Mogs for Women
Our speciality is you

# The Mumbai Obstetric & Gynecological Society

## MOGS MEDIA

**Vol.2**

## Anaemia and Nutrition in Pregnancy

**Dr Rishma Dhillon Pai**
President MOGS

**Dr Anahita Chauhan**
Secretary MOGS

**Dr Rajendra Sankpal**
Treasurer MOGS

**Dr Pratik Tambe**
Editor

**Dr Ganpat Sawant**
Co Editor

**Dr Priya Vora**
Co Editor

**Dr Vandana Bansal**
Co Editor

www.mogsonline.org

## President's Message

Dear Colleagues,

It gives me great pleasure to bring to you the second edition of 'MOGS MEDIA'. This is a series of focussed newsletters where we will be bringing to you an important subject discussed in detail with all the latest updates which will be relevant to you in your daily practice.

This issue is on the very common and extremely relevant problem of anaemia and nutrition in pregnancy and how we can best manage it. The editor Dr. Pratik Tambe and all the contributors have made a lot of effort to bring you concise and precise information and we are thankful to them.

The new MOGS team took over in May in the first ever digital installation in the history of MOGS. We followed that with the Evolve Conference where our Union Minister of State, Ministry of Health, Mr Ashwini Kumar Choubey was the Chief Guest. We have since had many digital CMEs on important subjects.

Now on 21st June we have the 'Focus on First Trimester' digital conference. On 28th June, 'Fresh Viewpoints in Infertility' an excellent focussed programme with an oration by the president of the World Endometriosis Society will be held online, all free for you to be enjoyed in the comfort of your home.

MOGS V Care & Share programme has been started by us to support our frontline workers and the women whose health we look after. PPE, N95 masks, face shields, fetal dopplers, thermal scanners etc have been donated by us to all major and many peripheral municipal and government hospitals. We need your help and support for this. You can donate by online payment on our website or by bank transfer.

We have many different academic and fun activities planned this year. Do visit our website for updates www.mogsonline.org.

Thank you once again for all your support over the years and we look forward to a wonderful year at MOGS.

*Stay Safe, Stay Healthy.*

***"They always say time changes things, but you actually have to change them yourself."***
***- Andy Warhol***

**Dr Rishma Dhillon Pai**
President MOGS.

**MOGS V Care & Share**

**MOGS extends a helping hand to our frontline healthcare workers and patients.**
**Support our efforts - contribute generously - if not now, when?**

| NEFT Details of MOGS | |
|---|---|
| Name as per Bank Account | The Mumbai Obstetric & Gynecological Society |
| Bank Account No | 24480100012858 |
| Bank Name | BANK OF BARODA |
| Bank Branch | JACOB CIRCLE BRANCH, Mumbai 400 011 |
| MICR Code | 400012092 |
| RTGS/NEFT/IFSC Code | BARB0JACOBC |
| Gstcertificate | 27AAATT4562C1ZL |
| Pan card | AAATT4562C |
| SAC CODE | 998599 |

Online payment gateway on MOGS website www.mogsonline.org

Mogs for Women
Our speciality is you

## Editors' Message

Dear MOGS Members,

The first MOGS Media newsletter on Preterm Birth was released last month and was widely appreciated. The MOGS Media newsletter is be themed on areas of practical interest with individual topics having relevance in day-to-day practice for practising obstetricians and gynaecologists. It is with great pride that we bring you the second issue of the MOGS Media newsletter.

This issue is centred around "Anaemia and Nutrition in Pregnancy" and highlights the current evidence on this subject. While this is something we all take for granted, it was felt that we need to re visit this area to brush up on the basics and look at the newer therapy areas. Hence, we have chosen articles which highlight practical management tips, current guidelines and emerging areas of patient benefit.

We thank the MOGS President Dr Rishma Dhillon Pai and the office bearers for giving us the opportunity to be part of such an innovative, important and immensely practical initiative.

We hope you enjoy reading the articles and find them useful. We would welcome any comments or suggestions regarding the same and encourage you to reach out to us with feedback.

Wishing you, your families and staff good health and safety in these difficult times!

Dr Pratik Tambe
Dr Ganpat Sawant
Dr Priya Vora Thakur
Dr Vandana Bansal
(Editors)

MOGS MEDIA
Vol.2
Anaemia and Nutrition in Pregnancy

Mogs for Women
Our speciality is you

# TABLE OF CONTENTS

# A PRIMER ON IRON METABOLISM

**Dr Bhumika Kotecha Mundhe MBBS DGO DNB MNAMS**

## Background

Iron is one of the essential trace elements. It has an important role in almost all living organisms, possibly dating back to the very origins of life.[1] It plays a vital role in several biological processes encompassing a full range of cellular activities, energy production, etc.

Despite its importance, it may be toxic because of its changing between +2 and +3 oxidative states. This reaction may elicit production of reactive oxygen species leading to cellular and tissue damage. During evolution,humans and other organisms have developed specialised proteins and tightly regulated homeostatic mechanisms for the uptake, transport, storage, and export of iron for essential biologic process and limit its toxicity. This review addresses some important physiological pathways involved in iron metabolism of human with its clinical relevance.

The term "Ironome"is the newer terminology used by the authors to describe iron metabolism and trafficking within cells and organelles.[2] For better understanding of this complex mechanism, we divide it into iron intake, absorption, circulation, storage and recycling.

## Iron intake

Adults have a total of 3-5 gm of iron. This corresponds to an average concentration of 50–60 mg of iron per kg of body weight

**Table 1 Distribution of iron in the body** [3]

| Site | % distribution |
|---|---|
| Hemoglobin in erythrocytes | 65% |
| Myoglobin, enzymes, | 10% |
| Macrophages of the reticuloendothelial system | 14% |
| Ferritin in hepatocytes | 5%-28% |
| Bone marrow | 4% |

Also, this iron store is available in the body in two forms–heme and non-heme. Non-heme iron is abundant in foods of both animal and plant origins and dominantly more from the plants. Most heme iron in the diet is from myoglobin and hemoglobin and is animal derived. Depending on their sequestration the absorption of these two forms are altered. Much of the non-haem iron is not tightly sequestered, and consequently its bioavailability can be affected by a range of dietary constituents and luminal factors.[4]

## Iron absorption [2,4,5]

The low pH of the stomach and proximal small intestine, small organic acids such as citric acid and ascorbic acid help to keep iron in a soluble form and increase its absorption. Other dietary components, notably plant derived phytates, tannins, and polyphenols, can bind non-hemeiron and impede its absorption. In contrast, heme iron is tightly sequestered within a protoporphyrin ring and is not accessible to the factors that influence non-heme iron.

Only one tenth of the consumed iron is absorbed, predominantly in the duodenum. The pathways for the two forms of iron absorption are given below:

**Heme iron** The mechanism responsible for heme uptake is not yet well understood, however it is known to occur by receptor-mediated endocytosis. The most probable receptor involved in this process are Heme-Carrier Protein 1 (HCP1) and Proton Coupled Folate Transporter (PCFT). Once in the enterocyte, heme is broken by Heme Oxygenase 1 (HO1) and iron is released in its ferric state as shown in Fig 1. This iron is now available in labile iron pool (i.e. follows same pathway with the iron obtained from non-haem iron)

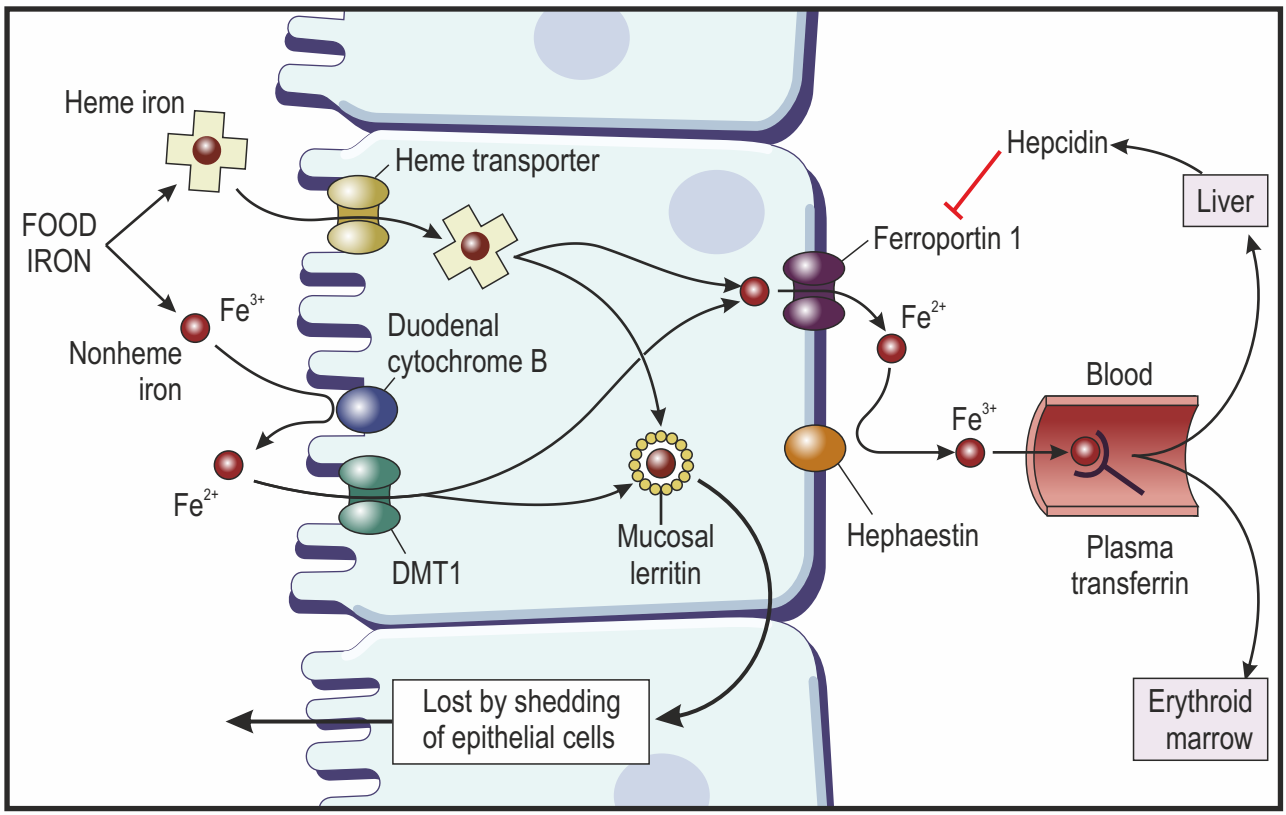



**Fig 1 Iron abosorption through the enterocyte**

**Non-heme iron**

Non-heme iron largely exists in the ferric (Fe3+) form. In order to be absorbed by the enterocyte, it is reduced to ferrous (Fe2+) by the low pH of the stomach together with ascorbic acid. At the apical membrane, facing the gut lumen, there are differentiated enterocytes which express on their surface various proteins required for entire process of dietary iron absorption.

Functions of these proteins are explained as follows:

Duodenal Cytochrome B (DcytB) and, most probably, Six Transmembrane Epithelial Antigen of The Prostate 2 (Steap2) proteins - facilitate iron reduction. Divalent Metal Transporter 1 (Dmt1), atransmembrane protein that performs the symport of Fe2+ coupled with H+ with the help of the proton gradient existing between the gut lumen and the enterocyte cytoplasm as shown in Fig 1.

Now, this iron which is taken up by enterocytes can be used directly for intrinsic cellular metabolic processes, stored as ferritin, or exported across the basolateral membrane for systemic delivery. The further use of this iron largely depends on body's need. The 'mucosal block' mediated by ferritin plays a crucial role on the effective absorption of iron. For instance, when ferritin levels are upregulated by the absence of the IRE(Iron Responsive Element) /IRP (Iron Regulatory protein) system, iron is mostly stored at the enterocyte instead of being delivered to the circulation.[5]

The export of iron from the enterocyte to the circulation is a crucial step for the entrance of iron in the body. The enterocytes express on their basolateral membrane the protein called Fpn1, the only known mammalian iron exporter. Fpn1 transports Fe2+ to the extracellular side of the basolateral membrane. As it enters circulation, it is oxidised by the ferroxidases named hephaestin (Heph) and ceruloplasmin (Cp) to Fe3+ in order to be associated with the circulatory transferrin (Tf).[2,5]

**Circulating Iron**[5]

Tf is found in the plasma in three states: apo-transferrin (apo-Tf), when no iron is bound; monoferric transferrin (bounded to a single iron atom); and diferric transferrin, also known as holo transferrin (holo-Tf; bounded to two iron atoms).The cellular uptake of Transferrin Bound Iron(TBI) is mainly mediated by the Transferrin Receptor 1 (TfR1), located at the cell membrane.

Diferric transferrin (holotransferrin) binds to transferrin receptor 1 at the cell surface. The complex is endocytosed via clathrin coated pits in the cytoplasm of the cell. The endosome pH of this cell decreases by the entry of H+ mediated by an ATP-dependent proton pump and Fe3+ is released. In the

endosome, free iron is reduced by six transmembrane epithelial antigen of the prostate3 (Steap 3) and transported to the cytoplasm by Divalent Metal Transporter 1 (Dmt1). Meanwhile, the Tf–TfR1 complex is driven to the cell surface where apo-Tf is released to the plasma as shown in Fig 2. Intracellular iron is used by ribonucleotide reductase for DNA synthesis, incorporated into heme or stored in ferritin.

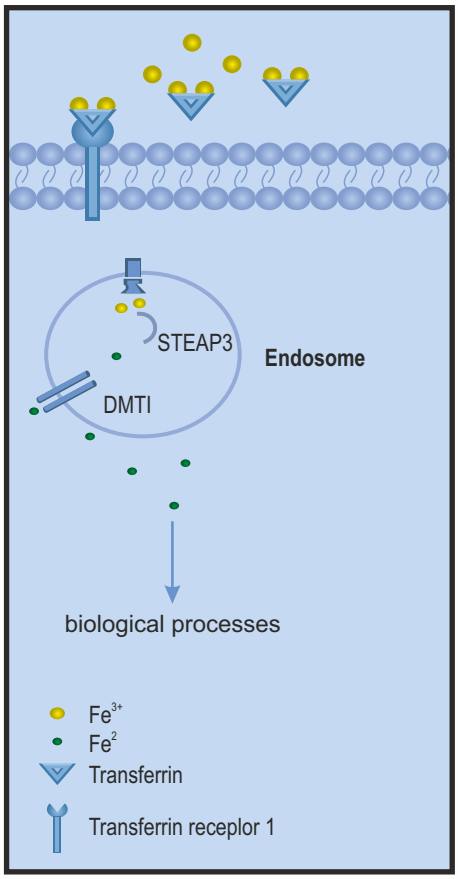



**Fig 2 Transferrin cycle and transferrin receptor 1 mediated cellular iron uptake**

However, Non-Transferrin Bound Iron (NTBI) species are also frequently found in the plasma. It has been suggested that the main form of NTBI is Fe3+ bound to citrate and recently acetate have also been detected. The origin and the mechanisms involved in the cellular uptake of plasma NTBI remain unclear. Erythrocyte precursors restrictively take up iron by using Tfr, notably Tfr1, whereas hepatocytes and other non-erythroid cells are also able to use NTBI.

## Hepcidin–Queen of Ironomics [2,3,5]

No review on iron metabolism can be complete without a mention of hepcidin. The pioneering work on hepcidin was done by Tomas Ganz and Elisabeta Nemeth. These two investigators, in collaboration with numerous other scientists, published about 100 scientific papers between 2003 and 2013. The liver is the major site of synthesis of hepcidin, but it also occurs on a smaller scale in other tissues, such

as alveolar macrophages, pancreatic cells and the kidneys. Its expression is regulated by several physiological conditions, such as systemic iron levels, hypoxia, anaemia, erythropoiesis, infection and inflammation. This 25peptide hormone is a master regulator of iron homeostasis. Hepcidin is encoded by the HAMP gene which codes for the precursor protein pro-hepcidin which then is cleaved into the activehepcidin.

## How is hepcidin expression initiated? [2]

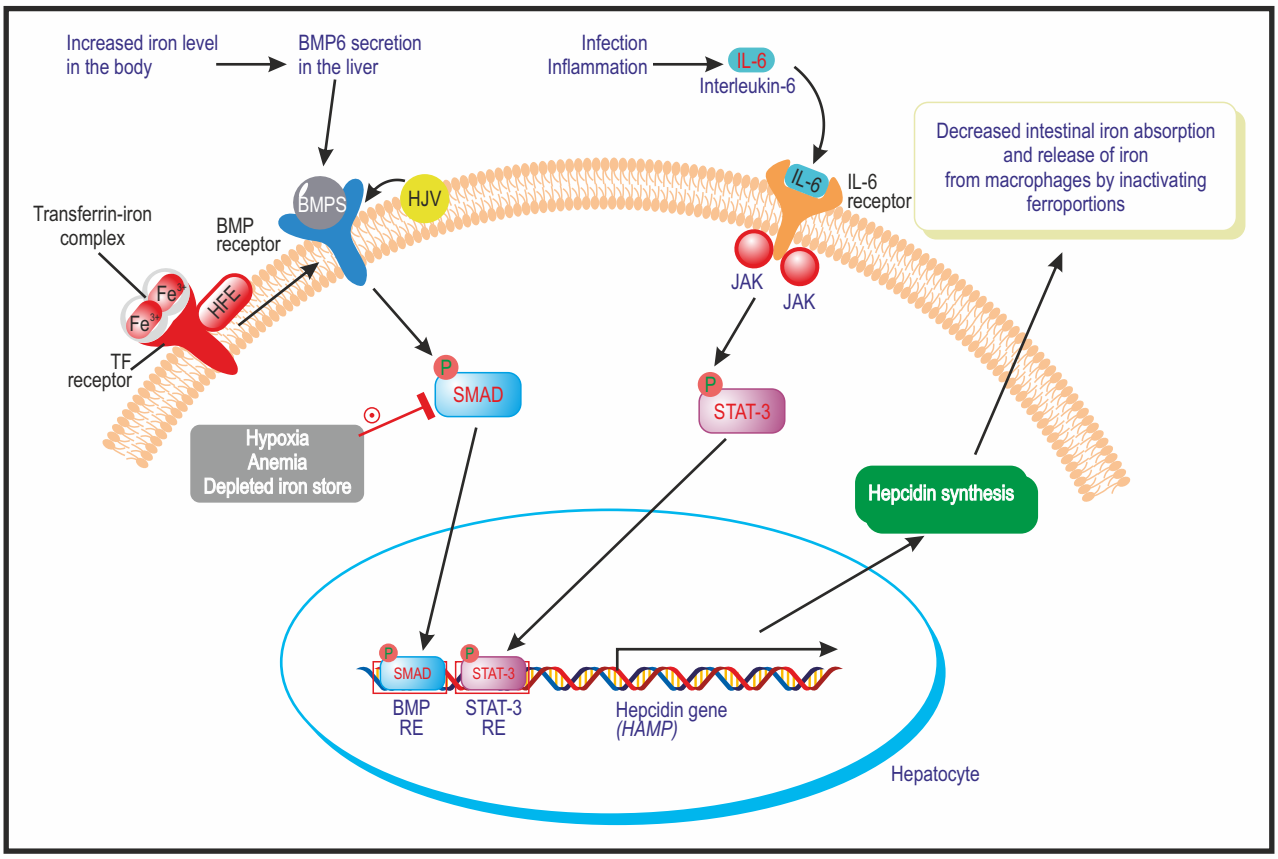



**Fig 3 Hepcidin expression in the body**

Hepcidin expression is basically initiated by two major mechanisms

1. IL-6-primary pathway for hepcidin regulation in inflammation which in turn triggers JAK-STAT3 pathway as shown in the Fig 3.

2. Depending on the iron saturation of Tf, iron-loaded Tf binds to Tfr1 which in turn binds to Tfr2 aided by the protein HFE. If iron-Tf is high, the Tfr2-mediated signalling by theBMP6 receptor complex is increased. After activation of the BMP receptor, the SMAD pathway is activated leading to over-expression of hepcidin as shown in Fig 3. Further more, at least 3 other proteins play roles by interacting between BMPs and the BMP receptor:

1. Hemojuvelin (HJV) a glycosylphosphatidy linositol-linked membrane protein,

2. Mt2, which regulates the levels of membrane-bound HJV, and

3.Neogenin, a ubiquitously expressed trans membrane protein with multiple functions

**How does hepcidin act at cellular level to alter the bioavailabity of iron?**[2]

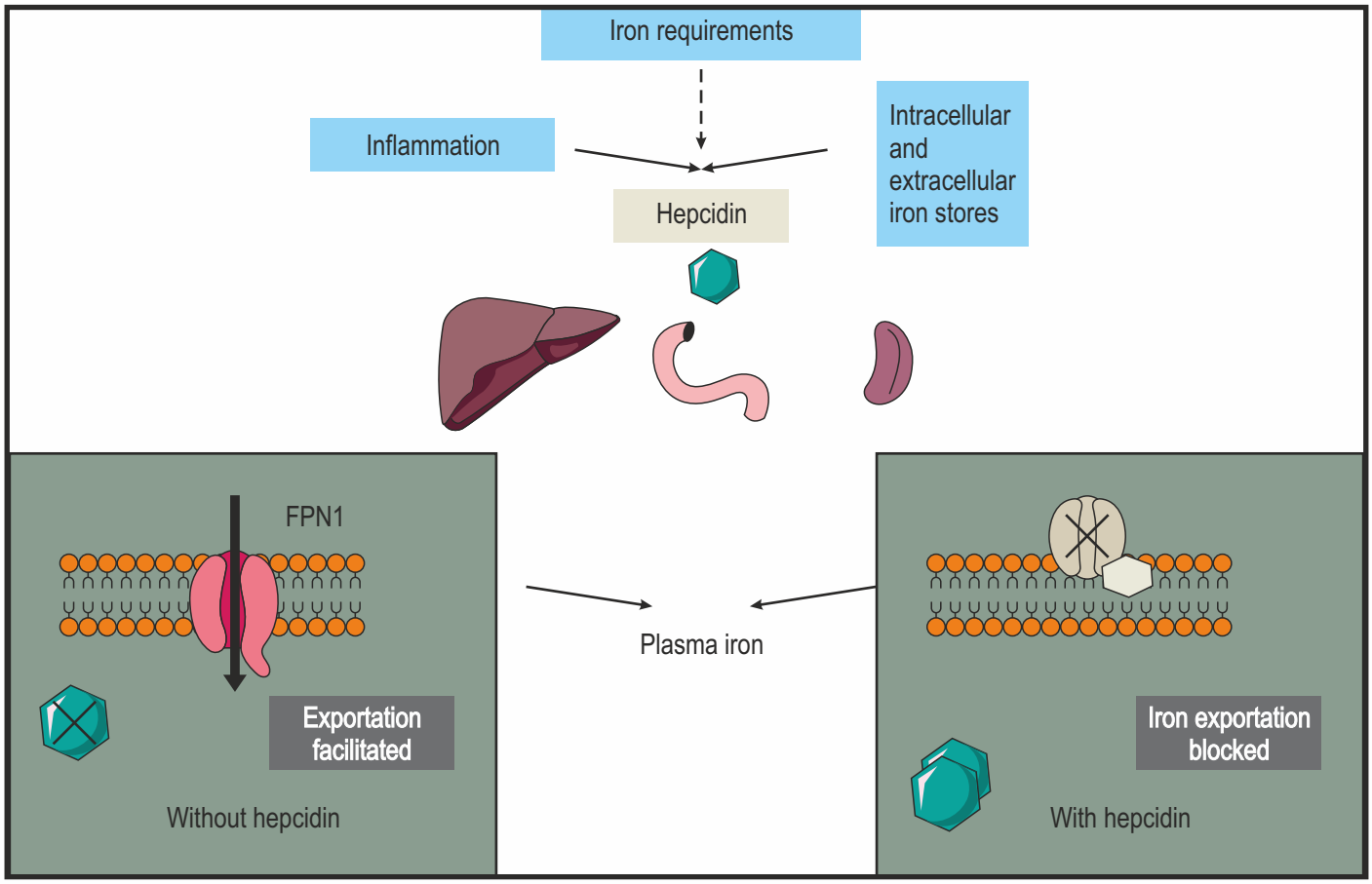



Fig 4 Hepcidin mediated regulation

**How does hepcidin act at cellular level to alter the bioavailabity of iron?**[2]

Once expressed, hepcidin is distributed via the circulation to its target sites where it binds to the iron export protein FPN1. Thus, FPN1 acts as the hepcidin receptor. This complex is ubiquitinated, internalized, and degraded with the consequence that the capacity of iron to be released into the circulation is impeded. Thus, when the body is iron replete, hepcidin concentrations are high and the iron supply to the plasma is reduced; however, when iron demands are high, hepcidin concentrations are reduced and more iron enters the circulation.

**Storage of Iron**[4]

The majority of iron in the body is used by erythroblasts for haemoglobin synthesis. The second large component of iron is stored in the body as ferritin. Iron storage is a critical component of cellular iron homeostasis, which enables iron to be sequestered in a nontoxic form but also provides a reservoir from which iron can be used for future metabolic needs. Ferritin is the major intracellular iron-storage protein.When high concentrations of iron-laden ferritin accumulate within the cell, the ferritin molecules aggregate.

These aggregates ultimately fuse with lysosomes. This process leads to the degradation of ferritin, and the resulting mixture of Fe3+ cores and peptides is known as hemosiderin.Iron can be efficiently mobilised from both ferritin and hemosiderin when it is required elsewhere in the body.

## Recycling [5]

One of the main functions of splenic and hepatic macrophages is to scavenge the senescent erythrocytes in order to release iron from the haemoglobin, rendering it available for another haemoglobin cycle.Recycling macrophages contribute to the major pool of plasma iron, exceeding the contribution of dietary iron absorption.

## Conclusions

Iron is one the essential elements, playing a vital role in various physiological processes. Taking into consideration its detrimental effects due to its reductive oxidative states, Ironomics plays a very important role. For almost a decade, hepcidin has been studied abundantly and is thought to be an important regulator of iron homeostasis. Although much research has been done on hepcidin for many years, we cannot neglect the vital importance of other mechanisms involved in iron metabolism regulation. In future, those mechanisms might be considered for the design of new effective therapeutic strategies able to greatly reduce iron-related disorders.

## References

1. Som Dev and Jodie L. Babitt. Overview of iron metabolism in health and disease. Hemodial Int. 2017 June; 21(Suppl 1): S6–S20. doi:10.1111/hdi.12542.

2.Sophie Waldvogel-Abramowski, Gérard Waeber, Christoph Gassner, et al. Physiology of iron metabolism. Transfus Med Hemother2014;41:213–221doi: 10.1159/000362888

3.AndronicosYiannikouridesand Gladys O. Latunde-Dada. A short review of iron metabolism and pathophysiology of iron disorders. Medicines 2019, 6, 85; doi:10.3390/medicines6030085.

4.Gregory J Andersonand David M Frazer. Current understanding of iron homeostasis. Am J Clin Nutr 2017;106(Suppl):1559S–66S.

5. Bruno Silva, Paula Faustino. An overview of molecular basis of iron metabolism regulation and the associated pathologies. Biochimica etBiophysica Acta 1852 (2015) 1347–1359.

# BIOCHEMICAL INVESTIGATION PATHWAYS

**Dr Siddesh Iyer MRCOG DGO DNB**

## Introduction

Iron deficiency is globally the most common nutritional deficiencyand is also the most common cause of anaemia.[1] Haemoglobin (Hb) levels may remain normal for a while after iron deposits are diminished, i.e., iron deficiency may be observed without anaemia and only the plasma ferritin level and the plasma transferrin saturation are reduced in this period. Once iron deposits are depleted, the haemoglobin level begins to drop. This means that this condition of diminished iron deposits in the body is referred to as iron deficiency (ID) and a continuation of this condition with the consequent development of anaemia is referred to as iron deficiency anemia (IDA).

## Biochemical Investigations

The time honoured tests for Iron deficiency anaemia are as follows:[2]

| Name | Rationale | Normal values |
|---|---|---|
| Haemoglobin | Measures oxygen carrying capacity of blood | 12-16 gm/dL |
| RBC | The RBC count and indices help in typing the anaemia | 4.5- 6.5 million |
| MCV | The average volume of red cells in a specimen. MCV is elevated or decreased in accordance with average red cell size; ie, low MCV indicates microcytic (small average RBC size), norm al MCV indicates normocytic (normal average RBC size) and high MCV indicates macrocytic (large average RBC size). | 80-95 fL |
| MCH | The average mass of hemoglobin (Hb) per red blood cell (RBC) in a sample of blood. MCH value is diminished in hypochromic anemias. | 27 - 31 picograms/cell |
| MCHC | It is a measure of the concentration of haemoglobin in a given volume of packed red blood cell. It is calculated by dividing the haemoglobin by the haematocrit. | 32-26 gm/dL |
| RDW | A measure of the range of variation of red blood cell (RBC) volume. Usually red blood cells are a standard size of about 6–8 µm in diameter. Certain disorderscause a significant variation in ell size. Higher RDW values indicate greater variation in size. | 11.5- 14.5% |
| Serum iron | Evaluating disorders of iron metabolism, low levels in IDA | 60–170 µg/dL (10–30µmol/L) |

| | | |
|---|---|---|
| TIBC | A test that measures the blood's capacity to bind iron with transferrin. It measures the maximum amount of iron that the blood can carry. Total iron - binding capacity: TIBC is less expensive than a direct measurement of transferrin. Although iron bound to transferrin is less than 0.1% (4 mg) of total body iron, it forms the most vi tal iron pool with the highest rate of turnover (25 mg/24 h). TIBC rises in IDA | 240–450 µg/dL |
| Serum transferrin | Iron transport protein; half life 8 -10 days, levels increase in iron deficien y | 170 – 370mg/dL |
| Transferrin saturation (TFS) | It is the value of serum iron divided by the total iron-binding capacity[ of the available transferrin. Tfs (%) = Fe (mg / L) x 70.9 / Tf (g/L) (11), where Fe is the iron concentration. Levels <20 % in IDA | Average 25% |
| Serum ferritin | A universal intracellular protein that stores iron and releases it in a controlled fashion. Plasma ferritin is used as a diagnostic test for iron-deficien y anemia. Levels <30 in IDA | 30–300 ng/mL (=µg/L) for males |

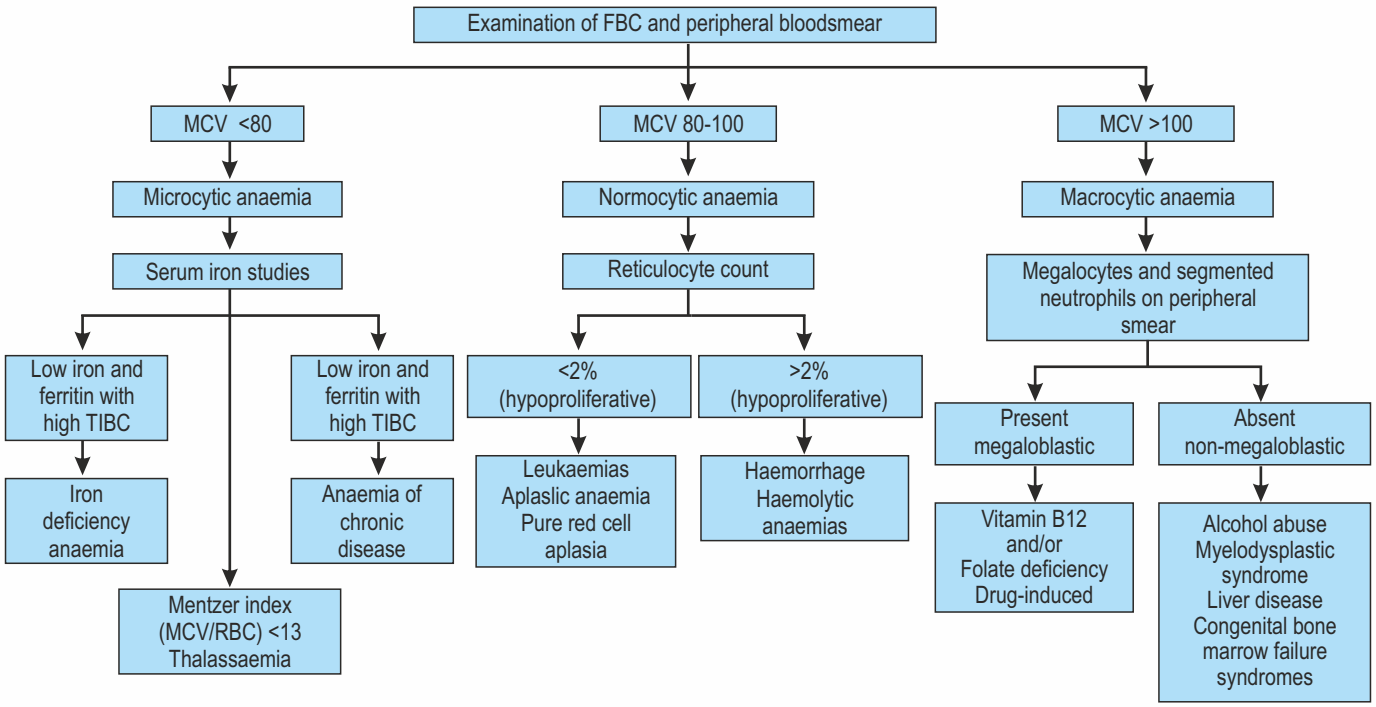
A simple peripheral blood smear and a detailed CBC on the coulter may give us RBC indices which have traditionally been the age old methods of diagnosing the type of anaemia. Serum ferritin concentration, serum iron level (Fe), total iron binding capacity (TIBC) and transferrin saturation (TSAT), are the most common biochemical tests, but these tests may be influenced by certain conditions. Serum iron falls in IDA, as well as in chronic disease anemia and also fluctuates during the day depending on the iron intake. Since TSAT is calculated based on Fe and TIBC, changes in these values are also reflected on TSAT. The serum ferritin level shows the iron deposited in the body and very low values indicate iron deficiency. However, serum ferritin is also an acute phase protein and may appear to be normal or high in cases such as infectious, inflammatory conditions and malignancy.

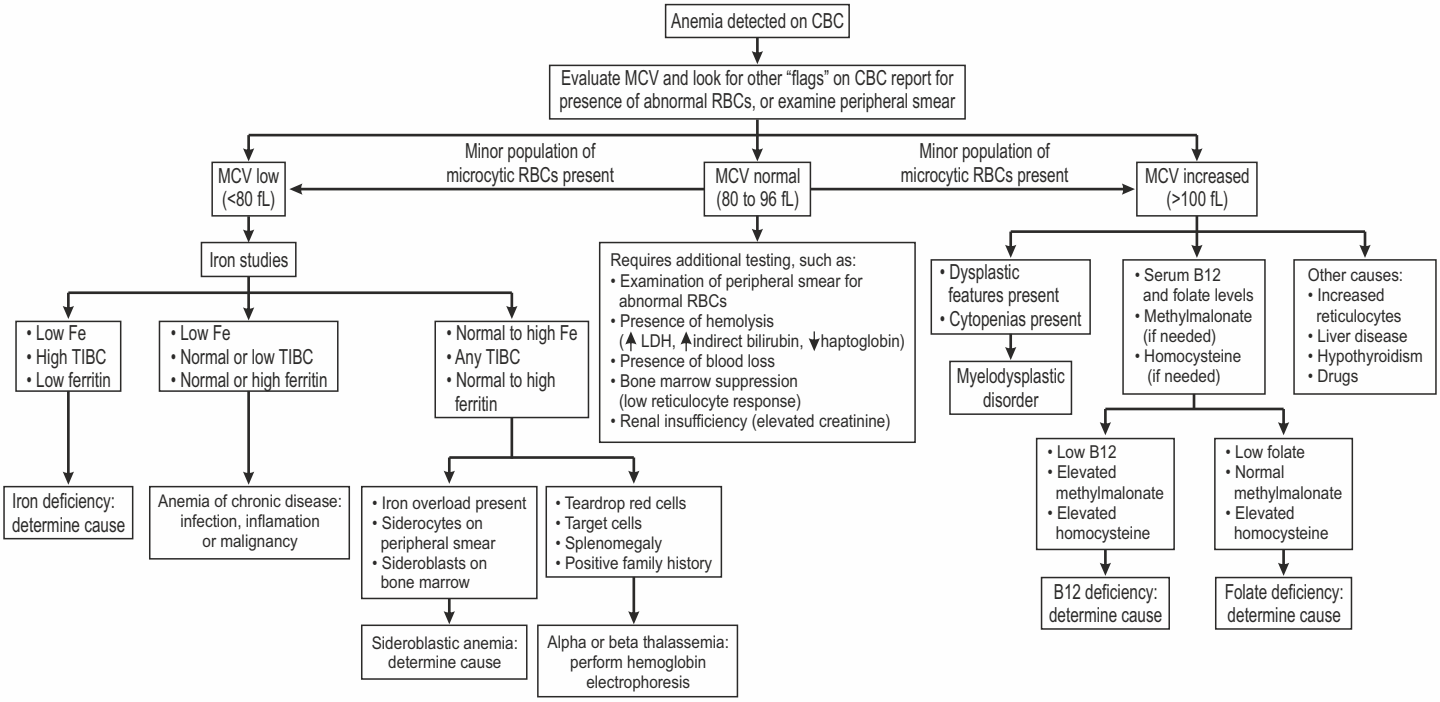
**Newer Tests**[3, 4]

| Name | Rationale |
|---|---|
| sTfR (soluble transferrin receptor) | Introduced as a promising new diagnostic tool for differentiating between iron deficiency anemia (IDA) and anemia of chronic disease (ACD). Levels increase in IDA and are normal in ACD. |
| (sTfR-F index) | Serum ferritin reflects the storage iron compartment and sTfR reflects the functional iron compartment, the sTfR/log ferritin index based on these two values, has been suggested as a good estimate of body iron compared with the sTfR/ferritin ratio |

| | |
|---|---|
| Hepcidin | Iron regulates hepcidin homeostasis. Increases in iron levels in the plasma and iron storage stimulate the production of hepcidin, which blocks iron absorption from the diet and its further storage. Hepcidin production is suppressed in the case of iron deficiency. Even in the absence of an aemia, hepcidin is a sensitive indicator of iron deficiency. Moreover, compared to hematocrit or hemoglobin, a decrease in hepcidin is an early marker of iron deficiency together with transferrin saturation and decreased ferritin. |
| Red blood cell Size Factor (RSf) | RSf joins the volume of mature red cells (MCV) and the volume of reticulocytes (MRV), both related to erythropoietic activity and hemoglobinisation |
| Low hemoglobin density (LHD%) | LHD % derives from the traditional mean cell hemoglobin concentration . MCHC is an all -inclusive measure of both the availability of iron over the preceding 90 –120 days and of the proper introduction of iron into intracellular hemoglobin.In the same way, LHD% is related to iron availability and the hemoglobinization of the mature red cells |

## Diagnostic algorithms[5]






Investigative approaches

1. Microcytic (MCV <80 fL) – check serum Fe, TIBC, ferritin

- iron deficiency – Low Fe and ferritin, high TIBC
- chronic disease / inflammation / malignancy – low Fe, normal TIBC, normal or high ferritin
- sideroblastic anemia – high iron, high ferritin, any TIBC; blood smear with siderocytes, BMA with sideroblasts
- alpha or beta thalassemia – high iron, high ferritin, any TIBC; blood smear with teardrop cells, target cells; abdominal ultrasound(splenomegaly)

2. Normocytic (MCV 80-96 fL)

- check blood smear
- check for haemolysis (high LDH, high IB, low haptoglobin)
- check blood loss
- check retic count (bone marrow suppression)
- check creatinine (CKD)

3. Macrocytic (>100 fL)

- B12 deficiency – low B12, high methylmalonate (MM), high homocysteine
- Folate deficiency – low folate, normal MM, high homocysteine
- Myeloplastic disorder – dysplastic features, cytopenias
- Others: reticulocytosis, liver disease, hypothyroidism, drugs

## Reticulocyte evaluation tests [6]

A detailed reticulocyte evaluation is the new kid on the block in diagnosing and planning iron therapy in IDA. Reticulocytes are immature erythrocytes which lack a nucleus and contain ribosomal RNA residues. Reticulocytes are generated in the bone marrow and released into the peripheral circulation after three days of maturation, becoming fully-matured erythrocytes the following day. They account for approximately 1% (0.5–2.5%) of erythrocytes in circulation. Measuring the reticulocyte count shows the erythropoiesis level in bone marrow and the response of bone marrow to anaemia. In case of iron deficiency, haemoglobin synthesis is firstly reduced in reticulocytes.

| Name | Rationale | Normal values |
|---|---|---|
| Percentage of reticulocyte count (% retic) | Measures erythropoiesis level in bone marrow and the response of bone marrow to anemia | 0.5 - 1.5 % |
| Ret-He(reticulocyte haemoglobin equivalent) | The RET -He level allows for the diagnosing of anaemia before it develops in a patient. RET-He is the first paameter to evaluate functional ID and show that the patient is benefiting fom the treatment. It can be identified long beore the increase in Hb and classical reticulocyte count and is useful for treatment follow-up. | 34 pg (Ret-He value of 28.5 predicts ID with >90% sensitivity) |
| CHr-Hb content per reticulocyte | (CHr = MCVr × CHCMr) Measurement of CHr provides an indirect measure of the functional iron available for new RBC production. | 27.5 for IDA <= 24.8 Thal Minor |
| Absolute reticulocyte count | (retic = red blood cells × % retic) | 50-100 x 10^9/ L |
| Immature Reticulocyte Fraction (IRF) | The rate of production of reticulocytes which is largely dependent on the bone marrow response to erythropoietin. Increases after a few hours as against 2 to 3 days taken by Ret He | 0.11-0.38% |

## Role of these tests

The clinical utility of reticulocyte parameters lies in differential diagnosis of iron deficiency anemia (IDA), vitamin B12 deficiency and beta-thalassemia minor and response to therapy. RET-He is helpful because it provides results in a very short period, does not incur additional costs, can be measured simultaneously in automatic blood count devices, and is not affected by other chronic diseases. RET-He has been shown to be useful in the diagnosis and treatment of IDA in infants, patients with chronic renal failure and pregnant women. Therefore, RET-He is an earlier measure of diminished hemoglobin production compared to hemoglobin and hematocrit.

## Diagnosis and assessment of ID/IDA[7]

### What's in?

A comprehensive reticulocyte evaluation: Three parameters can now be reported with every reticulocyte order. They can provide the comprehensive information needed by physicians to assess the rate of red cell production and hemoglobinisation. Reticulocyte count indicates the quantity of circulating reticulocytes. The Immature Reticulocyte Fraction (IRF) indicates the rate of production of reticulocytes and RET-He indicates cell hemoglobinisation, the reflecting quality of the newly produced reticulocytes. These three parameters enable "dissociation" of iron-dependent hemoglobinisation from erythropoiesis and the tests are rapid and inexpensive to perform

### What's out?

Age old hemoglobin and hematocrit as sole indicators of ID are out. They can provide a valuable snapshot, but they are relatively static parameters. The changes in patient therapy result in a significant change in haemoglobin after 2 – 3 weeks whereas Ret-He indicates a response after only 2 days.The biochemical tests for changes of iron status as sole indicators are out. The low biological variation demonstrated by RET-He makes it suitable for trend analysis. RET-He can give physicians information on a patient's response to therapy in days, not weeks.

## Conclusions

In conclusion, reticulocyte hemoglobin content (CHr) is an extremely valuable recent addition to an expanding list of biomarkers that can be used to differentiate iron deficiency from other causes of anaemia. In olden days, stainable marrow iron was used as a gold standard for diagnosing iron deficiency. After that, soluble transferrin receptor (sTfR)-Ferritin index was used for this purpose. Today, CHr can be called as the gold standard replacing both of these. It is a pity that despite its simplicity and utility, it is rarely used in clinical practice.

## References


1. Finc C. Regulation of iron balance in humans. Blood1994;84:1697-1702.
2. Gottfried EL. Erythrocyte indexes with the electronic counter. N Engl J Med. 1979;300:1277.
3. Ilenia Infusino, Federica Braga, Alberto Dolci, MD, Mauro Panteghini, MD Soluble Transferrin Receptor (sTfR) and sTfR/log Ferritin Index for the Diagnosis of Iron-Deficiency Anemia A Meta-Analysis. American Journal of Clinical Pathology Volume 138, Issue 5, November 2012, Pages 642–649. https://doi.org/10.1309/AJCP16NTXZLZFAIB
4. Brugnara C. Reticulocyte cellular indices: a new approach in the diagnosis of anemias and monitoring of erythropoietic function. Crit Rev Clin Lab Sci2000;37:93-130.
5. Matthew Short, Jason Domagalski. Iron Deficiency Anemia: Evaluation and Management. Am Fam Physician. 2013;87(2):98-104.
6. Urrechaga E. The new mature red cell parameter, low haemoglobin density of the Beckman-Coulter LH750: clinical utility in the diagnosis of iron deficiency. International Journal of Laboratory Hematology. 2010;32(1, part 1):e144–e150..
7. https://www.sysmex.com/us/en/Brochures/RET-He_A_Parameter_with_Well_Established_ Clinical_Value.pdf

# MATERNAL COMPLICATIONS

**Dr Ganpat Sawant MD DNB FCPS DGO DFP DICOG**

## Introduction

Anaemia is considered a severe public health problem if more than 40% of the population is diagnosed with anaemia. By that measure, anaemia in women and children has been a major problem in India for half a century.

Defining anaemia in pregnancy is not straight-forward given the physiologic plasma expansion, the ethnic variations of Hb values and the frequent use of iron supplementation in pregnancy. World Health Organisation (WHO) defines anaemia in pregnancy as Hb values less than 11gm/dL.[1,2] Anaemia in postpartum women is defined as Hb less than 10 g/dL by WHO.

**Table 1 WHO classification of severity of anaemia in adult women[3]**

| | Normal (Hb g/dL) | Anaemia (Hb g/dL) | | |
|---|---|---|---|---|
| | | Mild | Moderate | Severe |
| Non pregnant women(age>15 years) | >= 12 | 11–11.9 | 8–10.9 | <8 |
| Pregnant women | >= 11 | 10–10.9 | 7–9.9 | < 7 |

The prevalence of iron deficiency (ID) in pregnant Indian women is amongst the highest in the world. About 80% of non-physiologic anaemia during pregnancy occurs due to iron deficiency.[4]

## Consequences of IDA in pregnancy

It is estimated that maternal anaemia contributes to 18% of perinatal mortality and 20% of maternal mortality in South Asian countries including India as per a recent meta-analysis.[5] The exact level of maternal haemoglobin that is critical with respect to maternal mortality is not known. According to Child Health Epidemiology Reference Group (CHERG), the risk of maternal mortality significantly decreases for every 1 g/dL rise in Hb; however, the association becomes less clear at Hb levels above 8–9 g/dL.[6]

**Table 2 Complications following IDA in pregnancy**

| Antepartum | Intrapartum | Postpartum |
|---|---|---|
| Increased risk of preterm delivery | Prolonged labour | Postpartum depression |
| Premature rupture of membranes | Increased rates of operative delivery | Puerperal sepsis |

| | | |
|---|---|---|
| Preecclampsia | Postpartum haemorrhage, maternal mortality | Lactation failure |
| Intrauterine death | Abruption | Pulmonary thromboembolism |
| Intercurrent infection | | Subinvolution of uterus |
| Antepartum haemorrhage | | |
| Congestive heart failure | | |

**Antepartum complications**

**Increased risk of preterm delivery**

The role of maternal anaemia in preterm birth remains poorly defined, and the association between anaemia and preterm birth clinical subtypes remain unclear. These clinical subtypes include preterm premature rupture of membranes (PPROM), spontaneous preterm labour and medically indicated preterm birth. Preterm birth may occur through multiple pathways, with maternal infection, hypoxia and oxidative stress being the three major postulated biological mechanisms.

Iron deficiency may increase the risk of maternal infectionsand low haemoglobin may cause a state of low-grade chronic hypoxia that induces maternal and foetal stress. An activated immune system in the presence of infections and inflammation and corticotrophin-releasing hormone or cortisol that are released following stress responses, can activate the maternal or fetal hypothalamic–pituitary–adrenal axis. This, in turn, can initiate labour and eventually result in preterm parturition.[7]

Iron deficiency may also increase oxidative stress resulting in damage to erythrocytes and the feto-placental unit.[8] Maternal anaemia in early pregnancy is associated with increased risk of preterm PROM and anaemia in late pregnancy is associated with reduced risk of spontaneous preterm labour. Adequate physiological haemo-dilution during mid-to late pregnancy may be associated with reduced risk for preterm birth.[9]

**Premature rupture of membranes**

Preterm PROM is associated with low maternal haemoglobin and low socioeconomic status. A lower haemoglobin level may be a marker for subclinical infection.[10]

### Preeclampsia

Women with severe anaemia have a 3.6 times higher risk of preeclampsia than women with no anaemia. The susceptibility of women with severe anaemia to preeclampsia could be explained by a deficiency of micronutrients and antioxidants. Recent results indicate that reduction in serum levels of calcium, magnesium and zinc during pregnancy might be possible contributors to the development of preeclampsia.[11]

### Intrauterine death

Recent studies have shown that mothers with nutritional or iron deficiency anaemias tend to deliver prematurely with low birth weight babies and a high mortality rate or stillbirths, as compared to non-anaemic mothers. Some studies also show an increased risk of infant death immediately before or after birth.

### Intercurrent infection and antepartum infection

Anaemia during pregnancy increases the risk of infection by reducing the resistance of both the mother and the baby. Sub microscopic infections with P. falciparum during pregnancy reveals their associations with poor pregnancy outcomes. All of these elements combined lead to novel findings concerning the impact of sub microscopic infections with P. falciparum on maternal (anaemia) and foetal (premature birth, birth weight) health-related parameters.[12] Primigravidae are considered to be most susceptible to placental malaria and its consequences.

### Congestive heart failure

Anaemia itself can worsen cardiac function, both because it causes cardiac stress through tachycardia and increased stroke volume and because it can cause a reduced renal blood flow and fluid retention, adding further stress to the heart.

### Intrapartum complications

These include prolonged labour, increased rates of operative delivery, postpartum haemorrhage and maternal mortality. In multivariate analysis, women with moderate-to-severe anaemia at enrolment had a significantly greater total blood loss (91 mL average) compared to non-anaemic women ($p<0.01$). Greater blood loss was associated with an increased duration of the first stage of labour ($p=0.01$) and greater placental weight ($p=0.03$).

Several biological mechanisms are thought to play a role in postpartum haemorrhage. Higher blood loss may be attributed to impaired uterine muscle strength for labour when it is prolonged or decreased resistance to infection, as infection is suggested to contribute to uterine dysfunction or inertia.[13] Decreased uterine blood flow or low uterine muscle strength may contribute to inefficient uterine contractions and contribute to blood loss, potentially mediated by low body iron stores (serum ferritin <100 mcg/L). Severe anaemia may impair tolerance of postpartum haemorrhage and contribute to maternal death due to the failure of women to endure such excessive blood loss.[14]

**Abruption**

A higher frequency of abruptio placentae among anaemic patients has been documented in some, but not all previously published studies. Altered feto-placental angiogenesis during early pregnancy in anaemic women may partially explain this increased risk. Eleven percent of abruptio placentae cases and 3.3% of controls were diagnosed with iron deficiency anaemia. Maternal iron deficiency anaemia in early pregnancy was associated with a 3.60 - fold increased risk of abruptio placentae (95% CI 2.01-6.04).[15]

**Postpartum complications**

**Postpartum depression**

Anaemia during and after pregnancy significantly increases the risk of postpartum depression. Haemoglobin decline may change the function of neurotransmitters and subsequently alter the cellular, oxidative and thyroid hormone metabolism. The reduction of inflammatory cytokines such as interleukin 2 as causative agents for anaemia, can be an influencing factor.[16] In another study, bleeding more than 1000 ml after childbirth increased the incidence of anaemia and the risk of depression by 2.1 times.[17]

In other studies, fatigue has been mentioned as one of the causes of depression; fatigue indicates a decrease in body energy levels and the level of activity decreases to reduce energy consumption and to achieve balance. Increasing metabolic needs can explain the fatigue associated with pregnancy and postpartum period and the higher the fatigue of the mother, the greater the likelihood of depression.[18]

**Puerperal sepsis**

The most vulnerable time for maternal death is the postpartum period during which 60% deaths occur. Unfortunately, postpartum period is the most neglected period and sepsis is probably the most preventable of all postpartum complications. Vigilant attention to hygiene during delivery is of the utmost importance in preventing infection in anaemic patients.

### Lactation failure

Insufficient milk is a poorly understood problem that is often identified as a major reason for early discontinuation of breastfeeding. Anaemic mothers a higher incidence of insufficient milk production. There may be shorter period of exclusive breastfeeding and weaning at an earlier age. They identified not having enough milk, baby nursing too often, and baby not gaining enough weight as the main reasons for discontinuing breastfeeding.[19]

### Pulmonary thromboembolism

In retrospective study, Can et al. found that patients with PE had significantly lower mean haemoglobin levels than gender and age-matched controls. They proposed that this was due to decreased secretion of anti-thrombotic mediators, initiated by low blood viscosity in anaemic states, resulting in increased clotting. Other studies do not corroborate these findings.[20]

### Subinvolution of uterus

Uterine subinvolution is a slowing of the process of involution or shrinking of the uterus. Anaemia, endometritis and pelvic infection may cause uterine sub in volution. This is in contrast to uterine atony in which haemorrhage occurs immediately after delivery and is much more severe. Subinvolution is the most common cause of "delayed" postpartum haemorrhage.

### Conclusions

Anaemia in pregnancy is one of the most common public health issues in developing countries, affecting approximately 60% of pregnant women worldwide. Severity of antenatal anaemia has been robustly linked with complications in maternal and perinatal outcome. Prevention is not insurmountable; hence active intervention by all levels of health care providers is imperative in order to decrease poor maternal and perinatal outcome.

### References

1. CDC criteria for anemia in children and childbearing-aged women. MMWR Morbidity and mortality weekly report. CDC (1989)138(22):400–404.

2. WHO (2001) Iron deficiency anemia: assessment, prevention and control. WHO/NHD/01.3, Geneva. World Health Organization, Switzerland.

3. WHO (2011) VMNIS. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System, WHO, Geneva, World Health Organisation, Switzerland

4. Massot C, Vanderpas J. A survey of iron deficiency anaemia during pregnancy in Belgium: analysis of routine hospital laboratory data in Mons. Acta Clin Belg. 2003;58:169–77.

5. Rahman MM, Abe SK, Rahman MS, et al. Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis. Am J Clin Nutr. 2016;103(2):495–504.

6. Murray-Kolb L (2012) Maternal mortality, child mortality, perinatal mortality, child cognition, and estimates of prevalence of anemia due to iron deficiency. CHERG

7. Smith R. Alterations in the hypothalamic pituitary adrenal axis during pregnancy and the placental clock that determines the length of parturition. JReprod Immunol 1998;39:215–20.

8. Poranen AK, Ekblad U, Uotila P, Ahotupa M. Lipid peroxidation and antioxidants in normal and pre-eclamptic pregnancies. Placenta 1996;17:401–5.

9. Qiaoyi Zhang,Cande V Ananth, Zhu Li and John C Smulian.Maternal anaemia and preterm birth: a prospective cohort study.Perinatal Epidemiology 2ndJune 2009.

10. ISarah, EFerguson, Graeme Smith, et al.Preterm premature rupture of membranes: Nutritional and socioeconomic factors Obstetrics &Gynecology Volume 100, Issue 6 December 2002.

11.Jain S, Sharma P, Kulshreshtha S, Mohan G, Singh S. The role of calcium, magnesium, and zinc in pre-eclampsia. Biol Trace Elem Res. 2010;133(2):162–70.

12.Gilles Cottrell, AzizathMoussiliou, Adrian J. F. Luty, Michel Cot, et al Submicroscopic Plasmodium falciparum Infections Are Associated With Maternal Anemia, Premature Births, and Low Birth Weight, Clinical Infectious Diseases, Volume 60, Issue 10, 15 May 2015, Pages 1481–1488.

13. Kavle JA, Stoltzfus RJ, Witter F, et al. Association between anaemia during pregnancy and blood loss at and after delivery among women with vaginal births in Pemba Island, Zanzibar, Tanzania. J Health PopulNutr. 2008;26(2):232-240.

14.Allen LH. Anemia and iron deficiency: effects on pregnancy outcome. Am J Clin Nutr. 2000;71:1280S–4S.

15. Arnold, Dodie & Williams, Michelle & Miller, et al (2009). Iron Deficiency Anemia, Cigarette Smoking, and Risk of Abruptio Placentae. The journal of obstetrics and gynaecology research. 35. 446-52. 10.1111/j.1447-0756.2008.00980.x.

16. Corwin EJ, Murray-Kolb LE, Beard JL. Low hemoglobin level is a risk factor for postpartum depression. J Nutr. 2003;133:4139–42.

17. Paterson JA, Davis J, Gregory M, et al. A study on the effects of low haemoglobin on postnatal women. Midwifery. 1994;10:77–86.

18. Mayberry LJ, Gennaro S, Strange L, Williams M, De A. Maternal fatigue: implications of second stage labor nursing care. J ObstetGynecol Neonatal Nurs. 1999;28:175–81.

19. Henly SJ, Anderson CM, Avery MD, Hills-Bonczyk SG, Potter S, Duckett LJ. Anemia and insufficient milk in first-time mothers. Birth. 1995;22(2):86-92. doi:10.1111/j.1523-536x.1995.tb00565.x

20.Harringa JB, Bracken RL, Nagle SK, et al. Anemia is not a risk factor for developing pulmonary embolism. Am J Emerg Med. 2017 Jan;35(1):146-149. doi: 10.1016/j.ajem.2016.09.068. Epub 2016 Sep 30.

# FETAL COMPLICATIONS ARISING FROM ANAEMIA IN PREGNANCY

**Dr Vandana Bansal MD DGO DNB MNAMS MRCOG FICOG FNB**
**Dr Neha Singh MS DNB**

## Background

Anaemia is one of commonest medical disorders encountered during pregnancy accounting for about 40% cases globally. It has been associated with adverse feto-maternal outcomes e.g. higher maternal and perinatal mortality rates, preterm birth, preeclampsia, higher infectious morbidity, low birth weight, fetal growth restriction/ SGA and higher caesarean rates etc.[1]

WHO defines anaemia in pregnancy as haemoglobin level below 11 g/dL in all trimesters where as UK antenatal guidance and CDC define anemia when Hb falls below 11 g/dL in 1st and 10.5g/dL in 2nd /3rd trimesters.

ICMR classifies anaemia depending upon severity as mild (10-10.9), moderate (7-9.9), severe (4-6.9) and very severe (<4 g/dL). FOGSI has suggested a cut off of 10 gm/dL for India in pregnancy.

It can also be classified as

1. Inherited (haemoglobinopathies, inherited haemolytic anaemias)
2. Acquired (nutritional e.g. iron/B12/folate deficiency, haemorrhagic, anaemia of chronic disease, acquired haemolytic and aplastic anaemia)

As the severity of anaemia increases, there is a proportionate rise in fetomaternal complications. We focus on various fetal complications observed which are often caused by either anaemia itself or its treatment or both and their prevention. These include low birth weight, prematurity, infections, congenital malformations, neonatal anemia, abnormal cognitive development and increased risk of schizophrenia.

## Nutritional anaemias

### Iron deficiency anaemia

#### Pathophysiology of iron utilisation by the fetus

The maternal body requirement for iron during pregnancy increases to approximately 1000 mg on average. Of this 350 mg is associated with fetal and placental growth, 500 mg with expansion in the red cell mass and 250 mg with blood loss at delivery. To prevent anegative iron balance during pregnancy a mother requiresat least 300 mg of iron stores at the time of start of pregnancy. The developing fetus is entirely dependent on its mother for nutritional requirements and is relatively protected from the effects of iron deficiency. In thehierarchy of iron usage, the fetus takes the priority, followedby maternal hematocrit while the maternal ironstores are the poor last and are often depleted during thecourse of pregnancy.

**Fetal consequences of iron deficiency anaemia**

Untreated iron deficiency anaemia during pregnancy has been associated with an increased risk of adverseperinatal outcomes which include fetal growth restriction, prematurityand low birth weight, all with significant mortality risks.

**Biological mechanisms linking maternal anaemia to FGR**

Low haemoglobin levels restrict oxygen circulation in the body creating an environment of oxidative stress or chronic hypoxia, which could then cause fetal growth restriction. Iron deficiency causes an increased production of norepinephrine, which stimulates production of corticotropin-releasing hormone and in turn possibly restricts fetal growth.

Pre-pregnancy anaemia and iron deficiency anaemia in the first trimester is associated with greater than 2-fold increases in the risks of low birth-weight and preterm delivery. Fetal iron needs are compromised when maternal iron stores are suboptimal in pre-pregnant state. The negative impact on fetal growth is less significant when anaemia develops later in pregnancy.[4]

Moderate to severe anemia (<9 or 8 g/dL) is significantly associated with low birth weight due to increase in prematurity and fetal growth restriction, whereas there appears to be no relationship with milder form of anemia.[5] A fall in maternal haemoglobin below 11 g/dL is associated with a rise in perinatal mortality rate which increases with the severity of anaemia. There is a 2-3 fold increase in perinatal mortality rate with levels < 8 g/dL and 8-10 fold increase with levels <5 g/dL.[5]

**Neonatal consequences of anaemia**

Perinatal iron deficiency adversely affects the growth and functioning of multiple organ systems including heart, skeletal muscle, the gastrointestinal tract and brain. Altered immune function and temperature instability are also attributed to perinatal iron deficiency. The most significant adverse effects are neurodevelopmental impairments and predisposition to earlier onset of postnatal iron deficiency. With respect to birth weights, levels< 9 g/dL are associated with 2–3 times increased risk of small for gestational age neonates.[6]

**Effects of perinatal iron deficiency on neurodevelopment**

Perinatal iron deficiency anemia has been linked to abnormal fetal neurodevelopment including neurocognitive dysfunction and behavioural abnormalities. Therefore, infants and young children with iron deficiency anemia are at risk of developmental difficulties involving cognitive, social-emotional & adaptive functions as well as delays in both language and motor development and may

have an association with adult onset diseases. These long-term neurocognitive abnormalities are not reversed, despite adequate iron supplementation post-delivery.[6] Iron is essential for neurotransmission, energy metabolism and myelination in the developing brain. The exact mechanisms through which iron deficiency affects brain development and function are not completely understood.

**Predisposition to future iron deficiency**

Maternal iron depletion adversely affects fetal iron status and increases the risk of iron deficiency in the first3 months of life and may persist for up to one year. A maternal Hb concentration < 6 g/dL is associated with low cord serum ferritin concentrations <30 mcg/L suggestive of severe depletion of storage iron.[6] Breast feeding is usually protective, but not if the mother is iron deficient.

**Prevention of fetal complications**

Iron-folic acid supplements may need to be started before pregnancy in order to reduce the risk of adverse pregnancy outcomes and to improve the iron stores of the mother and the infant. Iron supplementation to the mother during pregnancy improves perinatal outcome. Mean weight, Apgar score and haemoglobin level 3 months after birth are significantly greater in babies of the supplemented group than the placebo group.[6] Delaying the time at which the umbilical cord is clamped after delivery has a significant impact on the net amount of iron stores transferred to the neonate at birth.

**Folate deficiency anaemia**

Folic acid is one of the B complex vitamins and is recognised as a major component of thepericonceptional care of women in the reproductive age group. Deficiency of folic acid can lead to megaloblastic anaemia in the mother.

**Need for folic acid supplementation**

The first few weeks of pregnancy are crucial for the development of the neural tube and this is the period when folic acid is most essential. Most women of reproductive age fail to get enough daily folates from diet alone. Most dietary folates exist aspolyglutamates, which are converted to the monoglutamateform and absorbed in the proximal small intestine. However, the body absorbs only about 50% of food folate. This problem is compounded by cooking practices such as prolonged stewing, processingand storage, which can destroy some of the folate innatural foods. About 5% to 15% of the general population have a variant of 5, 10-methylenetetrahydrofolate reductase enzyme

essential for catalysing the transfer of a methyl group to homocysteine to form methionine and the presence of this variant can compromise tissue folate levels.

**Fetal consequences of folate deficiency**

Embryopathies, especially neural tube defects, have been associated with folate deficiency and supplementation appears to decrease the percentage of such complications.[7] Maternal folate deficiency has also been associated with other fetal malformations like cleft lip, cleft palate, cardiac and limb defects and to a higher incidence of placental abruption, preeclampsia, spontaneous abortion, stillbirth, prematurity and low birth weight infants.

**Folate deficiency and birth defects**

The best documented congenital malformations associated with maternal folate deficiency are neural tube defects (NTDs) including anencephaly and spina bifida. The suggestion that folate deficiency might play a part in the aetiology of NTDs was made originally by Hibbard in 1964.[8] Periconceptional use of folic acid supplements reduces the risk of the first occurrence, as well as the recurrence of NTDs.

**Prevention of fetal complications**

Since the recognition of the link between folic acid and neural tube defects (NTDs) in 1960s, folic acid supplementation has remained the only intervention that can prevent serious congenital anomalies in the fetus. It is recommended that all women of child-bearing age should take 0.4 mg (400 mcg) of folic acid daily when planning a pregnancy. Women who have had a previous pregnancy affected by an NTD are recommended a higher dose of folic acid supplementation(5 mg folic acid daily) periconceptionally, starting at least 1 month before conception and continuing throughout the first trimester of pregnancy to reduce the risk of a subsequent NTD- affected pregnancy.[9,10] In folate deficiency anaemia daily FA supplementation in doses of 5-15 mg for 4 months causes haematological improvement and replenishes the body stores.[9,10]

**Vitamin B12 deficiency anaemia**

There is physiological fall in total serum cobalamin levels during normal pregnancy. Vitamin B12 deficiency has been associated with various fetomaternal complications like NTD, recurrent fetal loss, preterm birth and has also been linked to elevated homocysteine levels which may further increase the risk of preeclampsia. Increased demand during pregnancy, reduced intake (strict vegetarian diet), parasitic infestations may lead to Vitamin B12 deficiency during pregnancy. It may cause impaired myelination process during infancy, severe neurological disorders (i.e. subacute combined degeneration of spinal cord), failure to thrive, persistent long-term neurocognitive impairment and

poor intellectual development. Elevated total homocysteine levels secondary to inadequate folate or vitamin B12 levels may cause endothelial dysfunction due to increased oxidative stress. This results in placental dysfunction, thus giving rise to various obstetric complications like preeclampsia, spontaneous abortion, abruption, FGR, recurrent pregnancy loss and preterm birth.[10] FOGSI recommends oral vitamin B12 in dosage of 2.6 mcg and 2.8 mcg daily during pregnancy and lactation, respectively to avoid maternal and neonatal complications.[11]

**Haemoglobinopathies and fetal complications**

Haemoglobinopathies such as thalassemia and sickle cell disease should be screened for during pregnancy because of their impact on maternal and perinatal outcomes. They are genetic disorders of haemoglobin structure and synthesis and may transmit to the offspring, manifesting during pregnancy as anaemia. Usually the iron stores are normal, necessitating folate supplementation alone to avoid iron overload.

**Thalassemia**

A group of inherited haemoglobinopathies caused by partly or completely suppressed synthesis of one of the two polypeptide chains (α or β) as a result of missense/nonsense mutations or frames hift mutations of the globin genes leading to reduced Hb concentration, microcytosis and anaemia. They are classified based on which globin chain is deficient.

**Alpha thalassemia** is characterised by reduced synthesis of α globin chains and found in higher frequency in sub-Saharan Africa, Mediterranean, middle east and Indian subcontinent and southeast Asia. There are 4 genes responsible for α globin chain synthesis situated in two genetic loci in chromosome [16]. When all the 4 genes are affected in homozygous alpha thalassemia, unstable Bart's Hb (γ4) is formed due to excess of γ chains which is not capable of oxygen exchange. Fetuses having Bart's Hb suffer from severe anaemia, cardiomegaly, nonimmune hydrops fetalis and intrauterine fetal demise or early neonatal death. Homozygous α-thalassemia is one of the most common form of hydrops fetalis in Southeast Asia.

**Alpha thalassemia minor** (three normal alpha gene) is usually asymptomatic, but the patient may present with mild hypochromic microcytic anemia. Heterozygous α-thalassemia is difficult to detect by laboratory means because the affected globin chain is common to all types of haemoglobin. There is a reduction in the amount of hemoglobin A, F and A2, while the percentage of these compounds remains the same as in normal persons. This finding, combined with the fact that anaemia is usually mild, also complicates the detection process. Concomitant pregnancy reveals few adverse maternal or fetal effects, except a slight increase in spontaneous abortions.

**Beta thalassemia syndromes** are characterised by ineffective erythropoiesis resulting into increased extravascular hemolysis due to release of damaged RBCs and erythroid precursors into the peripheral circulation. It happens due to mutation in β globin gene which causes reduced β globin chain synthesis and inadequate Hb content.

**Thalassemia minor** or β thalassemia trait represents heterozygous state causing mild to moderate microcytic anemia. Beta thalassemia minor may also be asymptomatic but may present with iron deficiency anaemia with lowered MCV, MCH and MCHC. Hb electrophoresis will reveal elevation of the haemoglobin A2 (above 3.5%) and a mildly increased haemoglobin F (2% to 5%).

**Thalassemia major** or Cooley's anemia (β0β0/ β0β+) represents a homozygous state and results from the inheritance of a defective β globin gene from each parent. This results in a severe transfusion-dependent anaemia. Treatment involves repeated transfusions and pregnancy is infrequent since the patient usually dies in childhood. Beta thalassemia major in adults who survive present with iron overload and infertility.

**Thalassemia intermedia** is defined as a group of patients with β thalassaemia whose disease severity varies. At the severe end of the clinical spectrum of thalassaemia intermedia, patients are usually diagnosed between the ages of two and six years and although they survive without regular blood transfusions, growth and development are impaired. At the other end of the spectrum, there are patients who are completely asymptomatic until adulthood, when they present with mild anaemia and splenomegaly often found by chance during haematological examinations or family studies.

**Prenatal diagnosis**

Genetic counseling should be offered to any couple with a prior affected child or those at risk because of ethnic origin or pedigree. When both parents are carriers of the same trait (α-α / β-β), there is 25% risk of having a fetus affected with thalassemia major. Therefore, prenatal diagnosis should be offered to prospective parents. Parents not having an affected index child previously need verification ofcarrier status based on MCV, haemoglobin electrophoresis, pedigree analysis and parental mutation analysis before fetal assessment is undertaken.[12]

A sample of fetal deoxyribonucleic acid (DNA) can be obtained by either chorionic villus sampling, amniocentesis, or percutaneous umbilical (cord) blood sampling. Chorionic villus sampling has advantage of early diagnosis between 11-14 weeks, more DNA is obtained by placental biopsy, and it is perhaps safer to penetrate the placenta than the amniotic cavity. On the contrary, amniocentesis has

the drawback of being feasible only after the 16th week. The risk of miscarriage does not differ between these invasive procedures and is estimated to be less than 1%.

More than 200 thalassemic mutations have been reported. DNA analysis of that portion of the α-globin or β-globin gene containing the deletion or mutation can be identified using direct DNA sequence analysis by performing polymerase chain reaction amplification of fetal DNA. Using this method of detection, prenatal diagnosis can usually be confirmed within7-10 days of fetal sampling.

When both parents suffer from a certain homozygous hemoglobinopathy, use of donor gametes screened for haemoglobinopathies seems to be an option. If the partner of a homozygous parent is heterozygous, IVF/ICSI with a pre-implantation genetic diagnosis (PGD) should be considered at either the eight-cell stage (cleavage stage) on day 3 or at the blastocyst stage on day 5 by biopsy of the trophoblast cells.

**Other fetomaternal consequences of β thalassemia**

Modern treatment modalities in β thalassemia involve blood transfusion to combat anemia and iron chelation therapy to treat iron overload secondary to multiple blood transfusion resulting in hepatic, cardiac and endocrine dysfunction. Thalassemia complicates pregnancies by development of cardiomyopathy, new endocrinopathies e.g. diabetes mellitus, hypothyroidism, hypoparathyroidismin mother and fetal growth restrictionand spontaneous abortions due to chronic maternal anaemia. Iron chelators are considered to be potentially teratogenic during first trimester.

Gestational estrogen levels are elevated, leading to reduction of total immune function and an augmented infection risk. Apart from this, transfusion-transmitted viral infections can be a great risk. All women should be tested for hepatitis B virus (HBV), HCV, HIV, cytomegalo virus, rubella and human parvovirus B19, particularly in pregnancy.

**Prevention of fetal complications**

Preconceptional counselling for women with thalassemia includes genetic counselling and partner screening for hemoglobinopathy; should also involve discussion about fetal risk and methods of prenatal diagnosis and need for termination in case of fetal affection. All women with thalassemia have higher demand for folic acid, therefore RCOG recommends FA (5mg) daily to be commenced 3 months prior to conception. As diabetes is a common ailment in thalassemic women, it is ideal to

achieve euglycemia periconceptionallyin order to reduce the risk of early pregnancy losses and congenital anomalies. Iron chelators (e.g. deferasirox and deferiprone) should ideally be stopped 3 months before conception and converted to desferrioxamine for iron chelation after the first trimester if necessary.[13]

**Sickle cell disease**

SCD is an inherited single gene autosomal recessive disorder caused by sickle gene affecting structure of hemoglobin which results in polymerisation of the abnormal Hb in hypoxic conditions and formation of rigid fragile sickle shaped cells prone for increased breakdown and vaso-occlusion. Sickle cell disease is caused by the substitution of glutamic acid by valine at position 6 of the globin chain. It includes sickle cell anaemia (HbSS) and heterozygous conditions of Haemoglobin S (Sickle cell trait Hb AS) and other clinically abnormal haemoglobins (Sickle Hb C, Sickle Hb beta thalassemia, Hb D, Hb E). Sickling and crystallisation of the haemoglobin are induced by de-oxygenated states such hypoxia, acidosis and dehydration.

SCD has been associated with increased incidence of miscarriage, preterm labour, fetal growth restriction, preeclampsia, abruptio placentae, thrombosis, acute painful crisis, fetal distress, induction of labour, caesarean delivery and perinatal mortality. There is also an increased incidence of infection and the sickle cell crisis. All women (carrier/affected) should be encouraged to get their partner tested for haemoglobinopathies, ideally preconceptionally or early first trimester. If both partners are found to be carriers or one is affected by major haemoglobinopathy, they should have a discussion about prenatal diagnosis by invasive testing for mutation analysis and pregnancy termination in case of affected fetus.[14] The management of sickle cell disease in pregnancy should be in collaboration with haematologist.

**Prevention of fetal complications**

RCOG recommends daily folic acid intake for all women with SCD at risk of folate deficiency at dosage of 1mg in nonpregnant and 5mg during pregnancy so as to reduce the risk of neural tube defect in fetus and to meet the increased demand during pregnancy. Hydroxyurea / hydroxycarbamide (used to decrease the incidence of acute painful crisis and ACS in severe disorder) should be stopped at least 3 months before conception. As animal studies have shown teratogenic effects of this drug, a Level 3 ultrasound to rule out fetal structural defect is warranted if exposure takes place in periconceptional period or during the period of organogenesis. However, termination is not indicated only on the basis of exposure to drug alone. ACE inhibitors or angiotensin receptor blockers used routinely in SCD patients with significant proteinuria are not safe in pregnancy and should be stopped before planning pregnancy.[14]

### Haemolytic anaemia

This is an uncommon type of anaemia and is either primary or secondary. It is usually due to antibody production. Secondary haemolytic anaemia may be due to chronic infection, drugs or connective tissue disease. Typically, both direct and indirect Coombs tests are positive and spherocytosis and reticulocytosis are the typical characteristics of a peripheral blood smear.

### Hereditary spherocytosis

It is inherited as an autosomal dominant disorder predominantly but in 25% cases, it may occur due to de novo mutation or autosomal recessive inheritance. It must be considered while evaluating nonimmune hydrops fetalis. Genetic consultation is advised for risk of inheritance in the fetus.

### Autoimmune haemolytic anaemia

AIHA may result from presence of warm and cold autoantibodies which are directed against own red cell antigens. When it is associated with other auto-immune diseases like SLE, fetal complications like IUFD, haemolytic diseases of fetus and newborn and immune hydrops may arise due to crossing of IgG auto antibodies through placenta. Fetal surveillance for growth and MCA doppler flow for assessment of peak systolic velocity for identifying fetal anaemia is suggested so as to guide intrauterine transfusion for better perinatal outcome.[15]

### References


1. The global prevalence of anaemia in 2011. Geneva: World Health Organization; 2015 (http://apps.who.int/iris/bitstream/10665/177094/1/9789241564960_eng.pdf,29 September 2016).
2. Smith et al. Anemia in Pregnancy. Obstet Gynecol. 2019; 134:1234–44.
3. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 95: anemia in pregnancy. Obstet Gynecol.2008; 112 (1): 201- 207.
4. Scholl TO, Heldiger ML, Fischer RL, Shearer JW. Anemia vs iron deficiency: increased risk of preterm delivery in a prospective study. Am J Clin Nutr. 1992; 55:985–988.
5. Naoko Kozuki, Anne C. Lee and Joanne Katz. Moderate to severe, but not mild maternal anemia is associated with increased risk of Small-for-Gestational-Age outcomes; J. Nutr. 142: 358–362, 2012
6. Raghavendra Raoa, and Michael K. Georgieffa. Iron in fetal and neonatal nutrition: Semin Fetal Neonatal Med. 2007 February; 12(1): 54–63.
7. De-Regil LM, Ferna´ ndez-Gaxiola AC, Dowswell T, et al. Effects and safety of periconceptional folate supplementation for preventing birth defects. Cochrane Database Syst Rev.2010:CD007950.
8. Hibbard BM. The role of folic acid in pregnancy with particular reference to anaemia, abruption and abortion. J Obstet Gynaecol Br Commonw. 1964;71;529–542.
9. Talaulikar VS, Arul kumaran S. Folic acid in obstetric practice: a review. Obstet Gynecol Surv. 2011;66(4):240-247. doi:10.1097/OGX.0b013e318223614c.

10. Molloy AM, Kirke PN, Brody LC, Scott JM, Mills JL. Effects of folate and vitamin B12 deficiencies during pregnancy on fetal, infant, and child development. Food Nutr Bull. 2008;29(2 Suppl):S101-S115. doi: 10.1177/15648265080292S114.
11. Kriplani A, Sharma A, Radha A, Rizvi Z, et al. FOGSI General Clinical Practice Recommendations: Management of Iron deficency anemia in pregnanacy.2016.
12. Petrakos G, Andriopoulos P, Tsironi M. Pregnancy in women with thalassemia: challenges and solutions. International Journal of Women's Health. 2016;8:441-451. DOI: 10.2147/ijwh.s89308.
13. Royal College of Obstetricians and Gynaecologists. Management of Beta Thalassaemia in Pregnancy, Green-top Guideline No. 66: RCOG; 2014.
14. Royal College of Obstetricians and Gynaecologists. Management of Sickle Cell Disease in Pregnancy, Green-top Guideline No. 61: RCOG; 2011.
15. Ong MG, Hawthorne LM. Autoimmune Hemolytic Anemia in Pregnancy. Laboratory Medicine. 2010;41(5):264-266.

# PARENTERAL IRON THERAPY

**Dr Priya Vora Thakur MBBS DGO DFP DNB FCPS FICOG**

## Introduction

Iron deficiency anaemia in pregnancy is a common problem encountered in developing as well as developed countries. The WHO estimates 32 million pregnant women have anaemia and of those, half are due to iron deficiency and therefore responsive to iron supplementation.[1]

Iron deficiency anaemia is common in pregnancy because of fetal as well as placental requirements along the course of pregnancy. Experts recommend 40 –60 mg of iron daily. As this is lacking in the average Indian diet, supplementation of 100 mg of iron and 0.5 mg folic acid is the national recommendation.[2]

## Treating iron deficiency

Supplementation with oral iron is the first line treatment for pregnancy with iron deficiency anaemia. The Institute of Medicine recommends daily 32-120 mg of elemental iron for pregnant women, but nearly half of them suffer from side effects of oral iron therapy leading to non-compliance. Repeated doses of oral iron are less effective because of hepcidin feedback inhibition.

Intravenous iron formulations offer an alternative approach in the presence of moderate or severe anaemia. Intravenous iron is less commonly used due to fear of anaphylaxis with iron dextran formulations and long infusion time required with iron polymaltose.[3] The development of dextran free parental iron formulations, with an improved safety profile and a more rapid delivery time suggests that intravenous iron should be considered as a mainstay of treatment for moderate to severe iron deficiency anaemia.[4]

## Newer parenteral iron preparations

Iron sucrose and ferric carboxymaltose (FCM) are the dextran-free intravenous iron preparations that are currently available. Iron sucrose is preferred over oral iron because it increases haemoglobin with better replenishment and has a good safety profile.[5] Serious adverse effects are rare, however minor side effects occur in up to 18% of patients due to its non-physiological physical properties (high pH and high osmolality).

FCM is a newer Dextran free formulation with a near neutral pH, physiological osmolarity and increased bio availability which allows additional advantage of single high dose of 1000 mg in 15 minutes infusion time.[6] These properties make FCM an attractive alternative to iron sucrose in terms of risk profile, patient comfort and convenience. FCM therefore allows rapid and high-dose replenishment of depleted iron stores.

**Table 1 Parenteral iron preparations**

| Preparation | FDA category | Strength | Route of administration |
|---|---|---|---|
| Iron dextran Complex | C | 2 ml ampoule 50mg/ml 10 ml multidose vial | IM or IV |
| Iron sorbitol citric acid complex | B | 1.5 ml ampoule 50 mg/ml | IM |
| Iron sucrose | B | 5 ml ampoule 20 mg/ml | IV |
| Iron gluconate | N | 5 ml ampoule 12.5 mg/ml | IV |
| Ferric carboxymaltose | C | 2 ml and 5ml vials 50 mg/ml | IV |

**Indications for parenteral iron therapy**

- Intolerance to oral iron
- Non-compliance /unreliable
- Severe anaemia-(Hb between 5 g/dL and 8 g/dL)
- Chronic blood loss
- Patent seeks medical advice late in pregnancy
- Malabsorption & bowel disease
- Rapid replenishment required
- In combination with recombinant human erythropoietin

**Structure of FCM**

FCM is an iron complex that consists of a ferric hydroxide core stabilised by a carbohydrate shell. This design of the macromolecular ferric hydroxide carbohydrate complex allows controlled delivery of iron to the cells of the reticuloendothelial system and subsequent delivery to the iron- binding proteins ferritin and transferring, with minimal risk of large amounts of ionic iron being released into the serum. FCM is a stable complex and has a very low immunogenic potential. Therefore, the risk of anaphylactic reaction is very low with this molecule.[7]

**Dose calculation**

The total dose of parenteral iron needs to be calculated carefully to prevent the potential risk of overload and side effects.

- Total iron required= 0.3 x weight(lb) x (100-Hb%) + 500 mg
- Iron required (mg) = [2.4 x body weight(kg) x Hb deficit (g/dL)] +1000 mg to replenish iron stores.
- Iron required(mg) = 0.66 x body weight x [Hb g/dL x 100/14.8]

**Pre-requisites before administering parental iron**

- Correct diagnosis of iron deficiency anaemia should be established.
- No history of allergies- test dose recommended.
- Close supervision and adequate facilities to treat adverse reactions promptly.

Thalassemia needs to be excluded(Hb A2 levels and serum iron levels).

**Table 2 Dilution plan for FCM for intravenous drip infusion**

| FCM | Iron | Maximum amount of 0.9 % NaCl | Minimum administration time |
|---|---|---|---|
| 2 to 4 ml | 100 to 200 mg | 50 ml | - |
| >4 to 10 ml | >200 to 500 mg | 100 ml | 6 mins |
| >10 to 20 ml | >500 to 1000 mg | 250 ml | 15 mins |

**Scientific evidence**

Larger studies on the use of FCM and iron sucrose in pregnancy are lacking. Both the drugs should ideally be avoided in the first trimester. They can be used in the second and third trimester of pregnancy and during lactation.

Froessler et al performed a prospective observational study including 65 pregnant women in second and third trimester who received FCM. The haemoglobin values were assessed at baseline and then at 3, 6 and 8 weeks. All patients showed a significant rise in haemoglobin levels and 66% patients reported an improved sense of well being in postpartum period. No serious adverse effects were reported. Minor side effects were observed in 13(20%) patients. [8] The most recent study by Froessler also concluded that FCM infusion corrects various degrees of iron deficiency anaemia in pregnancy effectively and safely.

Christoph et al showed the superiority of FCM in comparison to other iron preparations in the second and third trimester in a retrospective study on 206 pregnant women.10 3 women were given FCM and 103 received iron sucrose. FCM showed higher increments in the haemoglobin levels (FCM 15.4 gm% and iron sucrose 11.7 gm%).[9] FCM was equally well tolerated as intravenous iron sucrose. The adverse effects were 8% in FCM versus 11% in iron sucrose. A recent Cochrane review concluded that large, good quality trials are required to assess the efficacy and adverse effects of FCM. [10]

A prospective study comparing the efficacy and safety of FCM versus iron sucrose was conducted on 100 pregnant women by Divyani et al.50 women were given intravenous FCM while 50 received intravenous iron sucrose. The mean rise in the haemoglobin with FCM was 2.92 gm/dL and with iron sucrose was 1.08 gm/dL. The mean rise in serum ferritin was also higher in the FCM group. There were no adverse effects reported in FCM group while 3 patients developed adverse reactions with iron sucrose. FCM was safer and efficacious in comparison to iron sucrose in the treatment of anaemia in pregnancy.[11]

Anouk Pelset al conducted a retrospective study on 128 pregnant anaemic women to study the safety and efficacy of FCM. Median FCM dose was 1000 mg and median gestational age at the time of first treatment was 34.6 weeks. Median haemoglobin increased from 8.4 gm/dL at the first FCM administration to 10.7 gm/dL at the time of delivery. No treatment related adverse effects were reported.[12]

**Table 3 Side effects**

| **Adverse event** | N(%) |
|---|---|
| Any adverse event | 13(20) |
| Local (irritation at injection site) | 5(8) |
| Slight burning sensation | |
| **Systemic** | |
| Hypotension | 1(1.5) |
| Headache | 4(6) |
| Nausea | 1(1.5) |
| Pruritis | 2(3) |

**UK recommendations**[13]

1. Parenteral iron should be considered from the 2nd trimester onwards and post-partem period in women with iron deficiency anaemia who fail to respond to or are intolerant to oral iron.
2. The dose of parenteral iron should be calculated at the basis of pre-pregnancy weight, aiming for a target of Hb 11 gm/dL.
3. The choice of parenteral iron preparation should be based on local facilities taking into consideration not only drug cost but also facilities and staff required for administration.
4. All centres should undertake audit of utilisation of intravenous iron therapy with feedback of results and change of practice where needed.

## National Guideline

The Ministry of Health and Family Welfare guidelines for treatment of iron deficiency anaemia in pregnancy continue to recommend intramuscular iron following a test dose as a cost-effective treatment for moderate to severe anaemia. However, the intramuscular route has essentially been replaced by intravenous route because of side effects of the former.

## Conclusions

The newer parenteral iron preparations are highly effective in treating iron deficiency anaemia in pregnancy. FCM has shown to be more effective and safe than iron sucrose in various studies. A large dose can be administered at one time. The haemoglobin rise is rapid along with replenishment of the depleted iron stores.

## References

1. WHO. The global prevalence of anaemia in 2011. Geneva: World Health Organization, 2015.
2. Ministry of Health and Family Welfare. Guidelines for the control of iron deficiency anaemia, 2019.
3. Chertow GM et al. The enlarging body of evidence: obesity and chronic kidney disease. J Am Soc Nephrol.2006;17(6):1501-2.
4. Khalaffallah AA et al.Iron deficiency anaemia in pregnancy and postpartum:Pathophysiology and effect of oral versus intravenous iron therapy.J Pregnancy2012:10.
5. Froessler B, Cocchiaro C et al.Intravenous iron sucrose versus oral iron ferrous sulphate for antenatal and postpartum iron deficiency anaemia:a randomized trial. J Matern Fetal Neonatal Med.2013;26(7):654-9.
6. Lyseng-Williamson et al.Ferric carboxymaltose: a review of its use in iron deficiency anaemia.Drugs2009;69(6):739-56.
7. Anker SD et al.Ferric carboxymaltose in patients with heart failure and iron deficiency.N Engl J Med 2009;361(25):2436-48.
8. Bernd Froessler et al.Intravenous ferric carboxymaltose for anaemia in pregnancy BMC Pregnancy and Childbirth 2014,14:115.
9. Christoph P et al.Intravenous iron treatment in pregnancy:comparison of high dose ferric carboxymaltose vs. iron sucrose.J Perinat Med2012:40(5):469-74.
10. CochraneDatabase Syst Rev.2019 Feb 21;2:CD007857.
11. Divyani et al.A study for efficacy and safety of ferric carboxymaltose versus iron sucrose in iron deficiency anaemia among pregnant women in tertiary care hospital.Int J Reprod Contracept Obstet Gynecol2019 Jun;8(6):2280-2285.
12. Anouk Pels et al.Safety and efficacy of ferric carboxymaltose in anaemic pregnant women: aretrospective case control study.Obstetrics and Gynaecology International2015.
13. UK guidelines on the management of iron deficiency in pregnancy.British Journal of Haematology.Jan 2012,Volume 5.

# NEWER FORMULATIONS

**Dr Pratik Tambe MD FICOG**

## Background

Anaemia is a near universal issue faced by obstetricians in daily practice.[1] Even in urban and metropolitan areas, the incidence of anaemia in pregnancy is as high as 50%. A persistently high level of anaemia among women in India (53% of all women as per the National Family Health Survey 2015–2016)[2] is of great concern, and the 2017 National Health Policy tabled by the Ministry of Health and Family Welfare, Government of India, acknowledges this high burden.[3]

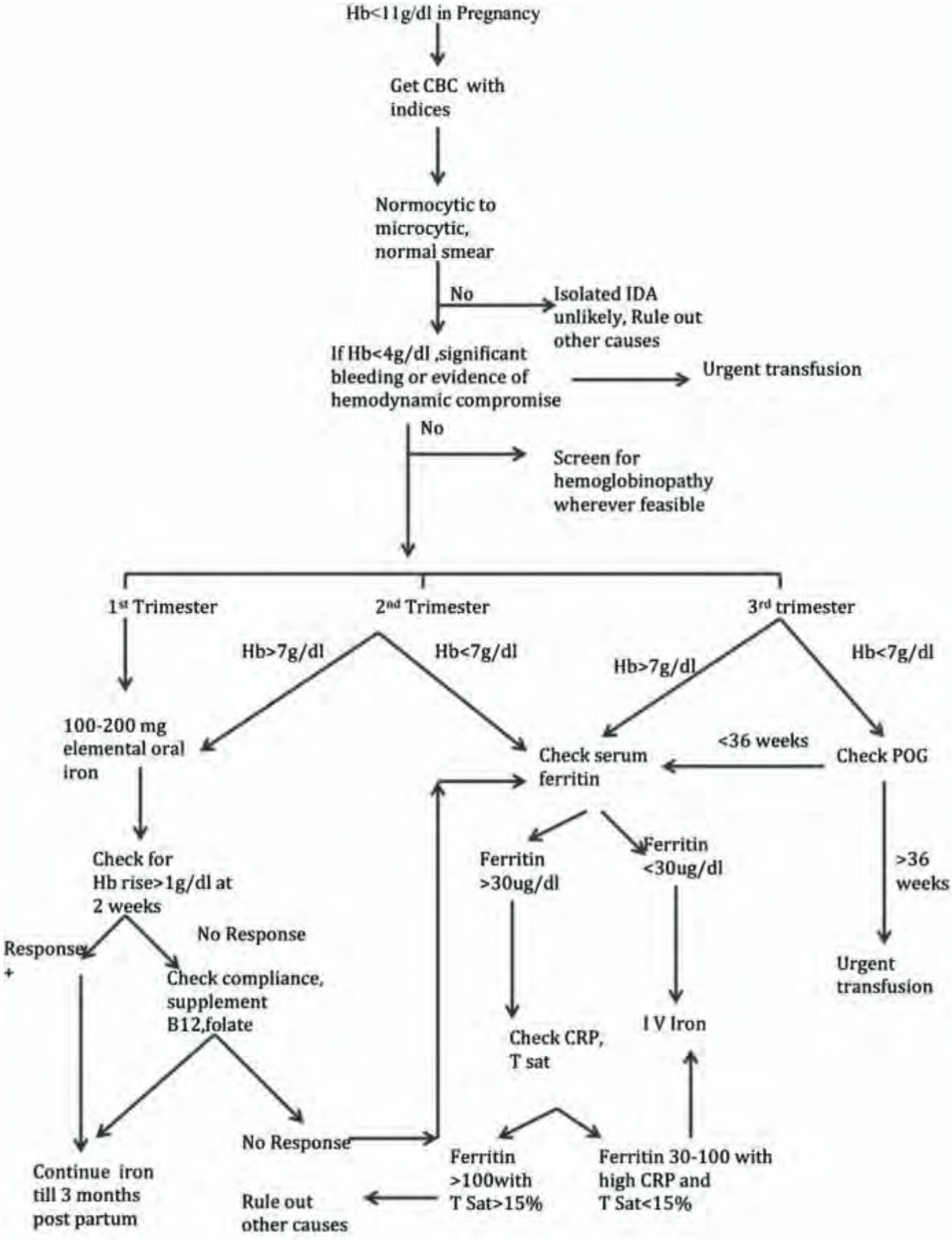
The most recent estimates reflect an unacceptably low consumption of iron (median 13.7 mg/day per person) among women in India aged ≥18 years and 51–83% of pregnant women in India are deprived of the recommended daily allowance of iron of 15–18 mg/day.[4] Women in India largely derive iron from non-haem, inorganic sources, including grains, plants, cereals, lentils and vegetables and to a small extent from iron supplements, such as iron or iron and folic acid (IFA) tablets for pregnant women and iron-fortified foods as compared to sources of haem iron such as meat and fish, which have a higher rate of absorption.[5,6]

Guidelines from the Ministry of Health and Family Welfare have noted that the burden of IDA is 3.0 times higher than the average globally for other geographies at a similar level of development, and that women are disproportionately affected.[7] While the previous articles in this issue have focused on iron metabolism, investigative pathways and the maternal, obstetric and fetal complications consequent to iron deficiency anaemia, in this article we highlight some of the newer, exciting formulations which are available to combat anaemia in the 21st century.

## Iron prophylaxis

Effective communication with pregnant women about diet and nutrition is an integral facet of preventing anaemia in pregnancy. As the extra demand of iron is often unmet by a routine diet, regular iron supplementation is recommended by most experts during pregnancy.

Recommendations for supplementation of iron vary from region to region and the CDC recommends that all pregnant women begin a 30 mg per day iron supplement at the first antenatal visit, while the WHO suggests 30–60 mg per day for all pregnant women. The equivalent of 60 mg of elemental iron is 300 mg ferrous sulfate heptahydrate, 180 mg ferrous fumarate or 500 mg of ferrous gluconate.[8,9,10]


Hb<11g/dl in Pregnancy
Get CBC with indices
Normocytic to microcytic, normal smear
No
Isolated IDA unlikely, Rule out other causes
If Hb<4g/dl ,significant bleeding or evidence of hemodynamic compromise
Urgent transfusion
No
Screen for hemoglobinopathy wherever feasible
1st Trimester
2nd Trimester
3rd trimester
Hb>7g/dl
Hb<7g/dl
Hb>7g/dl
Hb<7g/dl
100-200 mg elemental oral iron
Check serum ferritin
<36 weeks
Check POG
Check for Hb rise>1g/dl at 2 weeks
Ferritin >30ug/dl
Ferritin <30ug/dl
>36 weeks
Response +
No Response
Check compliance, supplement B12,folate
Urgent transfusion
Check CRP, T sat
I V Iron
Continue iron till 3 months post partum
No Response
Ferritin >100with T Sat>15%
Ferritin 30-100 with high CRP and T Sat<15%
Rule out other causes

## Oral iron formulations

Over a period of several decades, many iron formulations have been made available including ferric pyrophosphate, ferrous gluconate, ferrous sulfate, ferrous fumarate, ferrous carbonate and carbonyl iron. Depending on the type of preparation only 1–8% of iron is absorbed from the available oral iron preparations. There are considerable controversies regarding the optimal frequency, dose and type of oral iron preparation to be used. The absorption of oral iron increases with increasing doses of oral iron only up to 160 mg/day. Hence, the recommended dose of elemental iron for treating IDA in pregnancy is between 100 and 200 mg/day in the British guidelines and 120 mg/d in the WHO guidelines.[8,9]

Increasing the dose beyond this dose leads to increased gastrointestinal side effects without improving the efficacy. In contrast to traditional teaching, recent data on hepcidin kinetics comparing once daily with twice or thrice daily iron administration show little added benefit.[12]

## Gastrointestinal side effects

GI side effects are considerable in pregnancy and iron preparations are known to almost universally cause issues. Ferric salts usually have a superior GI tolerability than ferrous salts, but at the cost of reduced iron absorption. Gastrointestinal side effects such as nausea, constipation, diarrhoea, indigestion and metallic taste are reported in 70% of pregnant patients owing to the progesterone induced decreased GI motility and effect of gravid uterus. A significant proportion (>20%) of women stop oral iron irrespective of type of preparation and few patients adhere to the prescribed duration. Measures such as reducing the frequency, content of oral iron and changing it to an alternative preparation or taking the iron with meals may be employed to reduce GI side effects.[13]

Multiple studies comparing various oral preparations do not show conclusive evidence of superiority of one iron preparation over the other. The popular recommendation is to avoid the use of enteric coated and delayed release preparations as they have proven poor bio availability. All pregnant women should be instructed to take oral iron empty stomach or 1 h after meals for better absorption preferably with a vitamin C rich product such as orange juice or guava.[14]

## Newer formulations

Liposomes are a newer effective drug carrier system which carry different therapeutic substances to specific molecular targets. Their biocompatibility, biodegradability and low toxicity make them suitable for delivering drugs.[15]

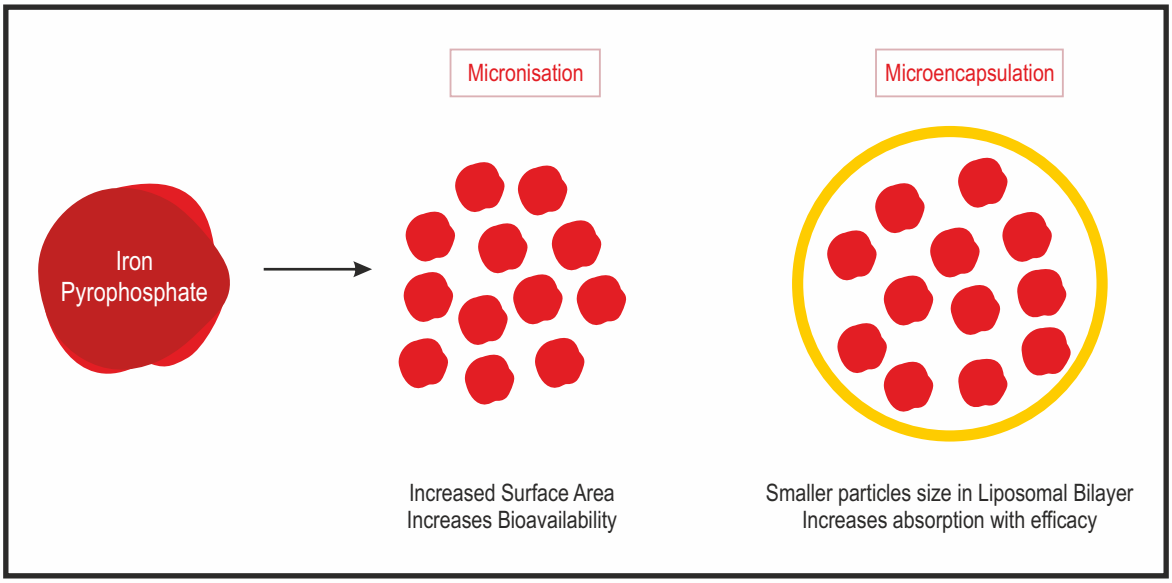
Micronisation is the reduction in particle size which increases solubility of the iron. Processing in to smaller particles increases the surface area to drug ratio and the dissolution rate of the drug. This results in increased bio availability of poorly aqueous soluble drugs.

Microencapsulation is the process where micronised iron is encapsulated by a lipid bilayer membrane like biological membranes. The formed liposome thus has outer bilayer membrane and inner core containing iron particles. Outer phospholipid bilayer confers resistance to degradation of iron from enzymes in mouth and/or stomach, interaction with alkaline juices, bile salts, intestinal flora and protection from free radicals. The protection offered from liposomes prevents oxidation and degradation of the iron content in the core and further assists in targeted delivery.[16]

**Safety issues**

Ferric pyrophosphate is the usual form of iron used for liposomal iron delivery. The USFDA states that ferric pyrophosphate is generally recognised as safe (GRAS) when used in accordance with good manufacturing practice. European Food Safety Authority (EFSA) also suggests that ferric pyrophosphate is safe for use as food additive. The current clinical evidence suggests that there are no major untoward effects in pregnant and non-pregnant women. Hence, ferric pyrophosphate contained in liposomal iron delivery is safe for treatment of iron deficiency.

**Fig 1 Schematic representation[15]**

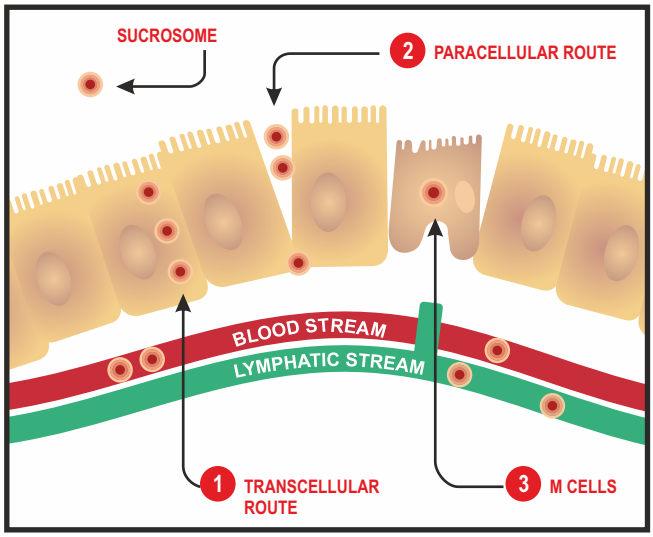


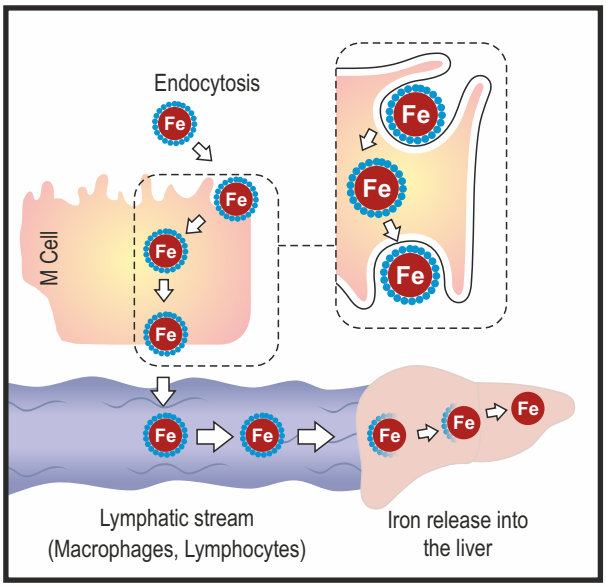

**Enhanced absorption**

The mechanisms of absorption are multiple:[16]

1. Simple adsorption which increases local concentration of liposomal contents at intestinal membrane with resultant absorption by diffusion or transporters
2. Endocytosis with subsequent breakdown of liposomal membrane by intra-cellular lysosomes
3. Fusion of lipid bilayer to plasma membrane with release of contents in to the cytoplasm
4. Exchange of lipids between liposomal bilayer and plasma cell membrane causing liposomal bilayer instability with subsequent release of contents intra-cellularly.

**Fig 2 Absorption characteristics**




Thus, liposomal (Sucrosomial) iron delivery may avoid protein mediated carrier transport of iron. This ultimately results in better bioavailability of iron. Additionally, direct absorption via microfold cells (M cells) in Peyer's patches bypassing the conventional routes of absorption may be involved.[17]

In experimental studies, administration of liposomal (Sucrosomial) iron was reported to be associated with significant increase in red cell count, haematocrit, serum iron levels and liver iron levels.[18, 19]

**Fig 3 Superior bioavailability vs traditional preparations**

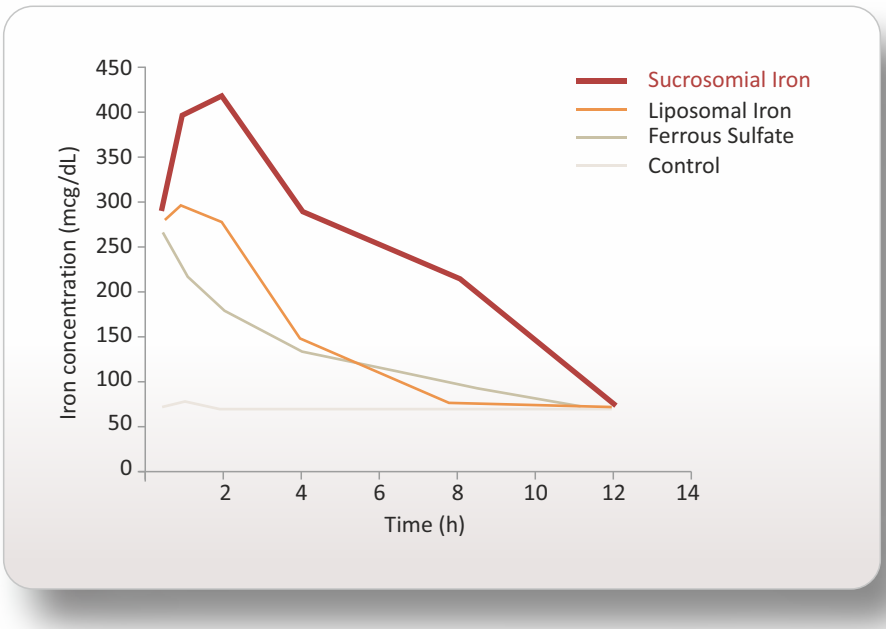


**Table 1 Summary of characteristics vs conventional preparations**

| Characteristic | Liposomal iron | Conventional Iron |
|---|---|---|
| Phospholipid Bilayer | Present | Absent |
| Effect of gastic acidity | None | Present |
| Oxidation of iron | No | Yes |
| Targeted iron delivery | Yes | No |
| Absorption of iron | Enhanced | Regular |
| Absorption via intestinal M cells | Yes | No |
| Food effect | No | Yes |
| Oxidative damage to intestinal epithelium | No | Yes |
| Gastrointestinal side effects | Minimal/Absent | Yes |
| Metallic taste | No | Yes |
| Chelation with other metals | No | Yes |

## Clinical trials

There have been several clinical trials on Sucrosomial iron preparations in various clinical scenarios including IDA subsequent to GI bleeding issues, cancer patients, non-pregnant women, etc. Almost universally, the liposomal / sucrosomial iron preparations have been shown to be better tolerated by different patient groups. Significantly, the increase in haemoglobin levels has been shown to be comparable to that with parenteral iron administration.

We discuss below some relevant trials where pregnant women and anaemic non-pregnant women were enrolled and Sucrosomial iron was found to be well tolerated and superior to conventional oral iron preparations.

## Pregnant women

Parisi et al in a randomised trial evaluated the effect of different doses of Sucrosomial iron in comparison to ferrous sulphate. They enrolled 80 non-anaemic pregnant women in 12 to 14 weeks of gestation and were randomised to one of the four treatments: Sucrosomial iron 14 mg/d (FF14), Sucrosomial iron 28 mg/d (FF28), ferrous sulphate 30 mg/d(SF) and controls (C).

Change in haemoglobin levels with 28 mg/d showed significant increase compared to ferrous sulphate ($p < 0.01$) and controls ($p < 0.05$) groups at 28 weeks and in post-partum period. Compared to control, FF28 treatment was associated with significantly higher ferritin levels at 20 weeks ($p = 0.05$), 28 weeks ($p < 0.01$) and in post-partum period ($p<0.01$). Dropouts were higher in control ($n = 6$), SF and FF14 (5 each) than FF28 ($n = 2$).Compared to controls, birth weight was significantly higher in FF28 group (3,479 ± 587 gm vs 3,092 ± 469 gm, $p < 0.05$).

Hence, 28 mg of Sucrosomial iron is associated with significant improvement in Hb and ferritin levels which may prevent maternal anaemia and improve birth weight. Changes in haematological parameters seen with 30 mg of ferrous sulphate were equivalent to that seen with 14 mg of Sucrosomial iron. This suggests that the use of Sucrosomial iron allows use of lower doses and thereby may help in ameliorating the adverse effects.[20]

**Nonpregnant women**

Giulio Giordano et al conducted a multicentric randomised study on 60 women with haemorrhagic gastritis, enteric bleeding or menorrhagia. Sucrosomial iron 30 mg four times a day was administered (Group A) vs IV iron 62.5 mg/day (Group B). The time required to increase Hb by 1 gm/dL was comparable (9 vs 7 days) and the time to achieve Hb 12 gm/dL (4 vs 3 weeks). The cost was one third that associated with parenteral iron.[21]

## Conclusions

Iron deficiency is a universal issue even in this modern era. While recommendations for oral iron therapy are issued regularly by various international organisations, there is little agreement as to the choice of iron preparation which is to be recommended. As practitioners, we have a vast array of choices available. Unfortunately, the majority of available salts of iron have gastrointestinal side effects which are pronounced especially in pregnant women where iron supplementation is most essential.

Liposomal (Sucrosomial) iron preparations have been studied over the past five years in several randomised trials in many groups of patients including cardiac, oncology, nephrology and now obstetric patients. They offer better bioavailability at a lower dosage, a consequent lower incidence of GI side effects, rapid increase in Hb levels both during and after pregnancy and on occasion have been shown to rival parenteral iron in terms of benefit at a lower cost.

More robust evidence and wider multicentric randomised trials are therefore, the need of the hour before we can recommend these as the first choice of iron preparation in pregnancy and the puerperium.

## References

1. Rai RK, Fawzi WW, Barik A, Chowdhury A. The burden of iron-deficiency anaemia among women in India: how have iron and folic acid interventions fared? WHO South-East Asia J Public Health. 2018;7(1):18–23. doi:10.4103/2224-3151.228423.

2. Ministry of Health and Family Welfare, Government of India. India National Family Health Survey (NFHS4) 2015–16. Mumbai: International Institute for Population Sciences; 2017 (http://rchiips.org/nfhs/NFHS-4Reports/India.pdf).

3. National Health Policy 2017. New Delhi: Ministry of Health and Family Welfare, Government of India; 2017 (http://cdsco.nic.in/writereaddata/National-Health-Policy.pdf)

4. Shankar B, Agrawal S, Beaudreault AR, Avula L, Martorell R,Osendarp S et al. Dietary and nutritional change in India: implicationsfor strategies, policies, and interventions. Ann NY Acad Sci.2017;1395(1):49–59. doi:10.1111/nyas.13324.
5. Deaton A, Drèze J. Food and nutrition in India: facts and interpretations. Econ Polit Wkly. 2009;44:42–65. (https://www.princeton.edu/~deaton/downloads/Food_and_Nutrition_in_India_Facts_and_Interpretations.pdf).
6. Mehta R, Platt AC, Sun X, Desai M, Clements D, Turner EL. Efficacyof iron-supplement bars to reduce anemia in urban Indian women:a cluster-randomized controlled trial. Am J Clin Nutr. 2017;105(3):746–57. doi:10.3945/ajcn.115.127555.
7. Guidelines for control of iron deficiency anaemia. National Iron+Initiative. Towards infinite potential in an anaemia free India. NewDelhi: Ministry of Health and Family Welfare, Government ofIndia; 2013 (http://www.pbnrhm.org/docs/iron_plus_guidelines.pdf)
8. Pavord S, Myers B, Robinson S, et al. UK guidelines on the management of iron deficiency in pregnancy. Br J Haematol. 2012;156(5):588–600.
9. Centers for Disease Control and Prevention (1998). Recommendations to prevent and control iron deficiency in the United States.MMWR Recomm Rep Morb Mortal Week Rep Recomm Rep 47(Rr-3):1–29.
10. World Health Organization (2016) WHO recommendations on antenatal care for a positive pregnancy experience. Geneva, World Health Organization, Switzerland.
11. Tandon R, Jain A, Malhotra P. Management of iron deficiency anemia in pregnancy in India. Indian J Hematol Blood Transfus. 2018;34(2):204-215. doi:10.1007/s12288-018-0949-6.
12. Moretti D, Goede JS, Zeder C, et al. Oral iron supplements increase hepcidin and decrease iron absorption from daily or twice-daily doses in iron-depleted young women. Blood. 2015;126(17):1981–1989.
13. Tolkien Z, Stecher L, Mander AP, et al. Ferrous sulfate supplementation causes significant gastrointestinal side-effects in adults: a systematic review and meta-analysis. PLoS One. 2015;10(2):e0117383.
14. Lloyd C. Medical disorders associated with pregnancy: Myles Textbook for Midwives. 15. Edinburgh: Churchill Livingstone; 2009. pp. 361–396.
15. Biniwale P, Pal B, SundariT, Mandrupkar G, Datar N, et al. Liposomal iron for iron deficiency anemia in women of reproductive age: review of current evidence. Open Journal of Obstetrics and Gynecology. 2018;8: 993-1005. https://doi.org/10.4236/ojog.2018.811100
16. Akbarzadeh A, Rezaei-Sadabady R, Davaran S, et al. Liposome: classification, preparation and applications. Nanoscale Research Letters. 2018;8: 102.
17. Brilli E, Lipinski P, Barnadas R, Camacho M, Fabino A, Giordano G, et al. Sucrosomial Iron Absorption Involve M Cells Interaction. Abstract No. 2217. 59th Annual Meeting and Exposition, American Society of Hematology, 2017.
18. Yu PP, Chang YZ and Yu P. Iron Liposome: A more effective iron supplement for sports anemia and anemia of inflammation. Journal of Pharmaceutical Care & Health Systems. 2015;84, 1-3.
19. Xu Z, Liu S, Wang H, Gao G, Yu P and Chang Y. Encapsulation of iron in liposomes significantly improved the efficiency of iron supplementation in strenuously exercised rats. Biological Trace Element Research.2014;162:181-188. https://doi.org/10.1007/s12011-014-0143-0.
20. Parisi F, Fusè F, Brunetti M, Mazzocco M, et al. Effects of different regimens of iron supplementation on iron status and pregnancy outcomes in a cohort of healthy pregnant women: arandomized control trial. The Journal of Maternal-Fetal & Neonatal Medicine. 2017;30:1787-1792. https://doi.org/10.1080/14767058.2016.1224841.
21. Giulio Giordano, Sandro Barni et al. 4th Mediterranean Multidisciplinary Course on Iron Anemia April 29th–30th 2016, Madrid, Spain, Expert Review of Hematology. 2016;(9)sup1, 1-42.

# ROLE OF CALCIUM AND VITAMIN D IN PREGNANCY

**Dr Pradnya Supe MS IBCLC**

## Introduction

Calcium and Vitamin D metabolism plays a very important role in pregnancy. A number of physiological changes occur during pregnancy which affect calcium and Vitamin D levels are falling of albumin level, expansion of extracellular fluid volume, increase in renal function and placental calcium transfer. Calcium homeostasis mainly involves calcium and three calcitropic hormones - parathyroid hormone, calcitonin and 1, 25-dihydroxyvitamin D 3 (1, 25(OH) 2D).

Total serum concentrations fall during pregnancy due to haemodilution. This fall mainly occurs in albumin bound fraction of the total calcium and due to fall in serum albumin. Ionised calcium levels do not differ from that in non-pregnant women[1]. However, constant blood levels of calcium are maintained by homeostatic control mechanism. Calcium homeostatic response during pregnancy includes increase in intestinal calcium absorption, increase in urinary excretion of calcium and increase bone turnover.

There is little information on vitamin D intake in pregnancy and lactation and few studies on clinical outcomes. Some have suggested that the requirement for vitamin D in these women may be up to 6000 IU/day and the ideal vitamin D regimen to prevent and treat vitamin D insufficiency in utero is unknown.[2] There are now many studies which mention that not only is vitamin D important for embryogenesis and fetal skeletal development, but the prevalence of vitamin D deficiency in pregnancy causes adverse maternal and fetal outcomes such as gestational diabetes mellitus (GDM), preeclampsia, small for gestational age (SGA), preterm births among others.[3]

## Requirement in pregnancy and lactation

WHO recommends 1.5-2 gm elemental calcium/day daily, with the total daily dosage divided into three doses, preferably taken at mealtimes from 20 weeks' gestation until the end of pregnancy in all pregnant women, particularly those at higher risk of gestational hypertension in areas with low calcium intake.[4] ICMR recommends 1200mg/day of calcium intake for pregnant women.[5]

Vitamin D supplementation is not recommended during pregnancy to prevent the development of pre-eclampsia and its complications.[6] The newest guidelines mention that "vitamin D supplementation is not recommended for pregnant women to improve maternal and perinatal outcomes".[7] But in cases of documented deficiency, vitamin D supplements may be given at 5 µg (200 IU) per day as recommended by WHO/FAO or according to national guidelines.

Vitamin D may be given alone or as part of a multiple micronutrient supplement, to improve maternal serum vitamin D concentrations. The benefit of this intervention for other maternal or birth outcomes remains unclear. In 2010, the Food and Nutrition Board at the Institute of Medicine of the National Academies established that an adequate intake of vitamin D during pregnancy and lactation was 600 international units per day. Most prenatal vitamins typically contain 400 IU of vitamin D per dose.[8]

However, the Scientific Advisory Committee on Nutrition (SACN)[9] and the UK Department for Health recommend 400IU while Endocrine Society Practice Guidelines[10] recommend 600 IU of vitamin D/day in pregnancy women. To ensure a sufficient vitamin D supply to their fetus or infant, an intake of a vitamin D supplement at a dose of 800 to 1000 IU per day during preconception or pregnancy is sufficient to achieve serum 25(OH)D target concentrations.[11]

**Natural sources of calcium**

**Table 1 Bioavailability of calcium from selected foods[12]**

| | |
|---|---|
| Cauliflower, waterc ress, brussels sprouts , kale, mustard greens, broccoli, turnip greens | |
| Milk, calcium-fortified soy milk, calcium-set tofu, cheese, yogurt, calcium fortified foods and beverages | ≈30% Absorbed |
| Almonds, sesame seeds, pinto beans, sweet potatoes | ≈20% Absorbed |
| Spinach, rhubarb, Swiss Chard | ≤5% Absorbed |

**Supplemental sources of calcium**

There are many sources of calcium available for treatment of calcium deficiency. Milk, minerals comprise mainly of calcium phosphate; organic salts like tricalcium citrate, calcium lactate, calcium lactate gluconate and calcium gluconate and inorganic salts like calcium chloride, calcium carbonate and calcium phosphate.[13]

The selection of the appropriate calcium source for a specific application is usually based on the consideration of a number of properties associated with the respective product such as solubility, calcium content, taste and bioavailability. Economic considerations are an additional important factor. Many patients have difficulty swallowing large tablets or do not want to take multiple tablets to achieve the desired dose of calcium.[14]

The most common forms of calcium available are calcium carbonate and calcium citrate. Other forms of calcium include lactate, gluconate and hydroxyapatite. Calcium supplements are available as capsules, tablets, chews, powders, and liquids.

**Table 2 Oral calcium salts comparison**[14]

| Formulation | % Elemental Calcium(w/w) | Comments |
|---|---|---|
| Calcium carbonate | 40 | Provides the highest amount of elemental calcium. Most widely used.<br>Well absorbed and well -tolerated specially when taken with a meal.<br>Limited solubility and absorption in patients with high gastric pH. |
| Calcium citrate malate | 21 | Better absorption than calcium carbonate in patients with higher gastric pH.<br>Recommend for those on H2 - blocker or PPI, those suspected with achlorhydria, inflammatory bowel disease, or absorption disorders.<br>Can be taken on empty stomach.<br>More doses necessary to get the equivalent elemental calcium compared to calcium carbonate.<br>Does not increase the risk of kidney stones. |
| Calcium phosphates | 31-38 | Low solubility compared to calcium carbonate. |
| Calcium gluconate | 9 | Multiple doses need to be taken to get sufficient amount of elemental calcium. More soluble than calcium citrate . |
| Calcium lactate | 13 | Multiple doses need to be taken to get sufficient amount of elemental calcium. Similar solubility as calcium gluconate. |

**Choice of compound**

Calcium carbonate is the least expensive salt. It provides greater quantities of elemental calcium and therefore require fewer tablets than other forms of calcium. This therefore improves its compliance as compared to other salts. Calcium citrate can be used in individuals who have absorption disorders. Additionally, busy individuals who find it difficult to supplement at meals should use calcium citrate, which can be taken with or without food.[15]

Calcium citrate malate is formed from the calcium salt of citric acid and malic acid. Calcium citrate-malate contains about 26% elemental calcium. Its bioavailability is as high as 42% and it also has the highest bioavailability (consistently over 35%) across human studies; which may be possible due to its water solubility and its method of dissolution. It is considered the most effective vegetarian form of calcium. The special structure of calcium citrate malate makes it 6 to 9 times more easily dissolved in the stomach than plain calcium citrate, with an absorption rate of 36-37% in tablets and capsules, or higher if dissolved in orange juice. It is well-absorbed taken with or without food.[16] Calcium lactate and calcium gluconate are not considered for practical use due to minimal amount of elemental calcium they offer.

Coral calcium (calcium carbonate matrix) is derived from coral exoskeletons. It contains primarily calcium carbonate (20%) and magnesium (10%). Calcium from a coral source has not been proven to be better than calcium from other sources.[17]

**Dosage**

The maximum dose of elemental calcium that should be taken at a time is 500 mg.[14] Calcium supplements are generally well tolerated. Iron and zinc preparations should be taken two hours before or after calcium supplementation as it may decrease their absorption.

**Vitamin D sources and metabolism**

Vitamin D is a fat-soluble vitamin obtained largely from consuming fortified milk or juice, fish oils, and dietary supplements. It also is produced endogenously in the skin with exposure to sunlight. Vitamin D that is ingested or produced in the skin must undergo hydroxylation in the liver to 25-hydroxyvitamin D (25-OH-D), then further hydroxylation primarily in the kidney to the physiologically active 1,25-dihydroxyvitamin D. This active form is essential to promote absorption of calcium from the gut and enables normal bone mineralisation and growth. During pregnancy, severe maternal vitamin D deficiency has been associated with biochemical evidence of disordered skeletal homeostasis, congenital rickets, and fractures in the newborn.[18]

**Vitamin D preparations**

Vitamin D is commonly available in two forms, Vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D3 is available in the form of alfacalcidiol (25 hydroxycholecalciferol), calcitriol (1,25 dihydroxycholecalciferol) or cholecalciferol (inactive vitamin D). It is available in the forms of tablets, capsules, granules/sachets, syrups and soft gel capsules.

**Summary**

Calcium supplementation is required in pregnancy from 20 weeks onwards in divided doses. Calcium from carbonate and citrate should be the preferred forms for supplementation. Calcium carbonate is cost-effective but should be taken with meals to enhance absorption.

As regards vitamin D, although there is no consensus regarding the dosage and there is insufficient evidence to routinely recommend vitamin D supplementation for the prevention of preterm birth or preeclampsia.19 We should continue to supplement this nutrient in all pregnant women from the 12th week of gestation onwards. Daily doses of 1000-2000 IU can be recommended in all antenatal women in South Asia, without estimating serum 25(OH) D levels. Higher doses can be used in symptomatic antenatal women, and in those with documented severe deficiency.[20]

## References


1. Kumar A, Kaur S. Calcium: A Nutrient in Pregnancy. J Obstet Gynaecol India. 2017;67(5):313-318. doi:10.1007/s13224-017-1007-2.
2. Hollis BW. Vitamin D requirement during pregnancy and lactation. J Bone Miner Res. 2007;22 Suppl 2:V39-V44. doi:10.1359/jbmr.07s215
3. Vitamin D and the Promotion of Long-Term Metabolic Health from a Programming Perspective.Palaniswamy S, Williams D, Järvelin MR, Sebert S Nutr Metab Insights. 2015; 8(Suppl 1):11-21.
4. WHO. Guideline: Calcium supplementation in pregnant women. Geneva, World Health Organization, 2013.
5. Dietary guidelines for Indians, ICMR, National Institute of Nutrition Hyderabad, second Edition – 2011.
6. WHO. Guideline: Vitamin D supplementation in pregnant women. Geneva, World Health Organization, 2012.
7. WHO recommendations on antenatal care for a positive pregnancy experience 2016.
8. Institute of Medicine of the National Academies (US). Dietary reference intakes for calcium and vitamin D . Washington, DC: National Academy Press; 2010.
9. Scientific Advisory Committee on Nutrition. Vitamin D and Health. London: The Stationary Office; 2016.
10. Holick MF, Binkley NC, Bischoff-Ferrari HA, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society clinical practice guideline. The Journal of clinical endocrinology and metabolism. 2011;96(7):1911–30.
11. Pilz S, Zittermann A, Obeid R, et al. The Role of Vitamin D in Fertility and during Pregnancy and Lactation: A Review of Clinical Data. Int J Environ Res Public Health. 2018;15(10):2241. Published 2018 Oct 12.
12. Sharon Rady Rolfes, Kathryn Pinna, Ellie Whitney Understanding Normal and Clinical Nutrition, Eighth Edition, Chapter 12, Page 418.
13. Dairy Products: Dairy Products: the calcium challenge http://www.jungbunzlauer.com/fileadmin/content/_PDF/Dairy_Products__the_Calcium__challenge__Jun02.pdf
14. Deborah A. Straub. Calcium Supplementation in Clinical Practice: A Review of Forms, Doses, and Indications. Nutrition in Clinical Practice 2007; 22:286– 296.
15. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium, Food and Nutrition Board, Institute of Medicine. Dietary Reference Intakes for Calcium and Vitamin D. Washington, DC: National Academy Press, 2010.
16. Trailokya A,Srivastava A et all. Calcium and Calcium Salts;Journal of The Association of Physicians of India Vol. 65 ,February 2017.
17. https:// www.medicines.org.uk/emc/ medicine/28505#Undesirable_effects access on 7 September 2016.
18. Gale CR, Robinson SM, Harvey NC, Javaid MK, Jiang B, Martyn CN, et al. Maternal vitamin D status during pregnancy and child outcomes. Princess Anne Hospital Study Group. Eur J Clin Nutr 2008;62:68–77.
19. Vitamin D: screening and supplementation during pregnancy. Committee Opinion No. 495. American College of Obstetricians and Gynecologists. Obstet Gynecol 2011;118:197–8.
20. Mithal A, Kalra S. Vitamin D supplementation in pregnancy. Indian J Endocrinol Metab. 2014;18(5):593-596. doi:10.4103/2230-8210.139204.