REVIEW PAPER

Appropriate Doses of Iron for Treatment of Anemia Amongst Pregnant and Lactating Mothers; Under Five Children; Children in 6-10 Years of Age; Adolescent Girls and Women in Reproductive Age Groups

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Abstract

Iron deficiency is the leading cause of anemia in India. In spite of implementation of a National iron supplementation programme of anemia control, the rate of decline in anemia prevalence has not been satisfactory. To address this issue, a National consultation meeting is being organized by the Ministry of Health and Family Welfare, Government of India, 23-24th April 2018, AIIMS, New Delhi. The purpose of the background document is to facilitate the deliberations of the above meeting. In this document, we discuss the latest progress in studies of iron metabolism, bioavailability, requirement and RDA for Indians. Hepcidin is a circulating peptide hormone secreted by the liver that plays a central role in coordinating the use and storage of iron with iron acquisition. Therefore, we considered it important to review and include trials on modulation of hepcidin during iron supplementation. Further, we made an attempt to review iron supplementation trials in Indian pregnant women and children to sequentially assess the basis for fixing iron dosage. Finally, we made an attempt to apply theoretical basis of computation of iron dose for the age/gender and physiological group for treatment of anemia due to iron deficiency. In the light of the role of hepcidin in iron nutrition, we consider it important to characterize the contextual determinants and establish the iron dosage. We believe that the systemic regulation may not allow the body to store adequate amounts of iron from oral doses in short duration of 100 days. Based on the biology of iron it appears that iron homeostasis leading to assimilation of storage of iron is a very slow process and may require practice of contextual food synergy systems to improve iron content (fortified foods) and bioavailability (vitamin C rich fruits) throughout life cycle.

Introduction

Iron occupies a central role among essential nutrients. Its deficiency can directly contribute to disease burden and productivity of a nation. Iron is the most versatile of all biologically active metals. It can exist as ferrous (Fe2+) or ferric (Fe3+) ions and ability to either accept or donate electrons in biological reactions and participates in free radical chemistry by donating electrons to oxygen. It is the major hemopoietic factor and participates in various metabolic processes including DNA synthesis, energy production, and oxygen transport. In biological systems it is associated with a large variety of biological molecules. In spite of its abundance and versatility, iron deficiency anemia is

the most common nutrient deficiency in the world today. It affects all age groups and both the genders. The prevalence of anemia is significantly higher especially in women of child bearing age, infants and children. The prevalence of anemia among children and women in India is around 50% (Table -1).

TABLE-1: ANEMIA PREVALENCE IN INDIA

Group	% Prevalence	Reference
Children 6-59 months of age Hb<11 g/dL	58.4	NFHS-4 (2016)
Non-Pregnant women age 15-49 years Hb<12g/dL	50.1	
Pregnant women 15-49 years Hb<11g/dL	50.3	

The physiology of iron and the factors leading to iron deficiency are well defined. A brief review of the distribution of iron with respect to the Indian context is described below. An attempt will be made to understand the storage compartment, which is very essential to establish a positive iron balance and to control iron deficiency in a population.

DISRTIBUTION OF IRON

The total amount of iron in human body is determined by intake, loss, and storage of iron. Healthy/non-anemic individuals have an optimum balance between iron intake and excretion. The total body iron content extrapolated from literature is about 50 mg/kg and 42 mg/kg body weight respectively, for a 60-kg reference Indian adult man and 55-kg reference Indian adult woman.

Conceptually iron is distributed in three compartments viz., functional, storage and transport compartments. About 70% of the total iron is present in the functional and 25% in storage compartment and is involved in iron homeostasis (Table 2). The compartment linking the storage and functional compartment is the transport compartment, transferrin which contributes to less than 1% of total iron. Thus, a decline of iron in functional compartment leads to deleterious functional consequences.

TABLE-2: DISTRIBUTION OF IRON IN VARIOUS COMPARTMENTS AND THEIR FUNCTIONS (NAIR, 2011)

Compartment	Indian adult man(60-kg)	Indian adult woman (55kg)
Total iron	3g or 50 mg/kg body weight	2.3 g or 42 mg/kg body weight
Functional compartments **Hemoglobin, myoglobin, respiratory enzymes, synthesis of DNA, dopamine D2 receptors** **1 g Hb =3.47 mg of iron** **Loss of 1 mL of blood (12g/dLHb)=0.52 mgof iron** **1g Hb transport 1.32 mL of oxygen** **> 80% of functional iron is found in RBC mass as Hb.**	70% of the total iron	70% of total iron (However, in reality entire iron will be present in this compartment in Indian women who have negligible storage iron)
Storage compartments **Liver, spleen and bone marrow**	25% or 750 mg of iron	25% or 580 mg
Transport compartments	Less than 1%	Less than 1%

RECYCLING OF RBC HEMOGLOBIN IRON

Iron recycling through the breakdown of hemoglobin in RBC is the predominant biological mechanism in humans to be in iron balance. About 90% of daily iron needs are obtained through this process and used for hemoglobin production. Due to the iron recycling, the major factors affecting iron requirements are the storage and the iron lost from the body. Sum of these two is equivalent to the iron requirement. Thus, what is absorbed from daily diet is used for meeting the requirements of functional compartment and that not utilized will be deposited in the storage compartment.

IRON LOSSES AND REQUIREMENTS DUIRNG LIFE CYCLE (ICMR, 2010)

Dietary iron requirements are highest in the second and third trimesters of pregnancy and in infants between 6 -18 months of age.

Women of child-bearing age

In women of child bearing age the total iron loss is 30 µg/kg/day which is the sum of the obligatory losses of 14 µg/kg body weight and the menstrual blood losses of 16 µg/kg/day. The iron requirement works out to be 1.65 mg iron per day for a 55 kg Indian woman.

Pregnancy

Total iron requirements during pregnancy include the following: (1) to replace basal loss of 210 mg (2) for expansion of the maternal red cell mass of 450mg (3) for the needs of the growing fetus 270-300mg and (4) for placental tissue of 50-90mg. The total iron loss during pregnancy is about 760-860 mg for a gestational weight gain (GWG) of 10-12 kg and the trimester –wise requirement is given in Table-3.

TABLE-3: IRON LOSS AND REQUIREMENT DURING PREGNANCY (TRIMESTER-WISE)

Trimester	Iron loss mg		Requirement mg per day	
	10 kg GWG	12 kg GWG	10 kg GWG	12kg GWG
First	130	138	1.44	1.53
Second	320	372	3.56	4.13
Third	310	351	3.44	3.90
Total	760	861	2.81	3.19

Lactation

The total iron loss during lactation is 23 µg/ kg/d (basal requirement and that required for making up the iron lost in breast milk of 9 µg/kg/d). However, considering the losses during pregnancy, iron requirement is fixed similar to that for NPNL women of 30µg/kg/d.

Adolescence: 10-18 years

Iron requirement for growth refers to the iron needed for expansion of blood volume and the need for increase in lean body mass. The additional requirement during adolescence for the growth spurt is estimated to be 12 µg/kg for boys and 8 µg/kg for girls. Girls of this age group require an additional 8 µg/kg to compensate for menstrual blood loss. Total iron loss during this period for boys is 26 µg/kg/d and for girls 30 µg/kg/d. In males, during adolescence, about 6 mg/kg of iron is stored, which increases to around 12-15 mg/kg. An allowance of 5 mg/kg for maintenance of stores throughout adolescence is needed for girls (Table-4)

TABLE-4:IRON REQUIREMENT DURING ADOLESCENCE

Age Group (y)	Body Wt (kg)	Gain in Body Wt (kg/y)	Basal Loss (mg/d)	For Blood Volume (mg/d)	Muscle Mass (mg/d)	Store (mg/d)	Blood Loss (mg/d)	Total Requirement (mg/d)
10-12								
Boys	34.3	3.5	0.49	0.27	0.13	0.16	--	1.05
Girls	35.0	3.7	0.49	0.27	0.13	0.16	0.28	1.33
13-15								
Boys	47.6	4.2	0.66	0.39	0.15	0.40	--	1.60
Girls	46.6	1.7	0.65	0.13	0.06	0.15	0.37	1.36
16-17								
Boys	55.4	1.5	0.78	0.14	0.05	0.40	--	1.37
Girls	52.1	--	0.73	--	--	0.15	0.42	1.30

Iron requirement during childhood (4-9 years)

The average iron requirement for growth would be about 17 µg/kg/d and body store of iron builds up to 5 mg/kg, which is maintained in girls until menarche. The requirement computed for the two age groups 4-6 y and 7-9 years is presented in Table -5

TABLE 5: IRON REQUIREMENT FOR CHILDREN 4-9 YEARS

Age Group (y)	Body Wt (kg)	Gain in Body Wt (kg/y)	Basal Loss (mg/d)	For Blood Volume (mg/d)	Muscle Mass (mg/d)	Store (mg/d)	Total Requirement (mg/d)
4-6	18.0	2.8	0.253	0.2	0.1	0.08	0.633
7-9	25.1	2.8	0.353	0.2	0.1	0.12	0.733

Infancy and preschool age children

Iron needs of infants from birth through 6 months of age (exclusive breast fed) are similar to the average iron intake of healthy infants fed breast milk. A full-term infant of 3.2 kg body weight needs 0.23 mg of iron per day to maintain hemoglobin at the normal concentration of 110 g/L and to replace excretory losses. Iron requirements increase markedly during later 6 months of life. Therefore, iron-enriched solid foods should complement breast milk from 7 to 12 months of age. During pre-school years the requirement decreases to 35 µg/kg/d (Table-6).

TABLE-6: IRON REQUIREMENTS DURING INFANCY AND PRESCHOOLERS

Age of Infant(mo)	Body Wt (kg)	Basal Loss (mg/d)	For Blood Volume (mg/d)	Skeletal Mass (mg/d)	Requirement	
					mg/d	µg/kg/d
0-6	5.4	Equal to Breast Milk Iron		--	0.23	46
6-12	8.4	0.12	0.4	0.21	0.73	87
1-3	12.9	0.181	--	0.27	0.451	35

RECOMMENDED DAILY ALLOWANCE (RDA) OF IRON FOR INDIANS

To maintain iron balance, the sum of the losses plus the iron required for growth in infants, children, and adolescents, and during pregnancy must be provided by the diet.

Recommended daily allowances (RDA) of iron for Indians have been computed taking into consideration iron absorption of 15% for 6- 12 months, 5% for men and children and adolescents and 8% for all women (Table-7).

DIETARY IRON

The iron density of a typical cereal-pulse vegetarian Indian diet is around 9 mg/1000 kcal. Non-heme iron contributes about 90-95 % of total daily iron. Heme iron consumption is minimal in India with majority of Indians obtaining non-heme iron from cereals, pulses, vegetables and fruits (in the absence of universal iron fortification). The NNMB surveys revealed that intake of dietary iron is inadequate in most of the states.

IRON ABSORPTION IN INDIANS

There are only two recent studies that assessed bioavailability of iron from a typical rice meal in Indians using stable isotopic method. The study among 18–35-y-old women have reported iron bioavailability ranging from 5.2–9.4% in non–iron-deficient and 15.6–19.7% in iron-deficient (Thankachan*et al* 2008). The study in adolescent boys and girls of 13-15 years reported an iron bioavailability of 9.7% (95%CI: 6.1, 13.3) in girls and 8.6% (95% CI: 6.2, 11.0) in boys, which is similar to the range of values reported in women above (Nair *et al*, 2013). Further, the study demonstrated that the bioavailability can be doubled with the inclusion of 100 g of guava fruit, a vitamin C rich fruit in habitual meals. Quantitatively, the ratio of phytate to iron should be <0.4:1 or ascorbic acid to iron of >4:1to significantly improve iron absorption in cereal- legume based meals (Hurrell and Egli 2010).

TABLE-7: RDA OF IRON FOR VULNERABLE GROUPS (ICMR 2010)

Age group	Body Wt kg	Requirement µg/kg/d	Absorption %	RDA mg/day
Infants				
0-6 mo	5.4	46	-	-
6-12 mo	8.4	87	15	5
Children				
1-3 y	12.9	35	5	9
4-6 y	18.0	35	5	13
7-9 y	25.1	31	5	16
Adolescent Girls				
10-12 y	35.0	38	5	27
13-15 y	46.6	29	5	27
16-17 y	52.1	25	5	26
Women NPNL	55	30	8	21
Pregnant	55	51	8	35
Lactating	55	30	8	21

REGULATION OF IRON ABSORPTION

The absorption of dietary non-heme iron, is carried out by mature villus enterocytes of the duodenum and proximal jejunum. The gastric acid production plays a key role in solubilizing iron. Abundance of dietary inhibitors or promoters modulates iron absorption. At the absorptive surface, iron must be in its ferrous form to be taken up by the enterocyte. A ferric reductase enzyme on the brush boarder does this function. A divalent metal transporter (DMT 1) transports the iron across the membrane into the cell. Once inside the cell, iron is either stored in ferritin or exported by the iron exporter ferroportin 1 (FPN) located on the basolateral side of the enterocyte. Iron is oxidized back to the ferric form by the ferroxidase, hephaestin and ceruloplasmin and loaded onto transferrin and circulated through the blood.

The amount of iron absorbed by enterocytes is influenced by a variety of factors including variations in body iron stores, changes to the rate of erythropoiesis, hypoxia, inflammation, and pregnancy. These factors result in induction of major enterocyte iron transport molecules, particularly DMT1, Dcytb, and ferroportin 1, at both the mRNA level and protein level.

HEPCIDIN: A REGULATIOR OF IRON ABSORPTION

The current scientific evidence suggests that hepcidin is a central regulatory hormone and its main action is to regulate systemic iron homeostasis. Therefore, hepcidin response to various iron stimuli will help us to design dosing schedule to treat iron deficiency anemia.

Hepcidin, a 25 amino acid cysteine rich peptide, is secreted by liver and its main site of action is cellular iron transporter, ferroportin. Hepcidin responds to various stimuli including hypoxia, and inflammatory response such as IL-6. It has an inverse relation with iron absorption. At the basolateral membrane of the enterocyte, hepcidin interact directly with ferroportin1 leading to its

internalization and degradation and prevent iron import into systemic circulation (under conditions of iron loading and during anemia of chronic disease/inflammation). Conversely, decreased hepcidin production leads to increased iron absorption (iron deficiency and increased erythropoiesis).

Circulating hepcidin is considered to affect iron metabolism by regulation of iron absorption in the gut, iron recycling from macrophages, and control of hepatic iron storage. It also appears to regulate iron transfer through the placental syncytiotrophoblast during pregnancy (Sun *et al*, 2012).

Therefore, understanding the association between circulating hepcidin and iron absorption under various dietary/supplemental and physiological and disease state will have direct implications in the treatment of iron deficiency anemia.

Relation between circulating hepcidin and iron absorption

Iron absorption from ferrous sulfate administered to fasted subjects in the absence of a food matrix was tested in healthy non-pregnant women (N=18). The absorption was found to be inversely associated with serum hepcidin (Young *et al* 2009). We reported that plasma hepcidin is a significant predictor of iron absorption (standardized β = -0.6, p = 0.001, R2 = 0.40) after adjusting for confounders (Nair *et al*, 2013).

Effect of different Fe doses on hepcidin and iron absorption

Two recent studies have assessed the duration and magnitude of plasma hepcidin, its dose dependence, and its effects on subsequent iron absorption (Moretti *et al* 2015 and Stoffel *et al* 2017).

Subjects were apparently healthy females aged between 18 - 45 years (N=41 and each study 13-16), with depleted iron stores (<20µg/L) but no anemia. The dose was administered under fasting state (Moretti *et al* 2015). The results are summarized below:

Acute effect of different Fe doses on hepcidin and iron absorption (40, 80, 160, 240 mg Fe)

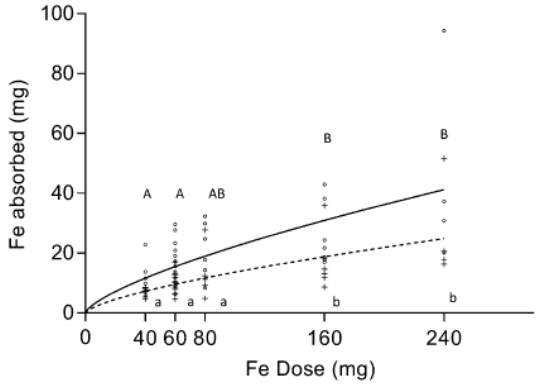
There was a significant increase in plasma hepcidin at 24 hours after the doses of 60, 80, 160, and 240 mg Fe, but not at 40 mg Fe. Plasma hepcidin was not significantly elevated 48 hours post–iron administration in the overall model or after any of the iron doses.

For the doses tested, fractional iron absorption decreased with increasing dose, whereas absolute absorption increased. Figure -1 shows at doses of 60 mg and higher, the first and second dose absorptions, differed significantly ($p<0.01$). Acute absorption is inhibited at dosages of 80, 160, and 240 mg within 24 hours.

Hepcidin increases and iron absorption decreases at 24 hours with 60 mg Fe

Fractional iron absorption decreased by 36%when 60mg iron was administered on the second day compared with the first day. Iron absorbed was 23.6 mg (~13% absorption) if 3 doses were administered within 24 hours compared with 22.6 mg Fe (~19% absorption) when only the 2 morning doses were given.

FIGURE 1 AMOUNT OF IRON ABSORBED IN RELATION TO THE FIRST AND SECOND DOSE ADMINISTRATION. LINE— FIRST ADMINISTRATION AND THE LINE---- SECOND ADMINISTRATION,(N=41). DATA WITH DIFFERENT SUPERSCRIPTS DIFFER SIGNIFICANTLY (CAPITALS: FIRSTDOSE; MINUSCULE: SECOND DOSE). ABSORPTION DATA ARE STANDARDIZEDTO A PLASMA FERRITIN LEVEL OF 15 µG/L. SOURCE: MORETTI ET AL. 2015.



A trial was carried to establish whether the short-term effects of hepcidin on fractional and total iron absorption persist during longer-term supplementation from single versus divided daily doses among iron-depleted women by Stoffel *et al* (2017). This was tested in two separate studies.

In study-1, a comparison of consecutive-day (60 mg Fe at 8am for 14 days, N=21) versus alternate-day (60 mg Fe at8 am alternate days for 28 days, N=19) and in study-2, a comparison of a single 120 mg Fe at 8 am doses versus divided 60 mg at 8am+60 mg at 5pm daily doses for 3 days (n=20).

Salient finding:

- Alternate day 60 mg Fe resulted in 34% higher Fe absorption than with consecutive-day supplementation (Table-8).
- Dividing a single oral dose of 120 mg Fe into two daily doses of 60 mg Fe does not improve iron absorption.
- During the first 14 days of supplementation in both groups, serum hepcidin was higher in the consecutive-day group than the alternate-day group. Twice-daily divided doses resulted in a higher serum hepcidin concentration than once-daily dosing.
- In iron-depleted women, providing iron supplements daily as divided doses increases serum hepcidin and reduces iron absorption.
- Providing oral iron on alternate days in single morning doses increases iron absorption in young women and is an effective regimen to optimize iron absorption. These findings should be confirmed in iron-deficient anemic patients.

Implications: By contrast with most current recommendations on iron supplementation, these findings suggest that providing oral iron on alternate days in single morning doses (Fasting) increases iron absorption in young women and is an effective regimen to optimize iron absorption. This regimen not only improves iron absorption but also, because of its simplicity, might increase compliance

TABLE-8: CUMULATIVE FRACTIONAL AND TOTAL ABSORPTION-STUDY-1

	Consecutive-day dosing for 14 d	Alternate-day dosing for 28 d	P value
Fractional absorption %			
Week 1 first 7 doses	16.1(8.9, 28.9)	21.2(13.2,34.3)	0.13
Week 2, second 7 d	16.6(9.4, 29.6)	22.3(13.2,34.3)	0.11
All 14 doses	16.3	21.8	0.0013
Total iron absorption, mg			
Week 1 first 7 doses	66.9(36.9,121.1)	88.0(54.8,141.4	0.13
Week 2, second 7 d	69.3(39.3,122.2)	92.7(58.8,146.2)	0.11
All 14 doses	131.0(71.4,240.5)	175.3(110.3,278.5)	0.0010
Geometric mean(±SD). Mixed –effect models with group as fixed and participant as random factor			

CHALLENGES

Plasma hepcidin negatively correlates with iron bioavailability and have reported that acute oral doses of iron increase serum hepcidin. This has implications in the current guidelines to treat iron deficiency recommend by WHO and the NIPI (2013). The expert group may deliberate on (1) frequency of dosing (2) dosage (3) and time of dosing fasting or fed state (4) co morbidities (sub clinical infection).

REVIEW OF INDIAN STUDIES:IRON ABSORPTION AND SUPPLEMENTATION INPREGNANCY

The IFA programme has undergone several transformations and currently life-cycle approach is adopted by the Ministry (National iron plus initiative, 2013). An attempt has been made to review the current literature pertaining to the various studies that have been carried out in India with respect to iron absorption and supplementation in pregnancy.

1. Report of Study Group of NSI 1968

The first publication in this area is the report prepared by the study group of the Nutrition Society of India which has recommended the use of 60 mg iron daily for the prophylaxis of anemia in pregnancy. The dose was determined after taking into consideration the poor iron stores of the mothers, the variability of iron absorption, and the possible irregularities in supply and intake of the iron tablets.

2. Dietary iron absorption (Apte and Iyengar,1970)

This is the only iron absorption study carried out in India among normal and iron-deficient pregnant women (n=12). A balanced diet that provided 22 mg of iron was fed throughout pregnancy and absorption estimated by using chemical balance method (Study design, Figure 2). It is demonstrated that dietary iron absorption is increased in the latter half of pregnancy in normal pregnant women and more so in iron-deficient pregnant women (Table-9). In order to meet the iron requirements of pregnancy, the predominantly cereal-based diets of the type used by poor Indian women should provide 40 mg of iron (with 8% absorption).

FIGURE- 2: STUDY DESIGN: DIETARY IRON ABSORPTION IN PREGNANT WOMEN

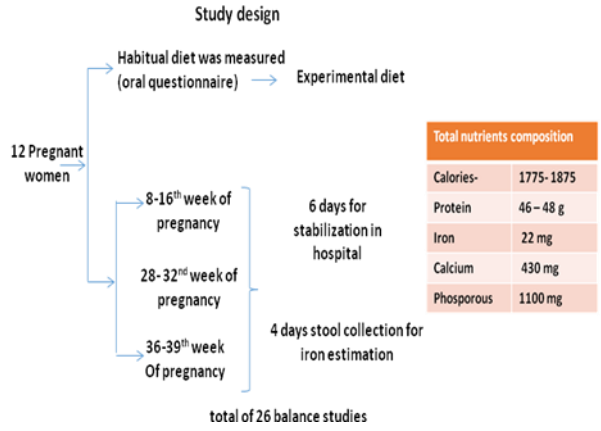


TABLE-9: IRON BALANCE DURING PREGNANCY

Weeks pregnancy	Subjects	Hb g/dL	Dietary Fe (mg)	Absorbed (mg)	Absorption (%)
8- 16 N=12	Normal	12.7	22.2	2.4	7.4
28- 32N=4	Normal	12.4	23.9	6.1	25.7
	ID	9.9	23.9	8.9	37.9
36-38N=2	Normal	12	27.6	8.7	31.3
	ID	10.5	28.7	9.9	34.5

3. Prophylaxis of Anemia in Pregnancy:A trial with 30 mg iron in pregnancy (Iyengar and Apte, 1970)

Based on the following considerations the dose was fixed at a level of 30 mg of iron for the trial. A woman requires additional 4-5 mg/day to meet the increased need for iron during the last 100 days of pregnancy (ie 400-500 mg iron). With absorption about 20%, the dietary allowance of iron would be about 25 mg/day. Normal pregnant women (N=800) in the first 24 weeks of pregnancy belonging to the low socio economic status were registered for the trial.

Study Design: Group I: received placebo tablets containing lactose; group II: received 30 mg of elemental iron daily as ferrous fumarate in a single tablet; group III: received 30 mg of iron and 500 µg of folic acid daily in a single tablet; group IV: received, in addition to iron and folic acid, 2 µg of B12 daily in a single tablet (Instructed to consume on an empty stomach).

The impact on hemoglobin level were categorized as "stable "if the change in Hb was not more than 1 g/dL ml from the initial level, "Fall" if a reduction in Hb of > 1 g/ dL ml from the initial level and "Rise" when an increase of Hb 1 g/dL from the initial level.

Results: Rate of follow up was 35%. More than 60% of the women who received iron with or without folic acid and B12 showed stable hemoglobin levels. In the three iron given groups, 29, 30, and 24%, respectively, showed a rise in hemoglobin levels from their initial values. It was observed that 60% of the women who received a placebo showed a fall in hemoglobin values as pregnancy advanced (Table-10).

It is suggested that daily supplements of 30 mg iron given during the last 100 days of pregnancy is adequate to maintain satisfactory hematological status during pregnancy.

TABLE 10 HEMATOLOGICAL STATUS 12-16 WEEKS AFTER SUPPLEMENTATION

Group	No. of Subjects	% Subjects Showing			% Subjects Showing Fall in Hb	
		Fall in Hb	Stable Hb	Rise in Hb	Below 8.5g/dL	Below 10g/dL
Control	89	60	33	7	11	41
Iron	77	6	65	29	0	1
Iron+ Folic Acid	69	10	59	30	0	4
Iron+ Folic Acid+ Vitamin B12	58	14	63	23	0	5

4. WHO collaborative programme on Iron, folate, and vitamin B12 nutrition in pregnancy: a study of 1000 women from southern India (Yusufji, et al, 1973)

The prevalence of anemia was studied among pregnant women from southern India along with the serum concentrations of iron, folate, and vitamin B12. The results showed high prevalence of iron, folate and vitamin B12 deficiency in the community (Table -11).

TABLE-11: PREVALENCE OF ANEMIA, FOLATE AND VITAMIN B12 DEFICIENCY IN SOUTHERN INDIA

Biomarker	Pregnant women	Non-Pregnant women
Hb (g/L)	104 ± 27	122 ± 15
Hb< 100g/L	32.7 %	
Hb< 110 g/L	57.4 %	
Hb< 120 g/L	82 %	
Serum Iron (mg/L)	0.31 ± 0.12	0.53 ± 0.21
<0.5 mg/L	96 %	
Serum Folate (µg/L)	5.6 ± 2.5	8.9 ± 3.9
< 6 µg/L	73%	
Serum B12 (ng/L)	159 ± 112	205 ± 67
<140 ng/L	52 %	

5. W.H.O. sponsored collaborative studies on nutritional anemia in India. 1.The effects of

supplemental oral iron administration to pregnant women (Soodet al 1975)

The success of two trials in Israel with 100 mg iron and 300 µg folic acid starting from 2nd trimester until term, formed the basis of this study. These trials showed a rise in Hb in 90%, and reduction of the prevalence of anemia from 50% to below 6%.

The design of the study is given in Table -12. The duration of intervention was 10-12 weeks.

Results: Hb increased in all the groups receiving iron. Greater rise in Hb was in women receiving iron, folic acid and vitamin B12. Also, the rise in Hb was greater in women with lower intial Hb. The regression line for final Hb values on intitial Hb values for different supplementation regimen were calculated and found to be heterogenous. The slopes of the regression line were found to be significant (P<0.001). Separate regression functions for each treatment groups were estimated and the expected final Hb concentration was calculated on the basis of the initial Hb value (Table -13).

The best results were observed with the groups receiving 120 and 240 mg of iron together with vitamin B12 and folate. It is concluded that in these women, a daily oral supplementation of 120 mg of elemental iron or more is needed.

TABLE-12:STUDY DESIGN: THERAPY GIVEN TO DIFFERENT GROUPS OF PREGNANT WOMEN IN SUPPLEMENTATION TRIAL STARTING AT 22±2 WEEKS OF GESTATION

	Stream A		Stream B				
	Group 0	Group 6	Group 1	Group 2	Group 3	Group 4	Group 5
Vitamin B12 *	placebo	placebo	100µg	100 µg	100 µg	100 µg	100 µg
Folic acid **	placebo	placebo	5 mg	5 mg	5 mg	5 mg	5 mg
Iron	--	100 mg	--	30 mg	60 mg	120 mg	240 mg

**Given by injection fortnightly*

***given daily, 6 d/wk, in 2 pills each containing half the amount of iron and folic acid as shown*

TABLE 13: ESTIMATED REGRESSION FUNCTIONS AND EXPECTED MEAN HB CONCENTRATION AFTER 10 WEEK OF THERAPY DEPENDING ON INITIAL HB CONCENTRATION

Gp	Source of variation	Estimated regression function	Expected Hb concentration after 10 weeks therapy					
			Initial Hb in Concentration (g/dL of blood)					
			6	7	8	9	10	11
0	placebo	Y=0.19+0.96 X	5.95	6.91	7.87	8.83	9.79	10.75
1	B12+FA	Y=2.08+0.76 X	6.64	7.40	8.16	8.92	9.68	10.44
2	B12+FA+30mg iron	Y=4.11+0.05X	8.01	8.66	9.31	9.96	10.61	11.26
3	B12+FA+60mg iron	Y=5.37+0.53X	8.55	9.08	9.61	10.14	10.67	11.20
4	B12+FA+120mg iron	Y=8.16+0.28X	9.84	10.12	10.40	10.68	10.96	11.24
5	B12+FA+240mg iron	Y=8.18+0.28X	9.86	10.14	10.42	10.70	10.98	11.26
6	120mg iron	Y=3.62+0.70X	7.82	8.52	9.2	9.92	10.62	11.32

X=initial Hb

6. WHO sponsored collaborative studies on nutritional anemia in India. The effect of parenteral iron administration in the control of anemia of pregnancy (Soodetal 1979)

In the previous trial a substantial proportion of pregnant women had a hemoglobin concentration of less than 110 g/L (Sood*et al*. 1975) which was attributed to an inadequate absorption of Fe. In this study, comparison of the effects of oral and parenteral Fe supplementation was tested.

Study design: Pregnant women of 26±2 weeks of gestation (Delhi & Vellore) were divided according to their hemoglobin concentration (50-79; 80-109; 110 or above g/L) allocated at random to one of the 5 treatment. Duration of treatment was 10-12 wks.

The result of the trial is given in Table -14.

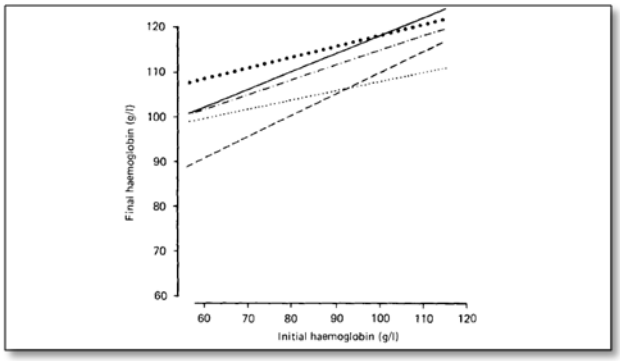
The results revealed that the magnitude of the rise in hemoglobin concentration after therapy was inversely related to the initial hemoglobin concentration also the regression line were found to be significantly different from each other (Figure -3). The differences in the effectiveness of various regimens were computed from the expected Hb concentration after therapy using the regression equation. Intramuscular Fe produced a greater increase in Hb compared to oral or IV. The percentage of women with persisting anemia at the end of period of supplementation in groups 1-5 was 33.3, 16.7, 20, 6.7 and 50, respectively.

In the present study, the greater rise in mean hemoglobin concentration obtained with intramuscular Fe, indicates that the unsatisfactory response to oral Fe therapy is attributable to the insufficient absorption of orally administered Fe to meet the body's needs in the available period of time.

TABLE-14: MEAN INITIAL AND FINAL HEMOGLOBIN VALUES IN FIVE GROUPS OF PREGNANT INDIAN WOMEN GIVEN DIFFERENT TREATMENTS

Treatment	No of women	Mean		Mean of individual difference	SE of mean of differences	Statistical sig. of diff. (paired t test)
		Initial	Final			
Oral iron (ferrous sulphate)	24	103.2	114.4	11.2	2.143	<0.001
Iron dextran complex (intramuscular)	30	100.5	120.7	20.2	2.539	<0.001
Oral iron +PGA+ Cyanocobalamin	35	109.9	120.5	10.6	2.698	<0.001
IV Oral iron+ PGA+ Cyanocobalamin	30	103.9	121.8	17.9	2.537	<0.001
Iron dextran complex(IM) +PGA+ Cyanocobalamin	32	97.2	108.8	11.6	2.638	<0.001

FIGURE 3: REGRESSION LINES OF FINAL HEMOGLOBIN CONCENTRATION (G/L) V. INITIAL HEMOGLOBIN CONCENTRATION (G/L) FOR THE FIVE GROUPS OF PREGNANT INDIAN WOMEN GIVEN DIFFERENT TREATMENTS. - - -, GROUP I ORAL IRON; -, GROUP 2 INTRAMUSCULAR IRON; - . -, GROUP 3 ORAL IRON, PTEROYLRNONOGLUTAMIC ACID AND CYANOCOBALARNIN; . . . , GROUP 4 INTRAMUSCULAR IRON, PTEROYLMONOGLUTAMIC ACID AND CYANOCOBALAMIN; . . . . . ., GROUP 5 INTRAVENOUS IRON, PTEROYLMONOGLUTAMIC ACID AND CYANOCOBALAMIN. SOURCE: SOOD ET AL. 1979.



7. *EFFECTS OF IRON SUPPLEMENTATION ON MATERNAL HEMATOLOGICAL STATUS IN PREGNANCY (Sloanet al, 2002)*

This comprehensive review, analyzed randomized controlled trials published between 1966 and 1998 from developed and developing countries, including from India.

Iron supplementation increased hemoglobin levels and is related to initial hematologic status (Table-15). The effect of iron supplementation is directly related to dose (Table-16). Substantial benefits, however, are evident only with supplemental doses of more than 91 mg/day compared to the recommended daily supplement dose of 60 mg but less than dose derived from the earlier studies from India (120 mg iron). The effect of duration of therapy was mediated by dose (Tables -17). The effect on Hb was the highest when the duration was >90 days (11-13 weeks). Iron with folate or vitamin C has not made any significant improvement in Hb.

TABLE-15: RELATIVE CHANGE IN HB BY INITIAL HB LEVELS: PREGNANT WOMEN 1966-1998

Initial Hb (g/dL)	Daily Dose (mg), Mean (SD)	Effect (g/dL), Mean (SE)	Experimental (n)	Comparison (n)
Developing Countries[a]				
<10	153 (543)	1.13 (0.120)*	147	108
≥10-<11	134 (76)	1.10 (0.045)**	148	128
≥11-<12	71 (33)	0.85 (0.018)***	140	135
≥12	NA	NA	NA	NA
Developed Countries[a]				
<10	NA	NA	NA	NA
≥10-<11	NA	NA	NA	NA
≥11-<12	64 (47)	1.17 (0.022)**	26	69
≥12	162 (49)	1.16 (0.045)***	61	57

NA= Not Available; [a]Comparison group received no iron supplementation; *p<0.05; **p<0.01; ***p<0.001

TABLE 16RELATIVE HEMOGLOBIN CHANGE BY DAILY DOSE OF IRON SUPPLEMENTATION, PREGNANT WOMEN 1966-1998

Daily Dose (mg)	Daily Dose (mg), Mean (SD)	Effect (g/dL) Mean (SE)	Experimental	Comparison[a]
≤60	42 (23)	0.41 (0.027)**	135	131
61-90	76 (4)	0.86 (0.018)*	140	135
91-120	117 (6)	1.87 (0.027)***	214	164
>120	223 (20)	1.78 (0.042)***	133	119
[a]Comparison group received no iron supplementation; *p<0.05; **p<0.01; ***p<0.001				

TABLE 17RELATIVE HEMOGLOBIN CHANGE BY DURATION OF THERAPY OF IRON SUPPLEMENTATION, PREGNANT WOMEN 1966-1998

Duration of Supplementation (weeks)	Daily Dose (mg), Mean (SD)	Effect (g/dL) Mean (SE)	Experimental	Comparison [a]
≤10	90 (24)	0.84 (0.017)***	292	249
11-13	124 (66)	1.37 (0.062)*	33	23
14-19	138 (87)	1.16 (0.022)***	182	161
>20	136 (62)	1.00 (0.039)***	74	82
[a]Comparison group received no iron supplementation; *p<0.05; ***p<0.001				

8. EFFECTIVENESS OF ORAL IRON IN REPENESHING IRON STORES IN PREGNANT WOMEN (Thane-Toe and Than 1982)

Ideally, iron supplementation during pregnancy should be aimed at not only correcting anemia and maintaining Hb levels throughout pregnancy but also in building up iron stores. Earlier studies have shown that a rapid rise in the ferritin concentration in the first week of treatment with ferrous sulphate in patients with iron deficiency anemia. It is suggested that repletion of iron stores in patients with uncomplicated iron deficiency anemia could be achieved after the correction of anemia.

The study was carried out in 99 healthy Burmese pregnant women between 22 to 28 wk receiving daily dose regimes of 60, 120, or 240 mg of ferrous sulphate. The tablets were given after meals. At baseline 54.8% of the pregnant women had serum ferritin < 10 µg/L (mean 14µg/L). Supplementation for 12 wk produced an increase in ferritin levels in all the groups, but significant increases were seen only in women given 240 mg of ferrous sulphate with or without folic acid (mean ferritin level rose to 27.9µg/L). However, there were no differences in final Hb levels among the supplemented groups. 240 mg dose could be taken as an effective one for building up iron stores in pregnant women (Table-18).

TABLE-18: SERUM FERRITIN AND HB LEVELS BEFORE AND AFTER SUPPLEMENTATION IN BURMESE PREGNANT WOMEN (MEAN ±SE)

Group	No	Serum Ferritin (µg/L)			Hb (g/dL)	
		Before [a]	After [b]	Difference [b-a]	Before	After
60mg+FA*	16	18.42 ± 5.75	26.03 ± 3.16	+7.61	10.9 ± 0.20	11.2 ± 0.27
120mg+FA	32	16.20 ± 2.94	25.44 ± 2.35†	+9.24	10.9 ± 0.14	11.3 ± 0.15
240mg+FA	33	14.30 ± 2.02	35.32 ± 2.79‡	+21.02	10.5 ± 0.34	11.1 ± 0.12
240mg-FA	18	7.88 ± 1.46	27.90 ± 2.77‡	+20.02	10.7 ± 0.24	11.3 ± 0.24
***Folic acid, 5mg/daily; †p<0.02; ‡p<0.001**						

HEMOGLOBIN AND SERUM FERRITIN CONCENTRATIONS (IRON STORES) IN LIFE CYCLE

Conceptually, the biomarkers hemoglobin and serum ferritin concentrations are on the opposite end of iron depletion scale. Therefore, it would be interesting to review the concentration of these two biomarkers in the vulnerable segments of our population and quantify iron stores. Information on these two is available throughout the life cycle starting from cord blood to pregnancy (Table-19).

It can be seen that there is a gradual decrease in the serum ferritin concentration as the age advances with a concomitant increase in the Hb level. The adolescent groups who had hemoglobin around 120 g/L had the lowest concentration of serum ferritin, Exclusively, breastfed infants of non-anemic and anemic mother (comparable birth weights) did not develop iron deficiency or iron deficiency anemia by six months of age, suggesting protective mechanisms of iron transfer. However, the total iron of fetus was found to be directly related to the weight of the fetuses (Figure-4), which signifies low iron stores among low birth weight babies in India

(LBW of 20% in India)-a concern that needs to be addressed. Whether LBW infants require a different iron dosage schedule is debatable?

Iron stores have a dominant role in regulating the rate of absorption and exhibit a close inverse relationship between serum ferritin and non-heme iron absorption. However, the proportionate effect of increasing iron stores is much smaller. These relationships have implications for prevention of iron deficiency in the life cycle as iron stores are always the first source for increased need. The body lacks the capacity to increase absorption and concurrently accumulate iron for later use (Lynch, 2007).

FIGURE-4: RELATIONSHIP BETWEEN BODY WEIGHT AND TOTAL IRON CONTENT. THE BODY COMPOSITION WAS ESTIMATED OF 41 FOETUSES OF DIFFERENT GESTATIONAL AGES BORN TO MOTHERS IN A LOW SOCIOECONOMIC GROUP OF THE INDIAN POPULATION SOURCE: APTE AND IYENGAR, 1972.

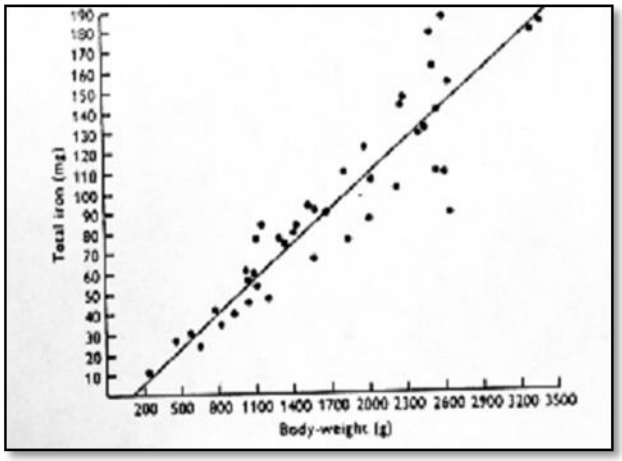


TABLE-19: COMPARATIVE ACCOUNT OF HEMOGLOBIN AND IRON STORES BASED ON SERUM FERRITIN IN VULNERABLE SEGMENTS OF THE POPULATION

Age group	Hb Mean±SD g/L	Ferritin Mean ±SD of 95% CI µg/L	Ref
Pregnant women 19 wk (n=73)	109 ±16.9	21.0 ±22.1	Nair *et al*, 2004
Non Anemia mothers (n=68)*	174 ± 16	132.8 ± 15.2	Raji *et al*, (2008)
Anemia mothers (n=61)*	166 ± 18	133.6 ± 9.0	
14 weeks NAM	125 ± 6	54.6 ± 9.7	
AM	116 ± 9	55.0 ± 8.5	
6 months NAM	115 ±5	17.8 ± 6.5	
AM	112 ±5	17.7 ± 6.4	
Infants 6-12 months n=108	118 (117, 120)	29·4 (25·7, 33·1)	Nair *et al* 2015
1-2 years n=401	97.5 (95.9,99.1)	10.97 (10.09,11.92)	Pasricha*et al* 2010
2 - 4 years n=103	121 (120, 122)	26·0 (21·9, 30·2)	Nair *et al* 2015
3 -5 years n=60	114.4 (111.5,117.3)	23.0 (19.7. 26.4)	Nair *et al*
Children 7-11 years, n=128	11.5 6 ±1.09	24.7 6± 15.30	Radhika *et al* 2011
Children 6-13 years, n=184	120 ± 12	17 ±17	Moretti *et al* 2006
Females 13-15 years(n=16)	129. 6 ±7.8	12.3 (9.3, 17.2)	Nair *et al* 2013

In cord blood, NAM-non-anemic mothers and AM-anemic mothers, Mean birth weight of infants of NAM 2.84 ± 0.25 kg and AM 2.82 ± 0.24 kg

EFFECT OF IRON SUPPLEMENTATION ON HEMOGLOBIN AMONG CHILDREN AGED 5–12 YEARS

The results of the literature survey are summarized in the Table -20. All these studies have been carried out in one location, Vadodra, Gujarat during 1982-2012 among 5-15 years old children and with the iron dose ranging from 20 mg -100 mg for a duration ranging from 60 days to 1 year.

The impact of daily vs once and twice weekly IFA tablet (100 mg Fe and 0.5 mg folic acid) and a placebo on Hb was tested among 9-13 y girls for 1 year (Sen and Kanani, 2012). The three groups that received iron showed significant rise in Hb from baseline and compared to placebo group. Though there was no difference in rise in Hb between daily vs once and twice weekly, twice weekly registered better Hb compared to once weekly. Whether these 3 dosage regimen has any superiority in building up iron store cannot be ascertained.

Daily dose of 60 mg iron was shown to increase Hb more than 2 g/dL in 60 days in girls 8-15 years. The impact of 60 mg iron for 60 days appears to be

better than the impact of 100 mg IFA for 1 year in a comparable age group from same area (Kashyap,1987; Sen and Kanani, 2012).

The two studies among 8-15 years boys with 30 and 40 mg iron for 60days also showed improvement in Hb to the same extent about 1.5 g/dL (Gopaldas and Kale, 1985a and b).

The two studies in the age group 5-8 years with 20 mg and 40 mg IFA showed greater impact in the higher age 6-7 (1.6 g/dL) and 7-8 (1.3 g/dL) category compared to 5-6 y (0.9 g/dL). Doubling the dose in this age group appears to double the response in Hb (2.2 vs 1.3 g/dL).The impact of iron in replenishing hemoglobin seems to be uniform in these age groups. An iron dose in the range of 20-60 for 60 days normalizes Hb, but its contribution to iron store is not known from the study. The iron replenishing process may possibly depend on the baseline Hb and the sequence of events leading to hemoglobin repletion from severe, moderate and mild anemia to normal status. Most likely storage iron repletion would take place only in normal and in mild anemia cases.

TABLE-20: EFFECT OF IRON SUPPLEMENTATION ON HEMOGLOBIN AMONG CHILDREN AGED5–12 YEARS –VADODRA, GUJARAT

Reference	Number /Site	Dose/Duration	Groups	Initial Hb (g /dL)	Final Hb (g/dL)
Sen and Kanani, 2012	60 per group (4 groups) (9-13y girls)	100 mg Fe+ 0.5 mg FA/1 y	Once weekly (E1) (n=65)	11.48 ± 1.06	12.09 ± 0.44
			Twice weekly (E2) (n=89)	11.09 ± 1.47	12.04 ± 0.41
			Daily(ED) (n=59)	11.26 ± 1.69	12.18 ± 0.55
			Control(CS) (n=41)	11.54 ± 0.67	11.54 ± 0.63
Kashyap and Gopaldas 1987	65 pairs (8-15 y girls)	60 mg Fe (FeSO4)/60 d	Treatment group	10.28±0.14	12.33±0.09, (p<0.001)
			Placebo group	10.39±0.14	10.68±0.11 (p<0.01)
Gopaldas *et al*, 1985(a)	70 per group (3 groups) (8-15 y boys)	30 mg and 40 mg iron/60 d	Placebo	10.72±0.15	10.81±0.13
			30 mg iron	10.90±0.16	12.07±0.12 s*
			40 mg iron	10.71±0.14	11.96±0.15 s*
Gopaldas, Kale, 1985(b)	16 per group (3 groups) (8-15 y boys)	30 mg and 40 mg iron/60 d	placebo	10.81±0.15	10.74±0.21
			30 mg iron	10.77±0.20	12.20±0.13
			40 mg iron	10.72±0.22	12.11±0.20
Seshadri *et al*, 1982	Exp 1: 94 (5-8y boys and girls)	Experimental (20mg Fe+0.1 mg FA) /60 d	5-6 y	10.4±0.16	11.3±1.8 ***
			6-7 y	10.2±0.23	11.8±1.3 **
			7-8 y	10.7±0.21	12.0±0.9 ***
		Control	5-6 y	9.9±0.40	9.7±0.47 NS
			6-7 y	10.8±0.32	10.7±0.33 NS
			7-8 y	11.0±0.29	10.9±0.30 NS
	Exp 2: 14 pairs in each group (5-6 y)	2x20mg Fe+0.1 mg FA)/60 d	Experimental	9.6±0.27	12.0±0.28, P<0.001
			Control	9.8±0.23	9.7±0.38, NS

**** P< 0.05** P<0.01, ***P<0.001, E1 versus CS: 5.07***, E2 versus CS: 6.89***,ED versus CS: 4.82***,E2 versus E1: 2.04*, ED versus E1: 1.45NS, E2 versus ED: 0.18NS, S diff from control and baseline***

APPROPRIATE DOSES OF IRON FOR TREATMENT OF ANEMIA AMONGST

a) Pregnant and Lactating Mothers
b) Under Five Children
c) Children in 6-10 years of Age-20 mg
d) Adolescent Girls
e) Women in Reproductive Age Group

A theoretical basis of computation of iron dose for the age/gender and physiological group is described below and in the Table-21.

The first step in the process is to quantify the amount of iron required to increase 10g/L hemoglobin (Hb) across all the age group. That is the iron requirement to support the synthesis of 10g/L Hb in 100 days has been calculated. This has been derived based on age specific reference body weight (ICMR 2010), corresponding blood volume, amount of iron present in Hb (3.47mg/gHb) and an incorporation efficiency of 90% of absorbed iron in red blood cells (RBC).

Additionally, 5-8% absorption is used for deriving iron RDA for Indians (Table7) and it is assumed that the rate of iron absorption from supplement reduces considerably; a stepdown approach of absorption has been followed. A conservative absorption of 3% has been considered.

CHALLENGES

Various principles have been followed to derive the dose of supplemental iron which includes i) level of iron deficiency, ii) maintaining circulatory Hb level, iii) improving circulatory Hb level, iii) improving iron

store or ferritin level. The determinants of iron absorption are many and characterizing them is a challenge. Solubility of iron at gastro-intestinal milieu, the diet matrix, the delicate pathway of iron transport across the enterocyte all needs to be in place. The role of hepcidin is being recognized and we need to address this in the Indian context. It is unlikely that the systemic regulation allow body to assimilate large amounts of iron from a single dose. Based on the biology of iron it appears that iron storage process is a very slow process and takes many years to substantially accumulate. This is possible only through contextual food synergies that improve iron bioavailability from a habitual diet.

A pre-pregnancy serum ferritin level more than 40 µg/L is required to balance the net iron loss in a single pregnancy which is about 400 mg of iron and would be sufficient to ensure their pregnancy needs without taking additional iron supplement.

Therefore the challenge is to establish strategies that enable women to acquire adequate stores before they enter into the pregnancy. Despite decades of national guidelines recommending universal iron supplementation among vulnerable segments, uncertainty about the benefits of iron supplementation still exists. Infections hamper the iron absorption and utilization. The role of hepcidin is emerging and requires in depth studies in the Indian context. Iron is also a potential catalyst of formation of hydroxyl radicals and other reactive oxygen species that can produce substantial damage to the gut (Paganini *et al*, 2017; Zimmermann, 2010; Srigiridhar and Nair, 1998).

TABLE-21: APPROPRIATE DOSES OF IRON FOR TREATMENT OF ANEMIA MG OF IRON REQUIRED TO IMPROVE 1 GRAM HEMOGLOBIN PER 100 ML IN 100 DAYS

S No	Age Group (y)	Body Wt (kg)	Blood Volume (mL)	Total Hb needed @ of 10g/L Hb (g)	mg Fe required to increase 10g/L Hb‡	Total mg Fe absorbed @ 90% incorporate in RBC	Considering 100days of supplementation mg Fe required per day	Fe dose (mg) @3% absorption	WHO Guideline Fe (mg)[1]	NIPI Fe (mg)[2]
1	0.6-2	9.1	†600.6	6.01	20.8	23.1	0.2	6.7	10-12.5	20
2	2-6	15.5	†1023	10.23	35.5	39.4	0.4	13.3	30	
3	7-9	25.1	†1656.6	16.57	57.5	63.9	0.6	20	30-60	45
4	10-12 B	34.3	†2263.8	22.64	78.6	87.3	0.9	30	30-60	
5	10-12 G	35.0	†2310	23.10	80.2	89.1	0.9	30	30-60	100
6	13-15 B	47.6	*3570	35.7	123.9	137.7	1.4	47		
7	13-15 G	46.6	†3075.6	30.8	106.9	118.8	1.2	40	30-60	100
8	16-17 B	55.4	*4155	41.6	144.2	160.2	1.6	53		
9	16-17 G	52.1	†3438.6	34.4	119.4	132.7	1.3	43	30-60	100
10	18-29 M	60.0	†3960	39.6	137.4	152.7	1.5	51		
11	18-29 F	55.0	†3630	36.3	125.9	139.9	1.4	47	30-60	100
12	Pregnant	55+12= 67	†4422	44.2	153.4	170.4	1.7	57	60	100

Blood volume in different age/gender and physiological groups *75mL/kg; †66mL/kg; ‡ 3.47mg/gHb
[1]WHO (2012), WHO(a) (2016) and WHO(b)(2016); [2]NIPI(2013).

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