ORIGINAL ARTICLE

Comparative Study of Effect of Calcium on Iron Absorption of Controlled Release Iron Preparation in Iron Deficiency Anemia in Pregnancy

Kishor A Bansod,[1] Mohammed Shakeel Mohammed Bashir,[2] NR Belorkar[3]

1. Associate Professor, Department of Pharmacology, Dr. Punjabrao Deshmukh Medical College, Amravati, MS
2. Associate Professor, Department of Pharmacology, Faculty of Dentistry, Taif University, Taif, KSA
3. Professor Emeritus, Indira Gandhi Government Medical College Nagpur, MS

Abstract

Background: *Iron deficiency anemia in pregnancy is a global health problem. To manage this iron supplementation along with other nutrients such as calcium are advised during pregnancy. But the possibility of calcium inhibiting iron absorption always remains. Moreover, iron has its own gastrointestinal adverse effects which discourage its use. In the present study, the effect of controlled-release and conventional iron preparation supplementation were compared for absorption and gastrointestinal adverse effects.* ***Materials & Methods:*** *A total of 72 pregnant patients in their second trimester of pregnancy participated in the study. They were divided into 2 groups. Group A received controlled release preparation of iron, calcium and folic acid while conventional preparation of iron, calcium and folic acid were given to group B. Hematological parameters were assessed at baseline (day 0), day 30, day 60 and day 90.* ***Results:*** *Initially inhibitory response of calcium on iron absorption was seen but it was lost gradually. Significantly lesser gastrointestinal adverse effects were observed in group A.* ***Conclusion:*** *There is a clear need for a combination iron-calcium supplement which delivers bioavailable iron with lesser degree of gastrointestinal adverse effects.*

Keywords*: Controlled release iron preparation, Iron deficiency anemia, Iron supplementation, Calcium supplementation*

Address for correspondence: Dr. Kishor Bansod. Associate Professor, Department of Pharmacology, Dr. Punjabrao Deshmukh Medical College, Amravati, MS. Email: kishorbansod74@gmail.com

Date of Acceptance: 14/03/2019

Introduction

Anemia is one of the leading causes of morbidity throughout the world and around one third world population suffer from it accounting more than 2 billion people. [1] Half of the anemia cases are due to iron deficiency. Pregnant women, childbearing age women and children under five years of age are more vulnerable for it. [2] According to WHO 1992 iron deficiency is one of the most common nutritional deficiency in the world among pregnant women. [3] It is estimated that prevalence is around 38% among pregnant women. [4] Pregnant women have increased risk of iron deficiency due to their higher iron demands. [5]

In women iron deficiency anemia has severe adverse consequence if occurs during pregnancy. It is associated with maternal low weight gain, preterm labour, placenta previa, premature rupture of membrane, cardiac arrest, and hemorrhage, lowered resistance to infection, poor cognitive development and reduced work capacity. It is also associated with premature baby, low birth weight and increased incidences of perinatal morbidity and mortality. [1]

Folic acid deficiency in early pregnancy leads to neural tube defects. On the ground of benefit and risk ratio it is advised that adequate folic acid supplementation including in diet should be given to all women during pregnancy. [6]

Calcium is one of the main nutrient supplements which is advised during pregnancy. In those populations which have traditionally low intake of calcium in that incidence of pregnancy induced hypertension is common. It is because of calcium deficiency is associated with pregnancy induced hypertension. Several studies indicate that supplementation of calcium in diagnosed cases of pre-eclampsia is responsible

for lower blood pressure. [7] Moreover, intakes of dietary or supplemental calcium during pregnancy also leads to lower incidence of osteoporosis.

There is inverse relationship between calcium intake and iron absorption. The inhibitory effect of calcium on iron absorption may increase the problem of iron deficiency anemia. It is more important during pregnancy as it can be one of the reasons for non-correction of iron deficiency anemia during pregnancy even after supplementation of iron. Most of the iron and calcium supplement advised during pregnancy, contains large amount of iron and calcium hence iron-calcium interaction may be especially significant in pregnant women and it is not unreasonable to suspect that iron bioavailability from such supplements is low. Early observations of the effects of calcium on iron absorption were based on animal studies. However, it is now known that small laboratory animals such as rats have a much lower sensitivity to the influence dietary facilitators or inhibitors of iron absorption than do humans. But human studies also indicate incidence of inhibition of inorganic iron absorption when some forms of supplemental calcium are given simultaneously. [8,9,10]

Those who are taking conventional oral iron therapy face the problem of gastrointestinal side effects such as nausea and epigastric discomfort. These symptoms are observed due to high concentration of administered iron in the gastrointestinal lumen. [11] The high concentration of iron preparations causes irritating effect on gastrointestinal mucosa leading to nausea and epigastric pain. Hence it can be expected that sustained release preparations will reduce such symptoms. But uses of such preparations are many times criticized because any reduction in gastrointestinal side effects is due to lower solubility and reduced absorption of such preparations. [12] Chapman DG and Campbell JA clearly demonstrated that calcium caused decreased iron absorption in rats fed mixed diets. [13]Subsequent reports have confirmed that in rats, increasing dietary calcium in a variety of forms results in decreased iron absorption. [14] An effect of calcium on iron absorption has been demonstrated in humans also. [15]

With this background, this study was planned to compare the effect of calcium supplementation on controlled-release and conventional iron preparation with reference to the serum iron, serum ferritin and the long term haematological responses with reference to the reticulocyte count, hemoglobin and red blood cell count. We also planned to compare the tolerability of the two preparations.

Materials and Methods

Present study was conducted at Indira Gandhi Government Medical College (IGGMC) Nagpur. Patients were recruited from antenatal clinic IGGMC Nagpur. In this clinical study a total of 72 patients recruited. Patients in their 2nd trimester of pregnancy with moderate degree of iron deficiency anemia were included. Patients were in between the age group of 19 to 35 years. Their gestational age was 12 to 16 weeks and hemoglobin concentration was between 7.0 to 9.9 gm/dl. Patient having a normal screening laboratory test of blood glucose, urine albumin and sugar were included.

Patients with the history of pre-eclampsia, sickle disease, seizure disorders, malabsorption and gastrointestinal disorders and any conditions known to impair iron absorption from the gastrointestinal tract were excluded from the study. Complicated pregnancy such as multiple pregnancy and diabetes mellitus were also part of exclusion criteria. Patients having other conditions including urinary tract infection, malaria, bleeding tendency, clinically significant renal, cardiac (including rheumatic or congenital heart disease), and hepatic disorders were also excluded from the study. Moreover, those patients who were on iron and calcium supplements at the time of enrolment were also not recruited for the study. For the present study permission was granted by the institutional ethics committee. Patients were well explained about the study and written informed consent was obtained from them at the time of enrolment.

Patients were divided into 2 groups. Patients of group A received fixed dose combination of calcium carbonate 875 mg (elemental calcium 350 mg), ferrous fumarate 100 mg (elemental iron 32 mg) and folic acid 0.5 mg. In the fixed dose combination, ferrous fumarate was in controlled release form while calcium carbonate and folic acid in immediate release form.

Fixed dose combination was formulated to provide iron and calcium in the same dosage form for maximum bio-availability of calcium and iron. This was made possible by coating the iron (as ferrous fumarate) with controlled-release coating material. Calcium carbonate is solubilised in the acidic environment of stomach and is rapidly absorbed through stomach and small intestine. The release of iron in the small intestine is delayed for at least one hour after the release of calcium. Such a delay in transit and release of iron in the intestine was made possible by making the use of the fact that only particles size 1 mm and less can pass directly from stomach. By keeping the size of iron coating greater than 1 mm, the gastric emptying of the same is delayed until the next peristaltic wave in the stomach arrives which occurs 2-3 hours after meal. Coating of iron pellet with controlled-release coating material restricts the dissolution of the iron in the stomach fluid.

Once the iron passes into the small intestine with the next peristaltic wave, the release of iron starts, thus releasing the iron which dissolves in intestinal fluid and get absorbed. Other ingredients in the formulation were folic acid which dissolves rapidly in stomach fluid and is readily available for absorption through the small intestine.

Patients of group B received conventional dosage of calcium carbonate 875 mg (elemental calcium 350 mg), ferrous fumarate 100 mg (elemental iron 32 mg) and folic acid 0.5 mg separately. Patients were instructed to take tablet simultaneously one after another with a glass of water orally three times a day. Drug dosage chosen represent the therapeutically as well as prophylactically used dose.

The total daily dose of supplemental iron, calcium and folic acid was same in both the groups. The total daily dose of iron in the form ferrous fumarate was 96 mg as elemental iron, total daily dose of calcium as calcium carbonate containing elemental calcium 350 mg was 1050 mg and total daily dose of folic acid was 1.5 mg.

Iron deficiency anemia was clinically detected and simultaneously hemoglobin concentration was determined by Acid Haematin method (Sahli's Hemoglobinometer) to correlate with the clinical findings. Baseline parameters such as hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell count (RBC), serum iron, total iron binding capacity (TIBC) and serum ferritin level were measured at day 0. The follow up of the study was at every 15 days for a period of 90 days in which blood sample were collected from each patient at 30, 60 and 90 days follow up visit to monitor the haematological response.

Patients were asked questions about tablet intake and any associated side effects, like any marked changes in their bowel habits (constipation, diarrhoea) or nausea, vomiting, heartburn, epigastric discomfort or other symptoms which they related to the therapy and were recorded.

12 patients discontinued the study due to abortion, sickle cell anemia, failed to co-operate and discontinued treatment. Thus 60 patients completed the study comprising 30 in each group. Mean values (at baseline, day 30, day 60 and day 90) were compared by using unpaired "t" test. Chi-square test was used for the statistical comparison of side-effects between two groups.

Results

At day 30, though serum iron concentration in group B slightly decreased there was a gradual increase later on in both the groups. In group A, TIBC increased at day 30, decreased at day 60 and returned to near baseline levels at day 90. In group B, mean value of TIBC increased over day 30, day 60 and day 90. In both the groups serum ferritin increased at day 30 and day 60. At day 90 serum ferritin concentrations returned to near baseline levels in both the groups (Table- 1).

In group A reticulocyte count was significantly more at day 30. In group B reticulocyte was significantly more at day 60. Mean hemoglobin was significantly more in group A at day 60. In group A, mean value of RBC was significantly more at day 60 (Table- 2).

In group B, mean value of MCV was significantly more at day 60. In group B, mean value of MCH was significantly more at day 60. In group A, mean value of MCHC was significantly more at day 60. There was continuous increase in mean value of PCV in

both the groups at day 30, day 60 and day 90 (Table- 3).

Table- 1: Mean values of serum iron-SI (µg/dl), TIBC (µg/dl) and Serum Ferritin-SF (ng/dl)

Group	Baseline (day 0)		Day 30		Day 60		Day 90	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A (SI)	76.906	55.840	78.984	34.049	79.021	26.441	81.261	22.125
B (SI)	74.982	31.858	63.970	35.523	70.020	27.598	70.876	31.640
p value	0.871		0.100		0.202		0.147	
A (TIBC)	440.501	88.426	448.050	75.344	416.625	73.252	441.589	62.358
B (TIBC)	433.768	93.034	440.262	60.357	447.707	80.831	452.810	56.020
p value	0.775		0.660		0.124		0.466	
A (SF)	6.073	1.978	14.307	8.414	11.193	6.698	6.530	2.055
B (SF)	5.933	1.792	13.103	3.524	10.710	3.810	6.900	2.220
p value	0.775		0.474		0.733		0.506	

TIBC- Total Iron Binding Capacity

Table- 2: Mean values of Reticulocyte count-RC (%), Hemoglobin-Hb (gm/dl) and RBC (million/cu.mm)

Group	Baseline (day 0)		Day 30		Day 60		Day 90	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A (RC)	0.633	0.292	1.790*	0.552	1.683	0.599	1.700	0.435
B (RC)	0.567	0.173	1.450	0.442	1.967*	0.453	1.670	0.589
p value	0.287		0.011		0.044		0.823	
A (Hb)	7.737	0.677	8.283	0.622	9.750*	0.932	10.200	0.879
B (Hb)	7.870	0.905	8.543	0.890	9.293	0.757	9.997	0.734
p value	0.521		0.195		0.042		0.335	
A (RBC)	3.721	0.476	3.313	0.389	4.231**	0.670	4.302	0.448
B (RBC)	3.806	0.403	3.480	0.394	3.616	0.336	4.125	0.413
p value	0.457		0.104		0.002		0.118	

RBC- Red Blood Cells

Table- 3: Mean values of MCV (fl), MCH (pg), MCHC (gm/dl) and PCV (%)

Group	Baseline (day 0)		Day 30		Day 60		Day 90	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
A (MCV)	72.347	9.020	86.111	11.370	73.866	10.596	75.460	6.748
B (MCV)	70.881	7.575	86.035	10.904	84.642**	8.348	77.040	8.102
p value	0.498		0.979		0.003		0.415	
A (MCH)	21.008	2.306	25.374	3.795	23.491	3.597	23.858	2.388
B (MCH)	20.837	2.831	24.811	3.475	25.874**	2.884	24.436	2.704
p value	0.799		0.552		0.006		0.384	
A (MCHC)	29.247	2.204	29.488	2.239	31.813*	2.204	31.700	2.739
B (MCHC)	29.352	1.900	28.824	1.447	30.592	1.900	31.755	1.859
p value	0.870		0.179		0.025		0.928	
A (PCV)	26.729	3.668	28.250	3.018	30.743	3.255	32.373	3.821
B (PCV)	26.830	2.813	29.617	2.450	30.410	2.053	31.489	1.523
p value	0.905		0.059		0.637		0.246	

MCV – Mean Corpuscular Volume, **MCH** – Mean Corpuscular Hemoglobin, **MCHC** – Mean Corpuscular Hemoglobin Concentration, **PCV**- Packed Cell Volume

Side effects observed during the study were constipation, diarrhoea, heartburn and nausea. The incidence of nausea was significantly less in group A in comparison to group B (table 4)

Table- 4: Side-effects reported by the patients

Side Effects	Group A	Group B
Constipation	14 (46.66)	16 (53.33)
Diarrhoea	1 (3.33)	1 (3.33)
Heartburn	9 (30.0)	8 (26.66)
Nausea	5 (16.66)*	14 (46.66)

*P ≤ 0.05 (Chi-Square Test)

Discussion

Iron deficiency anemia is one of the major causes of anemia in pregnancy which has severe adverse consequences for the mother, foetus and neonate. Routine iron supplementation is recommended during pregnancy because of it's potential interference with health for both mother and child. [16] Sufficient supply of iron during pregnancy is not only important but also essential to prevent iron deficiency anemia. It provides adequate increase of the hemoglobin mass during pregnancy which is a prerequisite for adequate hemoglobin mass in the mother. Hence, iron deficiency anemia prevalence among most of the countries is major world health problem. [17]

In clinical practice, it is common to supply iron and a number of other minerals such as calcium and folic acid to coup up the problem of iron deficiency anemia and other nutrient deficiency problems in pregnancy. It was found that iron from these supplements is absorbed in much amounts than when the same amount of iron was ingested alone. [10]

To access the effects of calcium supplementation on absorption of iron, the present study included parameters like serum iron determination, serum ferritin and other therapeutic efficacy parameters viz. hemoglobin concentration, packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell count (RBC).

Measurement of iron absorption from iron and calcium supplementation is difficult. Ekenved G et al; in their studies, demonstrated that the absorption of radioactive iron as measured with a whole –body counter is highly correlated with the increase in serum iron concentration 2 to 6 hours after ingestion. [18,19] One of the study, using histochemical bone marrow haemosiderin iron in pregnant women concluded that among a panel of iron status markers, serum ferritin displays the highest accuracy in the diagnosis of iron deficiency in pregnancy. [20] Various studies indicate that serum ferritin is the most frequently used indicator of iron status. [21] Seligman PA et al. suggested that studies involving haematological parameters in such types of study have greater importance since these studies are clearly feasible especially as serum ferritin measurements might be used in place of bone marrow examinations to evaluate iron reserves. [10] Iron deficiencies can be predicated even in early stages by measurement of hemoglobin and other red cell indices which are less expensive. [22]

In the present study, increase in serum ferritin level was observed at day 30 and day 60 follow up period in both the groups. But at day 90 serum ferritin decreased near initial levels in both the groups. Probable explanation for increase in serum ferritin levels is, it is due to the absence of some menstrual periods and the accompanying decrease in iron requirements during early gestation as well as supplemental iron status which rises serum ferritin level. Decrease serum ferritin level near initial level in both the groups at day 90 indicates that, as pregnancy advances, the need for iron increases and the iron from iron-supplementation and iron-stores in the body is utilized for hemoglobin synthesis which is responsible for decrease serum ferritin levels in both the groups. This phenomenon happens in all the pregnant women irrespective of the level of their iron stores and whether or not receives iron supplements. [23]

The present study indicated a shift of iron from the stores into the red cell mass. The fall in serum ferritin levels could in part be secondary to the increase in plasma volume during pregnancy. [24] Certainly the pattern of the fall in serum ferritin at 60 days and 90 days in both the groups support these explanations.

Serum ferritin concentrations are depressed during pregnancy. If serum ferritin is the factor controlling gut absorption of iron as suggested by Linder MC and Munro HN[25], then the low concentrations of serum ferritin could be the factor that enhances the absorption of iron from

the gut during pregnancy. [17] The fall in serum ferritin level is responsible for the physiological increase in transferring concentration that occurs during pregnancy (Taylor DJ et.al 1982) (79).[24]

In the present study, there was gradual increase in serum iron in group A. However, it was not statistically significant. There was no increase in serum iron in group B at day 30, day 60 and day 90. Availability of iron in pregnancy is indicated by the amount of storage of iron available and the amount of iron absorbed which is measured by the serum iron level. Hence decrease in serum iron level shows decrease iron store and when iron stores are filled, the serum iron will also increases. [17]

Though the serum iron levels in both groups showed no significant differences, the haematological parameters like reticulocyte count, hemoglobin and red blood cell indicated the inhibitory influence of calcium on iron absorption in group B, but this study did not address the mechanism by which calcium affects iron absorption. Studies in rats indicate that calcium inhibits mucosal uptake of iron and the subsequent delivery of iron into the circulation. [14] Roth DE et al. also indicated that calcium has inhibitory influence on iron absorption. [26]

In this study, increased levels of total iron binding capacity (TIBC) were seen only at day 30 in group A whereas it increased continuously throughout the study period in group B, suggesting that iron-stores in group B required more time to get filled up supporting the fact that calcium affects the absorption of iron. However, the changes in TIBC were not statistically significant. Total iron binding capacity (TIBC), indicates the transport protein for iron increases in iron deficiency and decreases when iron stores are filled. [27] Ahenkorah B et al. in their study found that TIBC decreased in pregnancy in iron deficiency state. [28]

In the present study, though hemoglobin concentration increased in both the groups, it was significantly more in group A at day 60. RBC count was also significantly more in group A at day 60. Reticulocyte count was significantly more in group A at day 30 but ay day 60 it was significantly more in group B. However, at day 90, there were no significant differences in hemoglobin, RBC, count and reticulocyte count in both the groups. This shows that, in group B, iron –stores required more time to get filled-up and start erythropoiesis, pointing towards the inhibition of absorption of iron by calcium.

Both the hemoglobin and red blood cell levels must be considered in evaluating the reticulocytes response to iron. For a given red blood cell level more the reticulocytes lower will be the hemoglobin and more the reticulocytes at a given hemoglobin level lower will be the red blood cell count. [29]

Minihane AM and Fairweather-Tait SJ[30] in their study over 6 months also found no significant changes in mean hemoglobin, hematocrit and plasma ferritin levels. One proposed mechanism to explain the difference between acute and chronic effects of calcium on iron metabolism is that of adaptive response in the intestinal mucosal cells. Single –meal studies show that iron absorption is reduced in presence of calcium and the lower supply of iron to the plasma may in turn modify the developing enterocytes in the crypts of intestinal villi, stimulating the production of specific proteins that favor a more efficient use of dietary iron once the developing cells reach maturity. [30]

We found increase in erythropoietic activity in both the groups as evidenced by increased reticulocytes and PCV in both the groups. A reduction in mean corpuscular volume (MCV) to less than 80 fl reflecting reduced hemoglobin synthesis has commonly been advocated as an index of iron deficiency. [31] In pregnancy, as a result of stimulated erythropoiesis there is a relative increase in circulating larger young erythrocytes and MCV is no longer thought to be an accurate index of iron deficiency. [31] This has been reflected in the present study, in which other parameters indicated early erythropoietic activity in group A but MCV was significantly more in group B at day 60.

This macrocytic response is not related to folate deficiency. In this study both groups had received folic acid. Taylor DJ and Lind T [31] observed a divergent trend in MCV between supplemented and unsupplemented groups which could have been secondary to differences in hormonal potentiation of erythropoietin, the fall in plasma colloid osmotic pressure or total osmolality. The results of present study did not show divergent trend in MCV between the two

groups supporting the view that though calcium inhibits iron absorption adaptive response occur on chronic administration which results in a more efficient use of supplemental iron. [32]

Taylor DJ and Lind T [31] found correlation between Mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV). They stated that mean cell hemoglobin concentration is a late sign of anemia. MCHC was maintained narrowly. There was direct relationship between Mean corpuscular hemoglobin (MCH) and mean corpuscular volume (MCV).

Barton JC et al. [14] found decreased iron absorption in a dose –related manner from the duodenum and jejunum with the use range of calcium chloride solutions of 1 to 100 mmlo/L. There were no significant changes in iron solubility, macromolecular structure or formation or ferrous-ferric shift were evident. They suggested that calcium decreases absorption of iron by reducing receptor uptake of iron or by affecting the metabolism of iron within the enterocytes and its subsequent delivery into the circulation. Present study also showed that the haematological response in terms of reticulocyte count, hemoglobin and red blood cell count was significantly slow in group B.

In the present study a range of known adverse effects was observed in both the groups, however the incidence of nausea in the study group (group A) was significantly less. It is well known that iron preparations may cause side-effects of such a severity that a patient does not follow the recommended dosage or discontinues the therapy. The use of the present controlled-release iron preparation obviously minimizes such side-effects which are attributed to irritating properties of iron on the mucosa of the upper gastrointestinal tract. The gastric delivery system (GDS) has the major advantage that gastrointestinal side effects occur less frequently with controlled-release iron therapy than with conventional iron therapy. Upper gastrointestinal symptoms are eliminated because it is released slowly over a period of hours. [11]

Conflict of Interest: *None declared*
Source of Support: *Nil*
Ethical Permission: *Obtained*

Conclusion

It is concluded that there was inhibitory influence of calcium on iron absorption initially. But on chronic administration this inhibitory influence lost because of some adaptive changes probably at mucosal level. The combination preparation has shown significantly early response with significantly less upper gastrointestinal adverse effects. As it contains both, iron in controlled-release form with calcium in a single tablet, the patient compliance may be better than to take iron and calcium supplements at different times, which is far from ideal. There is a clear need for a combination iron-calcium supplement which delivers bioavailable iron.

References

1. Kassa ZY, Awraris T, Daba AK, Tenaw Z. Compliance with iron folic acid and associated factors among pregnant women through pill count in Hawassa city, South Ethiopia: a community based cross-sectional study. Reprod Health. 2019;8;16(1):14.
2. Lopez A, Cacoub P, Macdougall C, Peyrin-Biroulet L. Iron deficiency anemia. The Lancet. 2016;387(10021):907-916.
3. Peña-Rosas JP, De-Regil LM, Dowswell T, Viteri FE. Daily oral iron supplementation during pregnancy. Cochrane Database Syst Rev. 2012;12: CD004736.
4. Juul E Sandra, Derman J Richard, Auerbach Maichael. Perinatal Iron Deficiency: Implications for Mothers and Infants. Neonatology 2019;115:269–274.
5. O' Brien KO, Zavaleta N, Caulfield LE, Yang DX and Abrams SA. Influence of prenatal iron and zinc supplements on supplemental iron absorption, red blood cell iron incorporation and iron status in pregnant Peruvian women. Am J ClinNutr 1999; 69: 509-515.
6. Mousa A, Naqash A, Lim S. Macronutrient and Micronutrient Intake during Pregnancy: An Overview of Recent Evidence. Nutrients. 2019 Feb 20;11(2):443.
7. Sackey DS, Larbie C, Mensah FO. Geophagia, nutrition and health of women with pregnancy-

induced hypertension. Afr Health Sci. 2018 Dec; 18(4):1243-1254.
8. Preziosi P, Prual A, Galan P, Daouda H, Boureima H and Hercberg S. Effect if iron supplementation on the iron status of pregnant women: consequences for newborns. Am J Clin Nutr 1997; 66: 1178-1182.
9. Hallberg L, Rossander- Hulthen L, Brune M and Gleerup A. Inhibition of haeme-iron absorption in man by calcium. Br J Nurt 1992; 69: 533-540.
10. Seligman PA, Caskey JH, Frazier JL, Zucker RM, Podell ER and Allen RH. Measurements of iron absorption from prenatal multivitamin-mineral supplements. ObstetGynecol 1983;61(3):356-362.
11. Cook JD, Lipschitz DA and Skikne BS. Absorption of controlled-release iron. Clin Pharmacol Ther 1982; 32(4): 531-539.
12. Middleton EJ, Nagy E and Morrison AB. Studies on the absorption of orally administered iron from sustained-release preparation. N Engl J Med 1966; 274(3): 136-139.
13. Chapman DG and Campbell JA. Effect of calcium and phosphorus salts on the utilization of iron by anaemic rats. Br J Nutr. 1957; 11: 127-133.
14. Barton JC, Conrad ME and Parmley RT. Calcium inhibition of inorganic iron absorption in rats. Gastroenterology 1983;84: 90-101.
15. Cook JD, Dassenko SA and Whittaker P. Calcium supplementation: effect on iron absorption. Am J ClinNutr 1991; 53: 106-111.
16. McMullin MF, White R, Lappin T, Reeves J and Mackenzie G. Hemoglobin during pregnancy: relationship to erythropoietin and haematinic status. Eur J Haematol 2003;71:44-50.
17. Svanberg B. Absorption of iron in pregnancy. Acta Obstet Gynecol Scand (Suppl) 1975;48:7-28.
18. Ekenved G, Norrby A and Solvell L. Serum iron increase as a measure of iron absorption-studies on the correlation with total absorption. Scand J Haematol (Suppl) 1976; 28: 31-49.
19. Ekenved G. Absorption from different types of iron tablets-correlation between serum iron increase and total absorption of iron. Scand J Haematol (Suppl) 1976; 28: 51-63.
20. Bye KE, Milman N and Agger AO. Correlation between iron status markers during normal pregnancy in women with and without iron supplementation. Haematology 2000; 4: 529-539.
21. Van Den Broek NR, Letsky EA, White SA and Shenkin A. Iron status in pregnant women: which measurements are valid? Br J Haematol 1998; 103: 817-824.
22. Rabindrakumar MSK[1], Pujitha Wickramasinghe V[2], Gooneratne L[3], Arambepola C[4], Senanayake H[5], Thoradeniya T[1]. The role of haematological indices in predicting early iron deficiency among pregnant women in an urban area of Sri Lanka. BMC hematol. 2018;22:18-37.
23. Fenton V, Cavill I and Fisher J. Iron stores in pregnancy. Br J Haematol 1977; 37: 145-149.
24. Taylor DJ Mallen C, McDougall N and Lind T. Effect of iron supplementation on serum ferritin levels during and after pregnancy. Br J Obstet Gyanecol 1982; 89: 1011-1017.
25. Linder MC and Munro HN. The mechanism of iron absorption and its regulation. Fed Proc 1977;36:2017-2023.
26. Roth DE, Pezzack B, Al Mahmud A, Abrams SA, Islam M, Aimone Phillips A, Baxter JA, Dimitris MC, Hawthorne KM, Ahmed T, Zlotkin SH. Bioavailability of enteric-coated microencapsulated calcium during pregnancy: a randomized crossover trial in Bangladesh. Am J Clin Nutr. 2014 Dec;100(6):1587-95.
27. Singh K, Fong YF and Kuperan P. A comparison between intravenous iron polymaltose complex (ferrumhausmann) and oral ferrous fumarate in the treatment of iron deficiency anemia in pregnancy. Eur J Haematol 1998;60:119-124.
28. Ahenkorah B, Nsiah K, Baffoe P, AntoEO. Biochemical and hematological changes among anemic and non-anemic pregnant women attending antenatal clinic at the Bolgatanga regional hospital, Ghana. BMC Hematol. 2018 Sep 17;18:27.
29. Minot GR and Health CW. The response of reticulocytes to iron. Am J Med Sci 1932; 183: 110-121.
30. Minihane AM and Eairweather-Tait SJ. Effect of calcium supplementation on daily nonheme-iron absorption and long-term iron status. Am J ClinNutr 1998; 68: 96-102.
31. Taylor DJ and Lind T. Haematological changes during normal pregnancy: iron induced macrocytosis. Br J Obstet Gynaecol 1976; 83: 760-767.
32. Van Den Broek NR, Letsky EA, White SA and Shenkin A. Iron status in pregnant women: which measurements are valid? Br J Haematol 1998; 103: 817-824.