Project Title: Once Daily versus Twice Daily Iron Supplementation to Treat Anemia in Pregnancy: A Prospective, Randomized, Placebo Controlled, Double Blinded, Clinical Trial

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Summary

Anemia is a common problem in pregnancy, affecting as many as 1/4 of pregnancies in the developed world in the absence of iron supplementation. It is defined as a maternal hemoglobin (Hgb) less than 11mg/dL in the 1st or 3rd trimester and Hgb less than 10.5mg/dL in the 2nd trimester [1].. Anemia is associated with low birth weight, preterm birth, and increased risk for transfusion at the time of delivery [1]. The most common cause of anemia in pregnancy is iron deficiency. Iron deficiency anemia in pregnancy is typically treated with oral iron supplementation in the form of once or twice daily ferrous sulfate, which contains 65mg of elemental iron.

Iron balance is complex, and iron demands are increased in pregnancy. The fetus, placenta, and increased maternal red blood cell volume require about 1g of additional iron during the entire pregnancy. The average American diet contains only about 15mg of elemental iron, which is insufficient to meet this need[1]. It is recommended that pregnant women consume about double this amount during pregnancy[1].

The absorption of oral iron is low, ranging from 2-28%. Taking iron without food increases absorption. Hepcidin is a key regulator of iron absorption in mammals, increasing hepcidin decreases iron absorption. Hepcidin is increased with morning fasting dosing, which may affect the efficacy of twice daily dosing (Morretti). Well-designed clinical trials describing the optimal dosing for iron to treat anemia in pregnancy are limited [2, 3].

A recent publication by Morretti et al [4] evaluated the effect of different doses of oral iron in non-pregnant iron deficient young women. They evaluated the effect of varying doses of daily ferrous sulfate and once and twice daily dosing of ferrous sulfate on hepcidin levels and fractional and total iron absorption. Women were treated with a single daily dose ranging from 40-240mg of elemental iron. This 6-fold increase in elemental iron dosage only resulted in a 3 fold increase in the amount of elemental iron absorbed, contradicting the notion that more iron is better. In a second arm of the study, women were treated with twice daily dosing for a total of 180mg of elemental iron. **The results of the study demonstrated that 24 hours after a dose > 60mg, hepcidin increased and fractional iron absorption decreased by 35-45%. Multiple daily doses did not increase iron absorption beyond single daily dosing.** The authors concluded that providing daily dosages less than 80mg of elemental iron and avoiding

twice daily dosing maximized iron absorption. **The results of the study by Morretti et al challenge the conventional wisdom that more severe iron deficiency anemia in pregnancy is best treated by increasing from daily to twice daily supplementation. Morretti et al also did not evaluate for gastrointestinal side effects or increase in hemoglobin.**

Given the high incidence of iron deficiency anemia in pregnancy and the associated complications, we propose a prospective, randomized double blinded clinical trial to evaluate the optimal dosing of oral iron supplementation in pregnancy. We will recruit mothers identified as having iron deficiency anemia at the time of routine prenatal blood work in the second trimester. These subjects will be randomized to daily or twice daily ferrous sulfate 325mg (65mg of elemental iron). The primary outcomes will be hemoglobin concentration at the time of admission for delivery and post-partum and assessment of gastrointestinal side effects. The secondary outcome will be frequency of blood transfusion at the time of delivery.

Hypothesis: Once daily dosing of ferrous sulfate 325mg/dL will be associated with increased maternal hemoglobin concentration at the time of delivery and post-partum and decreased gastrointestinal side effects.

Specific Aims

1. Determine whether once daily dosing of oral iron is more effective in raising maternal Hgb concentration than twice daily dosing.
2. Determine whether once daily dosing of ferrous sulfate 325mg has a lower side effect profile than twice daily dosing.
3. Determine the relationship between frequency of maternal iron administration and maternal hepcidin
4. Determine the relationship between frequency of maternal iron administration and fetal iron stores

Secondary Aim

Determine whether once daily dosing of ferrous sulfate 325mg is associated with a lower rate of maternal blood transfusion at the time of delivery than twice daily dosing.

Inclusion Criteria

Singleton gestation in the second trimester (14-28 weeks) who is not on iron therapy and has a normal hemoglobin electrophoresis if from a high risk group for hemoglobinopathy.

Exclusion Criteria

Multiple gestation, maternal hemoglobinopathy or hemochromatosis, subjects with irritable bowel disease or irritable bowel syndrome, history of bariatric surgery or extensive bowel surgery, or individuals already receiving iron supplementation aside from prenatal vitamins.

Study Design

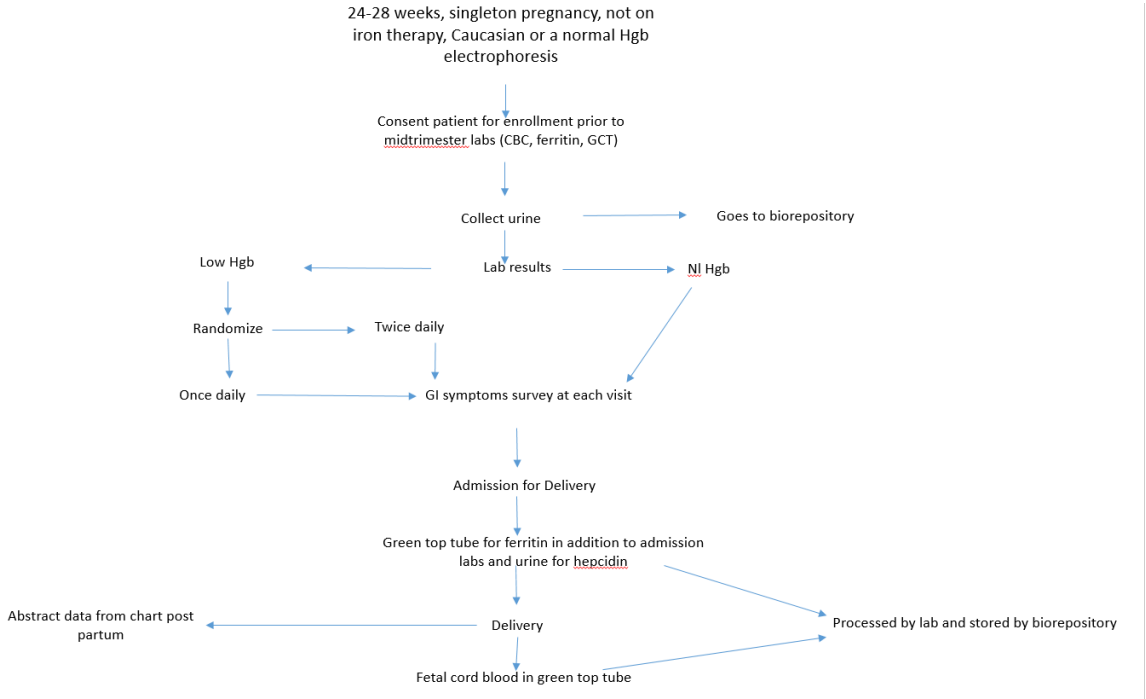
This will be a prospective, randomized, double blinded placebo controlled clinical trial. Subjects will be identified for possible enrollment during the second trimester during routine prenatal care. Criteria for enrollment will be a singleton intrauterine gestation as above. Subjects without anemia will have weekly questionnaires regarding GI symptoms during otherwise scheduled prenatal visits. Subjects with iron deficiency anemia as diagnosed by an Hgb concentration of less than 10.5-11mg/dL per ACOG guidelines and a ferritin of less than 15mg/dL will be randomized to once or twice daily iron supplementation by means of a computer generated random number list by the investigational drug pharmacy. The study drug will be provided by the University of Missouri Investigational Drug Pharmacy under the guidance of Sonja Grinfeld, the Investigational Drug Pharmacist for the University. Iron supplementation will be in the form of ferrous sulfate 325mg (65mg of elemental iron) once or twice daily. Anemic subjects will receive identical packs of drug with instructions to take one pill BID (twice daily). The morning pill will be ferrous sulfate 325mg. The evening pill will be randomized to contain either ferrous sulfate 325mg or placebo. The placebo will be produced by the investigational drug pharmacy and contain cellulose, a biologically inert plant fiber. The subjects and investigators will be blinded to assignment. As part of standard Obstetric care, the subject will have a hemoglobin repeated at the time of admission to labor and delivery and on post-partum day 1. Subjects who are not anemic will serve as controls and not receive a prescription. This is appropriate as the standard of care is not to treat non-anemic women with iron supplementation.

In addition to routine standard of care blood draws, subjects will have two extra samples of blood taken for research use at no cost to them. The first will be drawn at the time of enrollment, generally at the time of a clinically indicated blood draw for routine prenatal labs. The second will be drawn at the time of admission to the hospital for delivery or on post partum day 1, again generally at the time of an otherwise clinically indicated blood draw. At this time, a separate tube of about 10mL will be drawn for research purposes only. A sample of cord blood from the placenta will be obtained following delivery of the neonate as well. These samples will be stored for analysis of hepcidin and ferritin at the conclusion of the study. These values will be for research use only and will not be used in clinical care.

Subjects will be administered a validated questionnaire regarding gastrointestinal (GI) symptoms of iron usage at the time of follow up clinic visits [5]. This questionnaire will be administered via RedCap or via paper per patient preference. Compliance will be monitored by having the patient bring pill packs to her clinic visits.

Whether or not the patient required a blood transfusion in the peripartum period will be recorded. Due to the relative infrequency of this outcome, this will be evaluated as a planned secondary outcome with the understanding that the trial will not be powered specifically for this outcome.

All samples collected will be collected for, stored, and processed by the MU Biorepository. Following study related analysis, the MU Biorepository will de-identify what is left of the samples and store the excess for future non-specified research endeavors.



Risks and Benefits

1. Iron therapy is associated with mild gastrointestinal symptoms (upset stomach and diarrhea). Increased iron dosage may be associated with an increased risk of these symptoms. Significant gastrointestinal symptoms are rare with once or twice daily iron dosage. The additional iron in maternal diet (which is classically iron poor in the US) or in prenatal vitamins will not pose any additional maternal risk. There is no fetal risk to this study.
2. There are no individual benefits to enrollment in the study.

Subject withdrawal criteria and procedures specifying

a. When and how to withdraw subjects from the trial / investigational product treatment.
   If subjects are not compliant with the tasks required in the protocol.

b. The type and timing of the data to be collected for withdrawn subjects.
   All data collected from a withdrawn subject will immediately be destroyed.

c. Whether and how subjects are to be replaced.
   To replace withdrawn subjects, we will recruit another pregnant mother
d. The follow-up for subjects withdrawn from investigational product treatment/trial treatment.
   There will be no follow-up.

Data handling:

All data will be coded with an ID number. The key containing the ID numbers will be kept in a locked file separate from the data. Data generated from samples tested for this study will be stored by the MU Biorepository and by the research staff. . The data will be kept for 7 years after study completion. This data will not be shared with outside investigators or used for research other than this study. Additionally, data from this study will be included in the Prospective Obstetric Database (PODS) study for future use in de-identified fashion.

Data Analysis

Statistical Analysis: Statistical analyses will be performed using the IBM SPSS statistical software version 22 (IBM Corporation, Armonk, NY, USA). ANOVA will be used to assess the difference in mean hemoglobin at the time of admission and post-partum in the three arms for the study Regression analysis will be used to explore the relationships of iron supplementation on maternal hemoglobin at delivery and fetal hemoglobin, hepcidin, and ferritin while controlling for maternal ferritin and hepcidin at enrollment. A planned secondary analysis will be to use a Chi-square or Fisher's exact test as appropriate to examine the incidence of blood transfusion during the admission for delivery. Significance will be set at $P \leq 0.05$.

Power Analysis: The mean hemoglobin concentration in pregnancy is about 12mg/dL and the standard deviation is about 1mg/dL. At an alpha of 0.05 a sample size of 17 subjects in each arm would be required to have 80% power to detect a difference of 1mg/dL in maternal hemoglobin. In order to account for subjects lost to follow up due to delivery at other facilities, transfer of care and a 20% lost to follow up rate, we will continuously enroll subjects until 50 subjects are randomized to treatment in order to preserve adequate power to assess our primary outcomes and control for covariates in the setting of lost to follow up. The prevalence of anemia in pregnancy is reported to be between 8 and 27% in the second and third trimesters of pregnancy[1]. As such, we anticipate a total sample size of around 250 (50 subjects on treatment divided equally between once and twice daily iron and 200 controls receiving routine prenatal care. Our facility delivers around 2000 patients per year, most of whom receive care in our clinics. As such, we anticipate being able to recruit our target sample size, including accounting for patient drop out and replacement, in 12-18 months. Based on prior published data [5], 10 subjects per arm are required to identify a difference gastrointestinal symptoms.

Anticipated results and future directions

Based on the increased hepcidin and reduced fractional absorption seen with multiple daily dosing, I anticipate that once daily dosing will be superior to twice daily dosing in

the treatment of anemia diagnosed during the second trimester of pregnancy. In this case, I plan a follow up study to evaluate once versus twice daily dosing in anemia diagnosed in the first trimester (1-14 weeks gestation), which is less common but may represent a more severe depletion of iron stores. **If twice daily dosing is proven to be equivalent or superior to once daily dosing in pregnancy, I plan a study similar to that of Morretti to determine if the changes in hepcidin levels seen with multiple daily doses of iron in the non-pregnant patient are also seen in pregnancy. It is possible that pregnant women have a less robust increase in hepcidin in response to oral iron and can benefit from increasing dosage. It is also possible that they would have a more robust response and would be better served with less frequent (i.e.) every other day dosing**. I plan to use preliminary data from this study to apply for external funds to answer these questions.

The results of this study will be submitted for consideration at the annual meeting of the Society for Maternal-Fetal Medicine or the Society of Reproductive Investigation depending upon the timeframe in which the study is completed and subsequently submitted for publication.

References

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