Original Article

The Effectiveness of Single Versus Double Daily Dose of Oral Ferrous Bisglycinate on the Prevention of Iron Deficiency Anemia in Obese Non-Anemic Pregnant Women: A Randomized Clinical Trial

Mohammed K. Ali[1], Maher S. Mohammed[1], Ahmed A. Abd-Elrahman[1], Ali H. Ali[2], Ahmed M. Abbas[1] and Abdelrahman M. Abdelkader[1]

[1]Department of Obstetrics and Gynecology, [2]Department of Clinical Pathology, Faculty of Medicine, Assiut University, Egypt

ABSTRACT

Objective: To compare the effectiveness of 27 mg versus 54 mg oral ferrous bisglycinate on the prevention of iron deficiency anemia (IDA) in obese non-anemic pregnant women

Material and Methods: The study was a randomized clinical trial conducted from October 2019 to April 2022 at Assiut Woman's Health Hospital, Egypt included obese non-anemic pregnant women in the first trimester. The eligible women were randomized to 27 mg (group I) or 54 mg (group II) of oral ferrous bisglycinate taken daily until delivery. The primary outcome was the number of anemic women at the time of delivery. The level of serum hepcidin at 36 weeks was another outcome. Data was analyzed using an unpaired t-test, the Mann–Whitney U test, and the Chi-square test.

Results: Two hundred thirty women were divided equally into two groups. No statistically significant difference was observed between both groups in the number of anemic women at the time of delivery [15 women vs. 12 women; p= 0.445, respectively]. A significantly higher level of hepcidin was noted at 36 weeks in group II (30.1 ng/mL vs. 43.4 ng/mL; p=0.000, respectively). Other maternal and neonatal outcomes were comparable in both groups without statistically significant differences.

Conclusion: Double daily dose of oral ferrous bisglycinate is not superior to single-dose in the prevention of IDA in obese non-anemic pregnant women when taken from the first trimester till delivery. Consideration should be given to increased hepcidin level with high prophylactic oral iron supplementation stressing the need for longer-term follow-up.

Key Words: Hepcidin; iron; iron deficiency anemia; obesity.

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Corresponding Author: Abdelrahman M Abdelkader, Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Egypt, **Tel.:** +2010 9462 8728, **E-mail:** abdelrahmanmma@gmail.com

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INTRODUCTION

Anemia is known as a condition in which hemoglobin level is lower than normal[1]. Anemia is one of the most common complications during pregnancy[2]. It may lead to both maternal and fetal morbidity and mortality[3].

The pregnant woman needs more iron than a non-pregnant woman, so IDA is common during pregnancy[4]. In Egypt; IDA affects about one in every two pregnant women, especially in rural areas[5]. Pregnant women require about 27 mg/day of elemental iron to cover their increased need[6]. They should start taking a daily oral iron as a preventive measure against IDA from the first trimester to delivery, especially in poor countries[7].

Obesity is an excessive amount of body fat. The body mass index (BMI) determines the obese if the figure is more than 30 kg/m^2[8]. According to the World Health Organization, 46% of adult females in Egypt are obese[9].

There is a great relation between obesity and IDA because obesity is associates with imbalanced nourishment, an increase in iron requirements due to higher blood volume, and a decrease in myoglobin level that binds iron in the muscles due to a limitation in the activity in those women[10].

Hepcidin is an iron regulating hormone in the body[11]. Increases in iron levels in the plasma increase the production of hepcidin, which subsequently blocks iron absorption from the diet[12]. In contrast; hepcidin production is suppressed in the case of IDA and in normal pregnancy to meet the increased need for the iron[13]. The hepcidin level increases also in obese women leading to a decrease in iron absorption which adds more risk for IDA[14].

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Accordingly to the above evidence; there is a need to study the effect of high prophylactic oral iron on obese non-anemic pregnant women supposing that obese women may need more iron to meet their maternal and fetal needs. So; this study aimed to compare the effectiveness of daily 27 mg versus 54mg of oral ferrous bisglycinate on the prevention of IDA in obese non-anemic pregnant women and highlighting the changes in the hepcidin level. To our knowledge, no clinical trial has been registered or conducted to report on this topic.

MATERIAL AND METHODS

The study was a single-center, open randomized, parallel, and registered clinical trial (Clinical trial.gov-NCT04101461). It was conducted from October 2019 to April 2022 at Assiut Woman's Health Hospital, Egypt. The obese pregnant women who attended for antenatal care in our hospital in the first trimester were invited to participate. The study protocol was approved by The Assiut Medical School Ethical Review Board (IRB no: 17101138).

Eligible participants

Pregnant women in a singleton pregnancy between 12-14 weeks with BMI between 30- 40 kg/m^2 were included. Those women had normal hemoglobin levels, ferritin levels, serum iron, and total iron-binding capacity (TIBC) at the time of recruitment[15].

However; women who received a blood transfusion or blood donation within the previous 3 months, women with threatened miscarriage or known to have pathological blood loss, intolerant to oral iron form, history of the hematologic disorder, using oral iron, minerals, vitamins, or herbal supplements, had chronic medical/inflammatory diseases, lactating or vegetarians women were excluded.

Intervention

The screening phase of the study was done to assess the eligibility of the participants. The eligible women, after obtaining informed consent, were subjected to history taking including demographic and obstetric data. General and physical examinations were also done. A trans-abdominal ultrasound assessment of the fetal viability and the gestational age was performed. Baseline laboratory investigations included complete blood count (CBC), serum ferritin, serum iron, TIBC, and serum hepcidin were obtained.

After that; a blocked randomization was done using https://www.sealedenvelope.com and a table of random numbers and codes was generated. Women were randomized to either group I (Single oral iron group); those women received 27 mg oral ferrous bisglycinate (PharaFerro27; Devart Lab Company, Egypt) once daily until delivery and group II (Double oral iron group) were received 27 mg oral ferrous bisglycinate twice daily from the same drug for the same duration.

Follow-up schedule

The participants were asked to come to follow up every month till delivery. All the investigations and treatment were offered to the participating women for free without any charges. At each visit; BMI was recorded. Also; women were asked to report any side effects from the treatment. Adherence to the treatment was monitored by asking the women to get back the empty bottles and cautiously revise the intake of capsules .The women were advised to eat iron-rich foods and choose foods containing vitamin C to enhance iron absorption. The women were also advised to start 15 minutes of walking, three times per week and they increased it gradually to 30 minutes every day till delivery.

CBC was assessed at monthly intervals. The iron studies (serum ferritin, serum iron, and TIBC) were evaluated at 28 weeks and 36 weeks. The serum hepcidin level was estimated at baseline and at 36 weeks. A routine ultrasound anomaly scan was done at (18-21 weeks). Screening for diabetes was offered at early pregnancy and at 24-28 weeks of gestation by a two-step glucose screening test. At delivery; the maternal and neonatal outcomes were recorded.

The study outcomes

The primary outcome was the number of anemic women at the time of delivery. While the secondary outcomes included the mean difference in the serum hemoglobin, ferritin, iron, TIBC, and hepcidin during follow-up visits. The side effects of the oral iron, mode of delivery, and neonatal outcomes were also reported.

At the end of the study

At the end of the study; the participants were classified as “completed study,” or “lost to follow up.” Women who devolved severe IDA (≤ 7 g/dl) were subjected to therapeutic iron supplementation and or blood transfusion and were excluded from the final analysis. Women who opted to stop oral iron and women who developed hypertension or diabetes during the follow-up visits were also excluded.

Sample size

A previous non-randomized study by Phillips *et al*., 2014 reported that the incidence of IDA in obese pregnant women at the time of delivery is 34%[16]. Using a two-sided chi-square ($\chi 2$) test with α of 0.05, a total sample size of at least 230 patients (115 in each group) will have 80%

power to detect a 50% difference assuming a rate of loss to follow-up of 10% (Epi-info™, CDC, USA).

Statistical analysis

The data was collected and analyzed using the Statistical Package for Social Science (SPSS Inc., Chicago, version 21). Comparisons between the means in the groups were done using an unpaired t-test and the Mann–Whitney U test based on the normality of the data. Categorical data were shown by number or percentage. For dichotomous variables, the Chi-square test was used to estimate the significance value. The *p-value* <0.05 was considered statistically significant.

RESULTS

Two hundred sixty-five women were counseled for participation, however; 35 patients were excluded during the screening phase of the study. Two hundred thirty women consented to participate and were divided equally into two groups (115 women in each group). However, 32 women in the group I and 28 women in the group II were excluded during follow-up visits. So; 83 women in group I and 87 patients in group II were finally analyzed (Figure 1).

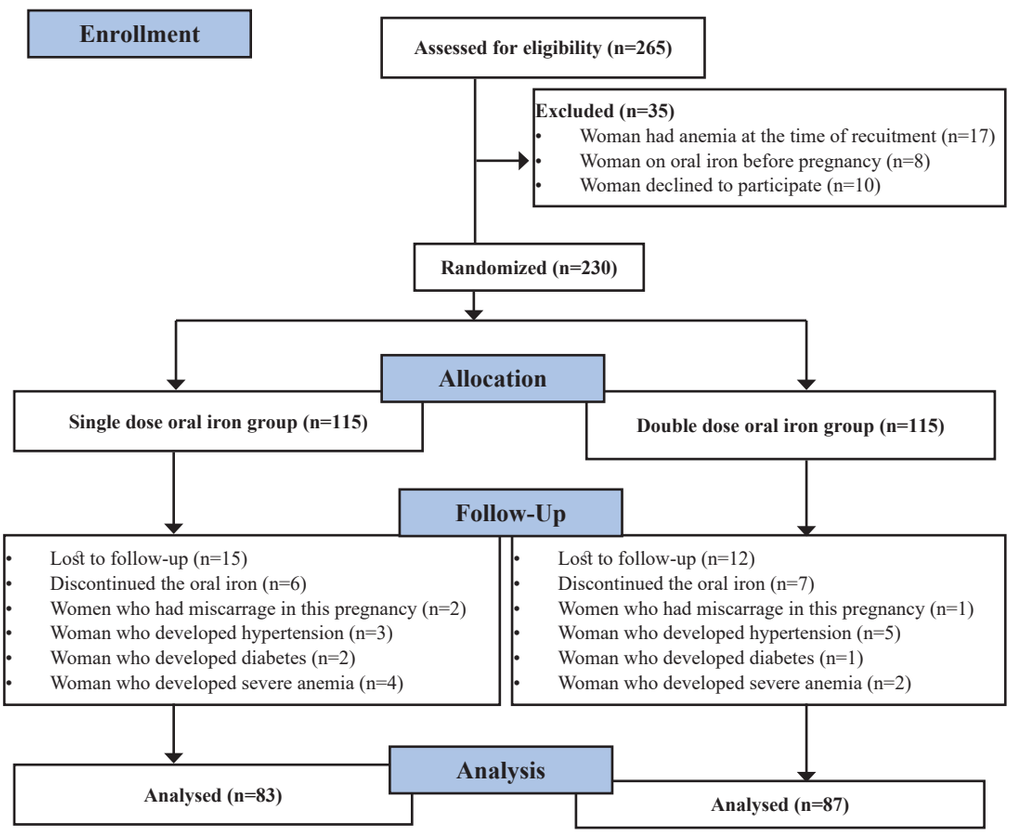

Fig. 1: Recruitment Flowchart

No statistically significant differences were noted between groups in the demographic and obstetrics data (Table 1). Table 2 shows the mean of different levels of hemoglobin in both groups during follow-up visits till delivery. No statistically significant differences were observed between groups regarding the hemoglobin levels throughout the pregnancy till delivery (Table 2).

No statistically significant differences were noted between groups in the respect to iron studies except for a significant increase in the serum iron level at 28 weeks in group II in comparison to group I with a statistically significant difference (61.58 µg/dl ± 22.70 vs. 70.14 µg/dl ± 22.08; *p*=0.009) (Table 3).

There was a significant increase in the serum hepcidin level at 36 weeks in group II (43.4 ng/mL) in comparison to group I (30.1 ng/mL) with statistically significant difference (*p*=0.000) (Table 4).

There was no statistically significant difference was observed between both groups in the number of anemic women at the time of delivery [15 (18.1%) women vs. 12 (13.8%) women; *p*=0.445). Again, no statistically significant difference was noted between both groups regards the mode of delivery (*p*=0.451). Seventy-five women in the group I and 82 women in group II had a term delivery without a statistically significant difference (*p*= 0.340). Moreover; no statistically significant difference

was observed between both groups in the number of babies with an Apgar score of more than 7, the number of babies who were admitted to NICU, the mean gestational age at the time of delivery, and the mean birth weight (p=0.565,0.466,0.883,0.078, respectively) (Table 5).

The reported side effects of oral iron in this study were constipation, nausea/ vomiting, abdominal or stomach pain, metallic taste, black stools, diarrhea, and heartburn. However, no statistically significant differences were noted between both groups regards the side effects (p>0.05).

Table 1: Demographic and obstetric data

	Group I (n= 115)	Group II (n= 115)	*P-value*
Age (years), mean ± SD	30.24 ± 7.61	30.34 ± 6.00	0.908
Residency, n (%)			
Urban	58 (50.4)	53 (46.1%)	0.509
Rural	57 (49.6)	62 (53.9%)	
Education, n (%)			
Illiterate	42 (36.5)	45 (39.1)	
Basic	47 (40.9)	43 (37.4)	0.861
Secondary or more	26 (22.6)	27 (23.5)	
Employment, n (%)	33 (28.7)	37 (32.2)	0.567
History of previous abortion, n (%)	12(10.4)	15(13.0)	0.539
Parity, n (%)			
Primigravida	12(10.4)	10(8.7)	0.947
Para 1	42(36.5)	40(34.8)	
2-3	39(33.9)	41(35.7)	
More than 3	22(19.1)	24(20.)	
Number of living children, n (%)			
no children	15(13.0)	13(11.3)	
1	36(31.3)	28(24.3)	0.588
2-3	42(36.5)	50(43.5)	
More than 3	22(19.1)	24(20.9)	
Duration from the last pregnancy (years), Median (Range)	2.8 (0.5-7.5)	3.1 (1.0-7.0)	0.499
Mode of last delivery, n (%)			
No previous delivery	12(10.4)	10(8.7)	0.904
VD	47(40.9)	48(41.7)	
CS	56(48.7)	57(49.6)	
Gestational age at time of recruitment (weeks), mean ± SD	12.83 ± 0.80	12.76 ± 0.78	0.508
BMI (Kg/m^2)	34.94±2.94	34.77±3.06	0.666

BMI body mass index, Cs cesarean section, Kg kilogram, m^2 square meters, n (%) number and percentage, SD standard deviation, VD vaginal delivery

Table 2: Serum hemoglobin levels during follow-up visits till delivery †

Hemoglobin levels (gm/dl)	Group I	Group II	*P-value*
	Mean ± SD	Mean ± SD	
At baseline	12.23 ± 0.81	12.19 ± 0.82	0.694
At 16 weeks	11.32 ± 0.72	11.29 ± 0.83	0.792
At 20 weeks	11.45 ± 0.69	11.60 ± 0.80	0.152
At 24 weeks	10.64 ± 0.76	10.72 ± 0.79	0.482
At 28 weeks	10.44 ± 0.75	10.25 ± 0.64	0.058
At 32 weeks	10.82 ± 0.84	10.73 ± 0.75	0.461
At 36 weeks	11.54 ± 0.85	11.57 ± 0.72	0.801
At time of delivery	11.57±0.83	11.63±0.65	0.554

gm/dl gram/deciliter, SD standard deviation
†The data are only for those women who completed the study.

Table 3: Serum iron studies levels at baseline, 28 weeks and 36 weeks in the participant women†

	Group I	Group II	*P-value*
Serum ferritin (ng/ml) median(Range)			
At baseline	28.0 (11.0-92.0)	30.0 (11.0-75.0)	0.453
At 28 weeks	21.0 (4.0-70.0)	23.0 (4.0-63.0)	0.151
At 36 weeks	29.0 (12.0-78.0)	31.0 (12.0-71.0)	0.150
Serum iron (µg/dl), mean ± SD			
At baseline	89.92 ± 11.90	87.50 ± 12.95	0.142
At 28 weeks	61.58 ± 22.70	70.14 ± 22.08	0.009*
At 36 weeks	62.39 ± 21.14	63.04 ± 19.26	0.825
TIBC(µg/dl), mean ± SD			
At baseline	350.08 ± 34.65	352.85 ± 30.51	0.521
At 28 weeks	330.19±77.65	345.50± 66.12	0.145
At 36 weeks	369.06 ± 55.11	373.69 ± 50.01	0.548

* Statistical significant difference (P < 0.05) ng/ml nanogram per milliliter, SD standard deviation, TIBC total iron binding capacity, µg/dl microgram per deciliter
†The data are only for those women who completed the study period.

Table 4: Serum hepcidin levels at baseline, and 36 weeks in both groups.

Hepcidin (µg/l)	Group I	Group II	*P-value*
	Median (Range)	Median (Range)	
At baseline	48.3 (14.5-82.3)	47.0 (18.5-78.4)	0.762
At 36 weeks	30.1 (6.3-60.4)	43.4 (10.7-73.3)	0.000*

* Statistical significant difference ($P < 0.05$)
µg/l microgram per liter

Table 5: The maternal and fetal outcomes in the present study

	Group I (n= 83)	Group II (n= 87)	*P-value*
Anemic women at delivery, n (%)	15(18.1)	12(13.8)	0.445
Degree of anemia, n (%)			
Hb 9.5-10(gm/dl)	2(13.3)	1(8.3)	0.681
Hb 10.1-10.9(gm/dl)	13(86.7)	11(91.7)	
Mode of delivery, n (%)			
VD	42 (50.6)	39 (44.8)	0.451
CS	41 (49.4)	48 (55.2)	
Number of term/ preterm babies, n (%)			
Term	75 (90.4)	82 (94.3)	0.340
Preterm	8 (9.6)	5 (5.7)	
Apgar score more than 7, n (%)	71 (85.5)	77 (88.5)	0.565
Admission to NICU, n (%)	6 (7.2)	4 (4.6)	0.466
Gestational age at time delivery, mean ± SD	38.57 ± 1.38	38.54 ± 1.36	0.883
Birth weight, mean ± SD	3340.36 ± 280.61	3258.04 ± 322.62	0.078

CS caesarian section, n (%) number and percentage, NICU neonatal intensive care unit, SD standard deviation, VD vaginal delivery

DISCUSSION

The present work demonstrated that both regimens were similarly effective in the prevention of IDA in non-anemic obese pregnant women when taken from 1st trimester till delivery. In addition, our results found a significant increase in serum iron at 28 weeks and an increase in the hepcidin level at 36 weeks in the double-dose iron group. However; other maternal and neonatal outcomes were comparable.

Anemia is common during pregnancy because there is an increase in the amount of plasma volume more than the RBCs[17]. IDA is the commonest type of anemia during pregnancy[18].

Egypt was ranked 18th with the highest prevalence of obesity worldwide[19]. Maternal obesity is associated with many adverse outcomes such as preeclampsia and IDA[20]. Obesity leads to an increase the interleukin (IL)-6 and IL-1 levels which elevate hepcidin levels[21]. There is also overexpression of hemojuvelin in adipose tissue of obese women which also increases hepcidin[22]. The elevation of hepcidin level leads to a decrease iron absorption and IDA[14].

In this study; we found that double oral iron dose in obese non-anemic pregnant women did not add more beneficial effect than single-dose when taken from 12-14 weeks till delivery.

Routine iron supplementation in well-nourished pregnant women is not recommended[23]. However; Pena-Rosas JP *et al*. in a Cochrane review concluded that routine daily antenatal iron supplementation may be beneficial where IDA is widespread[24]. So we agree with the Cochrane review results because Egyptian pregnant women are at a higher risk for the development of IDA and obesity is another risk factor.

Peña-Rosas *et al*. found that intermittent iron regimens produce similar maternal and infant outcomes as daily supplementation[25]. We are on the same track because we found that the high daily iron dose did not show a significant increase in hemoglobin level than single dose; it may be due to decreased iron bioavailability with a high daily oral iron dose[11].

The relationship between obesity and high ferritin level was previously reported[26]; however, serum ferritin cannot be considered a reliable indicator of iron stores in obese patients[27]. Lao TT et al. in a study found that an elevated ferritin concentration reflects maternal iron excess, and was associated with unfavorable pregnancy outcomes[28]. Despite a higher dose of oral iron in group II; the level of ferritin was comparable in both groups. This may be a result of diminished iron absorption due to a higher level of hepcidin due to obesity[29].

Hepcidin level decreases in pregnant women from the first to the second and third trimesters to undetectable levels compared with non-pregnant women[30]. In our study; we found that the level of hepcidin in the 1st trimester in both groups was 48.3 and 47 µg/l; respectively. The level was higher than reported by Finkenstedt A *et al* (16 µg/l) and Van Santen S *et al*. (17.5 µg/l)[31,32]. We think that obesity is the main cause of these higher figures. Guo Y *et al*. reported a higher level than we did (51.3 µg/l). But he included women with spontaneous abortion in the 1st trimester; the stoppage of transport of maternal iron through the placenta to the fetus may be the cause[33]. At 36 weeks; the hepcidin level dropped sharply in group I (30.1 µg/l) than in group II (43.4 µg/l). Moretti D *et al*. found that higher oral iron supplements increase hepcidin[11]. We think his finding can clearly explain the higher level of hepcidin in group II than in group I.

Finally; we observed in our study that both groups were comparable in the side effects throughout the pregnancy.

Using chelated oral iron which has few side effects[34] and increased hepcidin level in group II by blocking the extra iron absorption may explain this finding.

A major strength of this study was its design as a randomized study. The study was the first trial to explore the effect of the various prophylactic doses of oral iron on pregnant obese women which is very scarce in the literature.

The study of the effect of oral iron supplementation on hepcidin levels is another good point. Also, the long period of follow-up (± 6 months) in this study allowed the assessment of the side effects and iron studies parameters throughout the pregnancy.

However, the present work had some limitations. Blinding of the patients in our RCT was not done. We did not measure markers like interleukin (IL)-6 and IL-1 levels which may affect the hepcidin. We measured the hepcidin level only at two points during the pregnancy because of financial issues. The iron status of the neonates was not assessed. We did not measure the parameters of the lipid profile which may affect the anemia. Lastly; there are a considerable number of women who did not include in the final analysis.

CONCLUSION

Our findings suggest that a single oral dose of oral iron is effective as a double dose in preventing IDA in obese non-anemic pregnant women when taken from 12-14 weeks till delivery. More importantly, the results pull the care of the possible association between high dose oral iron and hepcidin which may have an important effect with long-term follow-up.

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CONFLICT OF INTERESTS

There are no conflicts of interest.

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