolb L.E.: "Iron and brain functions". *Curr. Opin. Clin. Nutr. Metab. Care*, 2013, 16, 703.
- [18] Beard J.: "Iron deficiency alters brain development and functioning". *J. Nutr.*, 2003, 133, 1468S.
- [19] Milman N.: "Postpartum anemia II: prevention and treatment". *Ann. Hematol.*, 2012, 91, 143.
- [20] Brannon P.M., Taylor C.L.: "Iron Supplementation during pregnancy and infancy: uncertainties and implications for research and policy". *Nutrients*, 2017, 9, E1327.
- [21] Goonewardene M., Shehata M., Hamad A.: "Anemia in pregnancy". *Best Pract. Res. Clin. Obstet. Gynaecol.*, 2012, 26, 3.
- [22] U.S. Department of Health and Human Services: "Healthy People 2020". Available at: [https://www.healthypeople.gov/sites/default/files/HP2020\\_brochure\\_with\\_LHI\\_508\\_FNL.pdf](https://www.healthypeople.gov/sites/default/files/HP2020_brochure_with_LHI_508_FNL.pdf)
- [23] Hoffbrand V.A., Moss P.A.H.: "Hoffbrand's Essential Haematology". Wiley-Blackwell, 2015.
- [24] Lazzari F., Carrara M.: "Overview of clinical trials in the treatment of iron deficiency with iron-acetyl-aspartylated casein". *Clin. Drug. Investig.*, 2005, 25, 279.
- [25] Petro De S.: "A controlled, double-blind study of Fe-ASP vs. Pernexin in the treatment of iron-deficiency anemia in pediatric patients". *Magis Farm SpA*. 1989.
- [26] Vito T.: "Clinical comparison of the efficacy and tolerability of Fe-ASP vs. Ferlxit in iron-deficiency anemia". *Magis Farm SpA*, 1991.

[1] Silla L.M., Zelmanowicz A., Mito I., Michalowski M., Hellwing T., Shilling M.A., et al.: "High prevalence of anemia in children and

- [27] Fortunato Z. "Evaluation of the efficacy and tolerability of oral Fe-ASP vs. IV Ferlixit in post-hemorrhage iron-deficiency anemia". *Magis Farm SpA*, 1991.
- [28] Giorgio P. "Controlled clinical study on oral Fe-ASP vs. Pernexin". *Magis Farm SpA*, 1990.
- [29] Ippolito D. "Clinical studies on the pharmacological activity and tolerability of Fe-ASP vs. Pernexin". *Magis Farm SpA*, 1988.
- [30] Maurizio D.M. "Treatment of iron-deficiency anemia in geriatric patients with oral Fe-ASP vs. Pernexin". *Magis Farm SpA*, 1988.
- [31] Chaud M.V., Izumi C., Nahaal Z., Shuhama T., Bianchi Mde L., de Freitas O.: "Iron derivatives from casein hydrolysates as a potential source in the treatment of iron deficiency". *J. Agric. Food Chem.*,

2002, 50, 871.

Corresponding Author:  
GEORGIOS DRYLLIS M.D., MSc., Ph.D.  
Hematology Laboratory-Blood Bank  
ARETAEIO Hospital  
National and Kapodistrian University of Athens  
76 Vasilissis Sofias Ave.  
115 28 Athens (Greece)  
e-mail: gdrillis@yahoo.gr