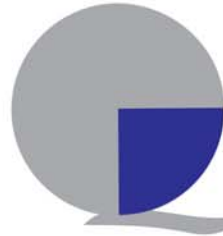


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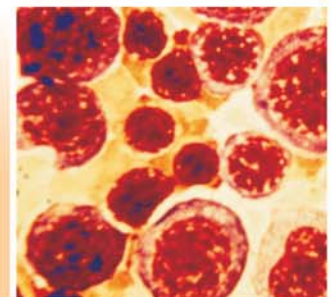
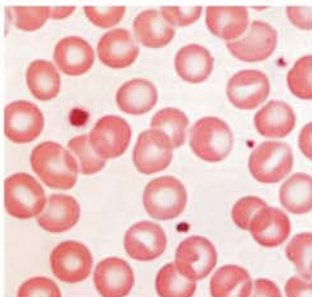
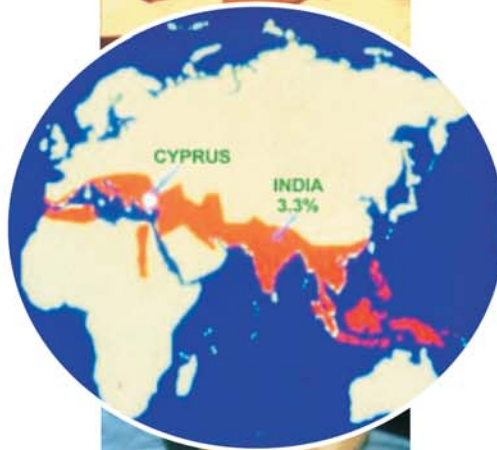
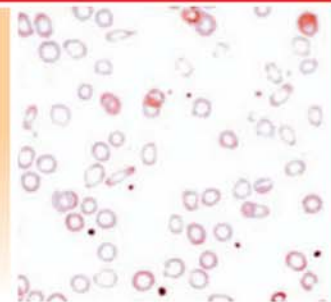


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Anemia in Children

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ANEMIA IN CHILDREN

Dr. M. R. Lokeshwar

INTRODUCTION

Anemia is global problem of immense public health significance. It is an ancient disease, and commonest chronic malady of mankind and seen all over the world. From public health viewpoint nutritional deficiencies account for the most anemic cases and iron deficiency is usually the most common cause of anemia

Anemia is defined as reduction of red blood cell volume or hemoglobin concentration, and Hematocrit below the range of values occurring in healthy persons, or two standard deviations below the mean for the normal population, age and sex.

Anemia is reduction in oxygen carrying capacity of blood as a result of :

- Decreased red cell mass,
- Decreased red Cell count / HCT
- Decreased Hb. concentration.

Anemia is not a specific entity but an indication of an underlying pathologic process or disease. Anemia is the commonest problem encountered in pediatric patients both in indoor as well as office practice.

- More than 30 % of the world population i.e. 1500 million people are suffering from anemia (1-6, 12-17). Most of them suffer from iron deficiency anemia which leads to tremendous loss of man power and productivity.
- The prevalence of iron deficiency anemia in developing countries like India has been found to be as high as 63% in 1-3 year age group and 44% in the age group of 3 to 6 years as per a study by ICMR in 1977.⁽³⁴⁾
- More recent report of the NFHS-2 shows that the prevalence has not much changed in 1998-1999 and is still 74% among children of 6-35 months of age.
- **In the adolescent period (10-19 years), in a multi center study, it has been found that the incidence of anemia is about 50% and increases from 10 years onwards and continues to remain high till 18 years of age.**^(18,26)

It has now been realized that iron deficient state without anemia is even more common. Besides anemia, iron deficiency leads to many other systemic changes; notable amongst them is its effect on growing brain where it has been shown to lead to cognitive dysfunction, which is sometimes permanent^(18,22,25,26). This is avoidable by simple treatment with iron which is cost effective and easily available, provided we realize the importance of treating infants with iron deficiency promptly and effectively.

Age specific indices

	Hgb (g%)	Hct (%)	MCV (fl)	MCHC (g/% RBC)	Retic (%)	WBC/ mm³ x 1000	P %	L %	M %	E %	Platelets (10 ³ /mm ³)
	Mean (-2SD)	Mean (-2SD)	Mean (-2SD)	Mean (-2SD)		Mean (+2SD)					
Term (cord)	16.5 (13.5)	51 (42)	108 (98)	33.0 (30)	(3-7)	18.1 (9-30)	61	31	6	2	290
1-3 days	18.5 (14.5)	56 (45)	108 (95)	33.0 (29)	(1.8- 4.6)	18.9 (9.4-34)	61	31	6	2	192
2 wks	16.6 (13.4)	53 (41)	105 (88)	31.4 (28.1)	-	11.4 (5-20)	45	41	9	4	252
1 month	13.9 (10.7)	44 (33)	101 (91)	31.8 (28.1)	(0.1- 1.7)	10.8 (4-19.5)	35	56	7	3	-
2 months	11.2 (9.4)	35 (28)	95 (84)	31.8 (28.3)	-	-	-	-	-	-	-
6 months	12.6 (11.1)	36 (31)	76 (68)	35.0 (32.7)	(0.7- 2.3)	11.9 (6-17.5)	-	-	-	-	-
6 mo-2 yr	12.0 (10.5)	36 (33)	78 (70)	33.0 (30)	-	10.6 (6-17)	32	60	5	3	150- 350
2-6 yrs	12.5 (11.5)	37 (34)	81 (75)	34 (31)	(0.5- 1.0)	8.5 (5-15.5)	42	50	5	3	"-
6-12 yrs	13.5 (11.5)	40 (35)	86 (77)	34 (31)	(0.5- 1.0)	8.1 (4.5-13.5)	54	38	4	2	"-

WHO cut off values for diagnosis of anemia at different ages (14).

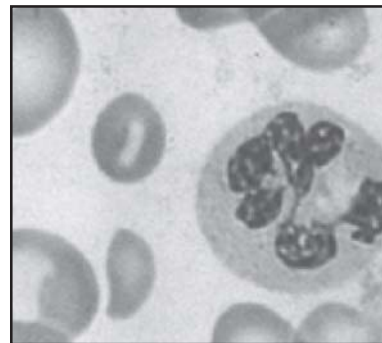
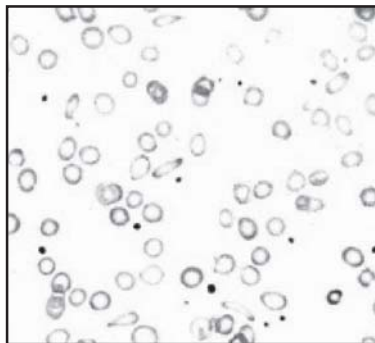
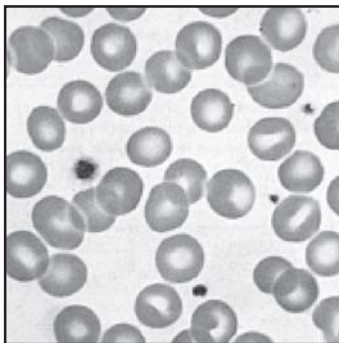
Age/Sex Group	Hb gm%
6 months - 6 yr	< 11
6-14 yr	< 12
Adult males	< 13
Adult females (non pregnant)	< 12
Adult females (pregnant)	< 11

W.H.O. CLASSIFICATION

- MILD ANEMIA - >10 gm%, BUT < NORMAL FOR AGE.
- MODERATE ANEMIA 7-10 gm%.
- SEVERE ANEMIA < 7 gm%.
- VERY SEVERE ANEMIA < 5 gm%

Morphological Classification	
• Normocytic Normochromic Anemia-	MCV- 80-94 m ³
• Hypo chromic Microcytic Anemia-	MCV- < 80 m ³ ,
• Macrocytic Anemia-	MCV-> 94 m ³ , MCHC < 32%

Normocytic Normochromic	Microcytic Hypochromic	Macrocytic(Hypersegmented) Neutrophils)
MCV-80-94 m³	MCV<80m³, MCH<20 Pg., MCHC < 32%	MCV > 94 m³, MCHC - normal



Anemia is not a specific entity but an indication of an underlying pathologic process or disease. A useful classification of the anemia of childhood divides them into two broad groups

Etiological (Pathological) Classification

<p>Those resulting from</p> <ul style="list-style-type: none"> • "Decreased or ineffective production of red blood cells or hemoglobin;" • "Increased Destruction or loss of red blood cells"
<p>Those resulting from</p> <p>"Decreased or ineffective production of red blood cells or hemoglobin;"</p> <p>◆ Marrow failure</p> <ul style="list-style-type: none"> • Aplastic anemia • Congenital • Acquired

- ◆ **Congenital-Decreased number of red blood cells in the marrow**
 - ◆ **One cell line affected**
 - Pure red blood cell anemias
 - Congenital – Congenital pure red bloodcell anemia (Diamond Blacktan syndrome)
 - Acquired – Acquired pure red blood cell anemia (e.g. TEC)
 - ◆ **More than One cell line affected**
 - Congenital
 - Fanconi's Anemia.
 - Dyscaratosis Congenita.
 - Acquired Aplastic anemia
 - ◆ **Marrow replacement**
 - Malignancies and infiltrative disorders,
 - Osteopetrosis,
 - Storage disorders (Gaucher, Nimennpick)
 - Myelofibrosis
 - ◆ **Deficiency of specific factors**

Megaloblastic anemia

 - Folic acid deficiency or malabsorption
 - B₁₂ deficiency - malabsorption or transport disorders,

Microcytic anemia

 - Iron deficiency
 - Pyridoxine responsive and x-linked hypochromic anemia
 - Lead poisoning, Copper deficiency.

Impaired erythropoietin production

 - Chronic renal disease
 - Hypothyroidism, hypopituitrism
 - Chronic inflammation, infection and malignancy
 - Protein malnutrition
- Increased Destruction or loss of red blood cells is the predominant mechanism".**
- ◆ **Blood loss**
 - Acute hemorrhage
 - Chronic hemorrhage
 - ◆ **Anemia due to increased destruction (Hemolytic anemias)**

Intrinsic defects - (Intra corpuscular defects)

Intrinsic abnormalities of the red blood cell Membrane defects

 - Hereditary spherocytosis / elliptocytosis / stomatocytosis
 - Defects in synthesis of hemoglobin Thalassemia syndrome-HbS, C, D, E etc alone and in combination Thalassemia.

- Enzymes of glycolytic pathway-pyruvate kinase, hexokinase, and others Enzymes of the pentosephosphate pathway and glutathione complex
 - o G6PD deficiency
 - o Paroxysmal nocturnal hemoglobinuria
- Immune hemolytic anemias.
 - o Extrinsic (Extra corpuscular) abnormalities-
 - o Allo & isoimmune hemolytic anemia
- Passively acquired antibodies (hemolytic disease of the newborn)
- Rh isoimmunization
- A or B isoimmune disorders
- Other minor blood group incompatibilities
 - o Active antibody formation
- Idiopathic autoimmune hemolytic anemia
- Evans' Syndrome
 - o warm and cold agglutinin diseases
 - o Symptomatic - lupus, lymphoma, Drug-induced
- Causes like (Miscellaneous)
 - o Microangiopathic anemia
 - o Infections
 - o Hypersplenism
- Non-immunologic disorders
- Toxic drugs, chemicals, Infections - malarial, clostridal

Modified from Nelson's Textbook of Pediatrics and Hematology of Infancy and Childhood Nathan & Oski

Symptomatology of anemia

Signs and symptoms are related to, not only Hb level but also rate of fall of Hb and haemostatic adjustment of various systems in the body. If fall of hemoglobin is gradual, the onset of symptoms is insidious as there is cardio respiratory adjustment. If the rate of fall of Hb is acute and rapid, child may be brought in serious condition, gasping, in congestive failure and this should be considered as medical emergency. Anemia is a manifestation of various diseases involving various systems.

Anemia may manifest as

- Bleeding from GI tract following ulcers, cancers
- Hepatosplenomegaly as seen in Thalassemia and abnormal hemoglobinopathies, autoimmune hemolytic anemia etc.

- CNS manifestations like hemiparesis as seen in sickle cell anemia
- May be associated with cardiac problems such as Bacterial Endocarditis and may manifest as congestive cardiac failure
- May be following involvement of kidney as in acute nephritis or hemolytic uremic syndrome

NUTRITIONAL ANEMIA IN INFANCY & CHILDHOOD

Nutritional anemia is anemia in which Hb concentration is below normal level due to deficiency of one or more nutrients needed for hematopoiesis and supplementation of involved nutrients increases the hemoglobin level to normal value.

Nutritional anemia is a major problem of immense public health significance, particularly in the developing world. It affects nearly two billion people, persons of all ages & economic groups, all over the world. Of these, 90% are in the developing world^(2,3).

Types of nutritional anemia - deficiency of

Iron	Proteins
Folate	Vitamin E
Vit. B ₁₂	Ascorbic acid

Iron deficiency anemia (IDA)

Prevalence

Iron deficiency is the most frequent and widespread nutritional deficiency in the world. It plays a vital role in formation of hemoglobin which is important for oxygen carriage in multicellular organisms like man. Iron is important in a number of iron dependent enzymes including the catalase, peroxidase, cytochromes and ribonucleotide reductase.

It is estimated that iron deficiency affects nearly 2170 million persons worldwide and 30% of the world population i.e. 1200 million of them suffer from nutritional anemia, of these, 90% are in the developing countries. Infants, pre-school children, adolescents and women of childbearing age are at greatest risk of developing anemia. **The reported prevalence of nutritional anemia in children varies from 44 to 74%**^(5-7, 10-17). Young children and pregnant women are most affected, with an estimated global prevalence of 43% and 51% respectively. The condition is more prevalent in developing countries (36%) than in industrialized developed countries (8%). India falls in the category of high prevalence for nutritional anemia In the adolescent period (10 -19 yrs), in a multicentric study, it has been found that the incidence of nutritional anemia is about 11 to 90% and increases from 10 years onwards and continues to remain high till 18 years of age.

With increasing age, the prevalence rate declines in males, and not in the females In pregnant women, the incidence of nutritional anemia varies from developed countries- Europe 18% to developing countries - South Asia 75%, South-East Asia 63% and in over 50% in Africa.

In India, anemia prevalence among pregnant women may be as high as 88% (38-88%). Iron requirements are higher during the second and the third trimester and iron balance during this period depends more on the adequate intake of bioavailable iron rather than the store at conception⁽¹⁷⁾.

The condition is more prevalent in developing countries (36%) than in industrialized developed countries (8%). Intestinal parasites are additional aggravating factors particularly for those from rural areas. Chakma T et al reported intestinal parasite in 50% of adolescents (Hook worm 16.3%, lumbricoid 18.5%) from Madhya Pradesh. Food fads, lack of knowledge of nutritional factors further adds to the problem⁽²¹⁾. **1624 babies Die Every Day Due to Iron Deficiency Anemia(The World HealthReport -Preventing Risks, Promoting Life, 2002)**

<p>Prevalance of IDA in children < 5 years:</p> <ul style="list-style-type: none"> • 75% India (NFHS- 2 1998-99) • 55% Bangladesh • 56% Pakistan • <5% Canada 	<p>Incidence in children</p> <p>1 - 3 years 63-74%</p> <p>3 - 6 years 44%</p> <p>School children 60 - 80%</p>
--	--

Normal full term infants at birth have 75 mg iron/kg body weight.

Trans placental blood transfusion occurs immediately after birth. Amount of blood contained in placenta is about 75 to 125ml, about 1/3rd of blood volume in normal child. After delivery, during the first 15 seconds about quarter and at the end of minute about half of the placental blood is transfused from placenta to newborn. Transfer of iron from mother to the child transplacentally occurs largely during 3rd trimester of pregnancy and hence premature babies have poor iron stores. Full term newborns exclusively on breastfeeding for first 4-6 months of life are unlikely to develop IDA due to better bioavailability of iron from the breast milk.

Table: Causes of Iron Deficiency

<ul style="list-style-type: none"> ◆ Decreased Iron Stores Preterms, small for dates, twins ◆ Decreased Intake/assimilation Inadequate intake of iron containing foods- Diet containing low iron or non-bioavailable iron. <ul style="list-style-type: none"> • Delayed weaning, malnutrition • Non breast-fed infants on cow's milk • Malabsorption syndromes, chronic diarrhea, Giardiasis etc., celiac disease, cow's milk allergy, GI surgery • Cow's milk allergy ◆ Increased requirement/Increased demands During periods of growth <ul style="list-style-type: none"> • Preterm infants, / LBW
--

- Toddlers, /Puberty, &Adolescence
- During reproductive age in females
- Pregnancy and lactation
- Recovery from P.E.M. (Protein Energy Malnutrition)

◆ **Increased losses**

Malabsorption of iron:

- Chronic diarrhea, /celiac disease
- Feto-maternal hemorrhage
- Repeated Blood sampling
- Intestinal parasites - hookworm infestation

◆ **Inadequate Transport**

- Atransferrinemia
- Anti transferrin receptor antibodies

STAGES OF IRON DEFICIENCY

The spectrum of iron nutrition status can be divided into 3 stages.

First stage:

It consists of a depletion of iron storage -

Iron Deficiency State.

This is characterized by

- Deficient iron store
- Decrease in the concentration of serum ferritin.
- The stores in liver, spleen and bone marrow are decreased.

Second stage Stage of iron-limited erythropoiesis -

Iron Deficiency without anemia:

- Milder form of iron deficiency
- Hb has not dropped enough to meet the criteria of anemia.
- Decrease in iron stores / Low serum iron levels
- Reduced transferrin saturation / Increase in total iron binding capacity (T.I.B.C.)
- But no anemia. Hb concentration is normal or in the low normal range

Third stage:

Stage of Iron deficiency anemia:

- Severer form of iron deficiency,
- Hemoglobin has dropped sufficiently to meet the criteria of anemia.
- Reduced iron stores /Low serum iron concentration &Low transferrin saturation

- Hb & hematocrit values also drop.
- FEP (free erythrocyte protoporphyrin) is increased;
- Microcytosis & hypochromia on peripheral smear.
- Associated with a low MCV (mean corpuscular volume)
- MCH (mean corpuscular hemoglobin)
- High RDW (red cell distribution width).

Sources and Contents of Iron

Major source of iron in diet are two types -

- Heme iron is present in meat, fish, poultry. Heme iron is better absorbed than non-heme iron.
- Non-heme iron.

Table : Sources of iron:

Foods rich in iron are

- Green leafy vegetables, (10-18 mg)/80-100Gm
 - Jaggery,
 - Cereals especially ragi, Cereals (4-11 mg/ 80-100Gm)
 - Pulses (9-11 mg)/80-100 gms
 - Dates, Almonds, Nuts,
 - Ripe banana (0.9 mg), Mango (1.3 mg) Melon (7.5 mg))/80-100 gms
 - Non-vegetarian sources such as Pork and Red meat, Liver. -Meat, fish, poultry (10-25mg/80-100 gms
 - Human milk (0.5 mg), Cow's milk 0.02-0.3 mg/litre
 - Though breast milk contains small amount of iron, the bioavailability of this iron (lactoferrin) is about 50 to 70% and hence, it is adequate for the first 4 to 6 months of life.
 - Additional sources - Cooking in ironpots, water
-

Factors affecting iron absorption :

The bioavailability of iron from a particular dietary source affects the amount absorbed. Heme iron is not affected by presence of any factors in the gut.

The absorption of non-heme iron is retarded by an alkaline pH, presence of phosphates, phytates, bran, starch, tannins, calcium, antacids, other metals (Co, Pb) etc. Phytates, which constitute 1-2% of many cereals, nuts and legumes, play a major role in the causation of nutritional anemia in the developing world. It is estimated that in the wheat millet based diet, iron absorption is around 2% and in rice-based diet, iron absorption is around 5-8%.

Calcium in the form of milk, cheese or (calcium added to the bread), depresses iron absorption and hence iron & calcium should not be given together. Iron absorption is enhanced by ascorbic acid, free hydrochloric acid, presence of sugars and amino acids in the diet, presence of heme iron (non-vegetarian source of iron) and EDTA. Ferrous iron is better absorbed compared to ferric iron. Fish, meat and poultry are good sources of iron and bioavailability is around 20-30%. Increasing the dietary intake to meet the caloric needs will also increase the dietary intake of iron by one third.

Mucosal Cell Control theory: The iron molecule that is taken into the mucosal cell across the brush border, can bind either to the apoferritin molecule or the ferroportin molecule in the mucosa. This is determined by the iron status of the body. If iron in the plasma is adequate, iron binds to apoferritin, which remains in the mucosal cell and gets denuded with the cell within 3 to 4 days. If iron is required in the body, it is bound to the ferroportin, which is then transferred to the transferrin (produced in the liver), which carries it across the mucosa. It is then utilized in the bone marrow for hemoglobin production, in the muscle tissue for myoglobin and in the body for various other enzymes. Any excess iron is stored in the form of ferritin in the liver. The RBCs circulate for their life span of approximately 120 days and are then destroyed in the spleen, liberating the free iron, which is then re-transported to the bone marrow and other tissues for its re-utilization. Thus, most of the iron is cycled continuously in the body, with only 1 to 1.5 mg/day of iron being excreted through the intestinal epithelial cells after completion of their life span. Since 10% of ingested iron is absorbed and the daily loss is only 1 to 1.5 mg, one needs to ingest about 10 mg of iron daily, except during periods of extra needs. Maximum absorption of iron occurs from the duodenum and it decreases as the food passes down the small intestine.

DAILY IRON REQUIREMENTS IN DIFFERENT AGE GROUPS

Pregnant females	30 mg/day
Females 11 yrs. - 30 yrs.	15 mg/day
Adult males	10 mg/day
Males 11 yrs.	12 mg/day
Up to 10 years	10 mg/day
Full term infants*	1 mg/kg/day from 4 months of age
LBW babies*	2 mg/kg/day from 2 months of age
Babies < 1000 gms*	4 mg/kg/day from 2 months of age
1000-1500 gms*	3 mg/kg/day from 2 months of age

* In the form of daily iron supplements.

Clinical Features of IDA

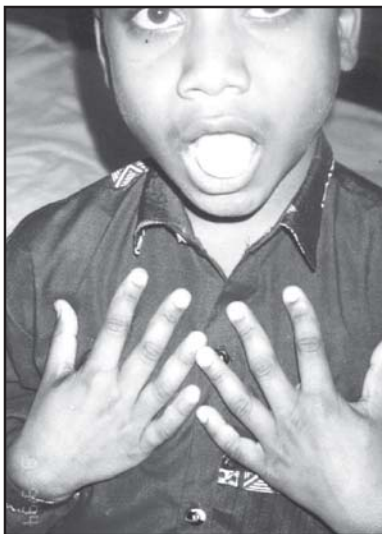
Common symptoms seen in infancy in childhood are vague Symptoms like

- Pallor, loss of appetite, anorexia,
- Failure to grow,
- Recurrent infection,
- Perspiration over the forehead, lethargy,
- Lack of interest, irritability,
- Mild degree of splenomegaly and hepatomegaly.
- As anemia progresses or in acute anemia, due to hyper dynamic circulation may lead to cardiac manifestation like tachycardia, dyspnoea, palpitation, shortness of breath, and symptoms of congestive failure
- Decreased exercise tolerance
- Pedal edema in IDA may be due to congestive heart failure, impaired renal function or associated protein deficiency.

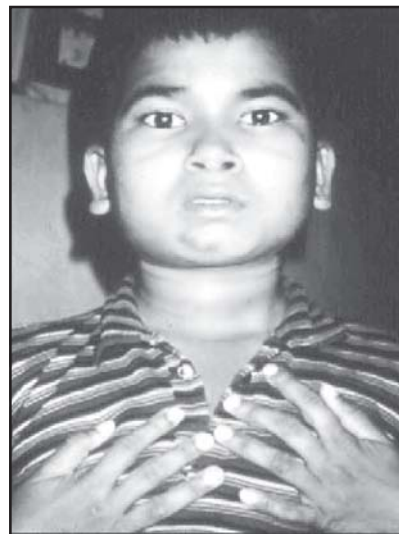
Non-hematologic consequences of iron deficiency -

It has been shown that iron deficiency per se even in the absence of anemia leads to several morphological and biochemical changes at tissue level with deleterious effect on various systems. Functional impairment of various tissues such as the **myocardium, peripheral nerves, intestinal mucosa, cerebral cortex, kidney and liver** etc. have been demonstrated in patients of iron deficiency, which have been corrected by iron therapy before a significant rise in the hemoglobin level

Pale Tongue in Child with Anemia



Koilonychia in Anemic Adolescent



School/growing children & adolescents with iron deficiency are at high risk of long-term impairment scores in IQ test. There are studies to suggest that children with iron deficiency have lower IQ scores, lack of concentration, distractibility, short attention span and impaired mental and motor development. Such deficits in cognitive functions may eventually result in scholastic backwardness and school dropouts. Unfortunately, the developmental deficits that occur due to iron deficiency in infancy have been shown to be irreversible. Thus prevention of IDA in infants and growing children is an urgent need for improving mental abilities and cognitive functions of our children. **Neurological changes that occur due to iron deficiency may be long-term or even irreversible.**

Systematic review of randomized controlled trials has shown that iron supplementation improves mental development score in children above 7 years of age and in initially anemic or iron deficient anemic subjects. However, there was no convincing evidence that iron treatment has an effect on mental development in children below 27 months of age or on motor development⁷.

Iron deficiency also adversely affects the immune system, thus increasing susceptibility to infection. It is believed that IDA children have increased susceptibility to infection due to immunosuppression. Humoral, cell-mediated and non-specific immunity and the activity of cytokines which have an important role in various steps of immunogenic mechanisms are influenced by iron deficiency anemia.

Another area of special significance is poor endurance and physical fitness even with mild anemia, probably due to lower myoglobin production. Anemic children have poorer endurance capacity and lack physical fitness³ In a study on effect of anemia on physical performance of children done at Baroda, it was seen that anemic children were using higher heart rates for the same level of work compared to normal children and were having a poorer endurance capacity.⁽²⁵⁾

Other well documented feature associated with IDA is Pica

- Geophagia (eating mud),
- Amylophagia (eating starch),
- Phagophagia (eating ice),
- Altered appetite like increased desire to eat salt, cardboard, coal are seen in 60-80%
- Cured by iron therapy.

Mild splenomegaly is seen in 10 to 15 percent of patients. Pedal edema in IDA may be due to congestive heart failure, impaired renal function or associated protein deficiency. Rarely increased intracranial tension with papilledema may occur. Skull changes similar to those seen in congenital hemolytic anemia may be seen in children with iron deficiency since early life. These skeletal changes do not reverse with iron therapy.

DIAGNOSIS OF IRON DEFICIENCY ANEMIA-

Practical and commonly used tests for diagnosing IDA are based on

- Evaluation of Red cell indices & P.S. Examination
- Evaluating the iron in the plasma compartment, RBC compartment and iron stores.

Evaluation of Red cell indices & P.S. Examination

Automated red cell indices:

Cell counters have made the estimation of red cell indices easy to perform as well as accurate and reproducible. They analyze a host of indices including **Hb, hematocrit, and RBC count, MCV, MCH, MCHC and RDW** (Red Cell distribution width) **which are important in the diagnosis of IDA.**

Hb, RBC count and indices including reticulocyte count, peripheral smear examination are initial tests.

- Low hemoglobin / Low hematocrit
- Reduced red cell count
- MCV, MCH, MCHC are reduced in IDA, whereas RDW (Red Cell Distribution Width) increased unlike in anemia of chronic infection and thalassemia where they are normal (table).

Red cell indices may not be altered in mild iron deficiency anemia, however, with ongoing iron deficiency, these are reduced as follows:

MCV < 80 fL, MCH < 27 pg and MCHC < 33 % is the last of the indices to be affected and is the least important in diagnosis of IDA. MCV is more sensitive than MCH in diagnosis of IDA.

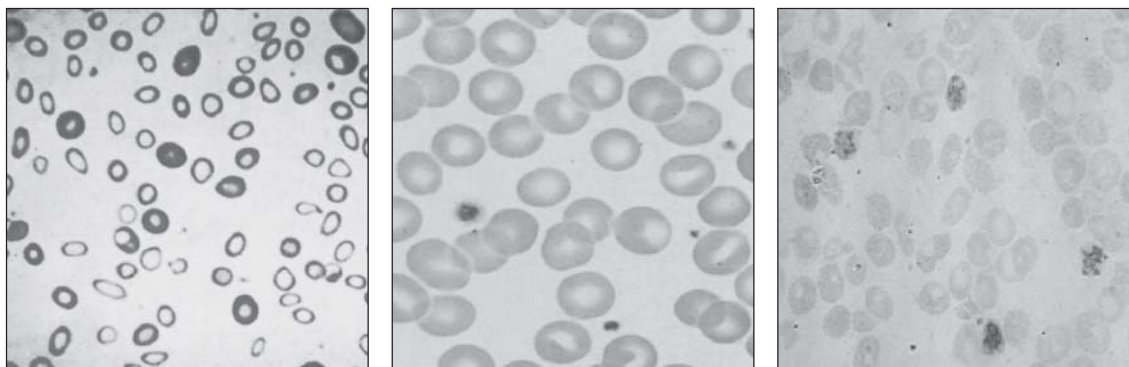
RDW is the quantitative measure of anisocytosis i.e. variability in RBC size. Increased RDW > 14 % by CV (Normal 13.4 + 1.2%) is observed. Normal or low RDW values are unlikely to be present with IDA.

Following iron therapy there is reticulocytosis which peaks at 1-2 weeks. However, mild cases are far more difficult to diagnose in which Hb may not be more than 1 gm/dl below the reference range. Such mild anemia may be seen in other conditions too and thus there is need for a screening test which is both sensitive and specific. Reticulocyte count is usually normal, however it may be increased / normal or decreased depending upon whether patient's on treatment, had recent blood loss which was acute or long standing. Screening tests are more useful in populations with high incidence of anemia. White cell counts are normal and platelet count may be increased or normal.

Table : Screening Test for Iron Deficiency

Age (Yrs) / Sex	Hb gm%	HCT %	MCV μ^3	MCH Pg.
0.5 - 4 (Female)	11.0	32.0	72.0	24.0
5 - 10 (Male)	11.0	33.0	75.0	25.0
11 - 14 (Female)	11.5	34.0	78.0	26.0
(Male)	12.0	35.0	78.0	26.0
15 - 19 (Female)	12.0	35.0	79.0	27.0
(Male)	13.0	39.0	79.0	27.0
20 - 24 (Female)	12.0	35.0	80.0	27.0
(Male)	13.5	40.0	80.0	27.0

Peripheral blood smear: shows microcytic, hypochromic anemia with significant anisocytosis and Poikilocytosis. Hb, RBC count and indices including retic count, peripheral smear examination are initial tests



Microcytic Hypochromic
red blood cells

Normal R.B.Cs

Reticulocytosis

When to suspect IDA?

- Anemia during age of rapid growth i.e. first 6 months - 3 years of life, premature babies and adolescence and in pregnant or lactating women.
- Child not breast fed but fed with faulty diet, especially improper weaning, prolonged breast feeding, bottle feeding.
- Irritable, cranky child, breath holding spasm, H/o pica, worms infestation, chronic bleeding.
- Microcytic, hypochromic anemia on Peripheral Smear.

How to prove IDA?

- CBC showing Microcytic Hypochromic anemia with low MCV & MCH with Increase in RDW on counter
- Decrease in serum iron, increase in TIBC, decrease in TS (< 16%), decrease in serum ferritin, increase in FEP.
- Therapeutic test: Increase in reticulocytes at 1-2 weeks, Increase in Hb so as to reach normal Hb levels by 2-3 months.

TESTS FOR PLASMA IRON COMPARTMENT:

Serum iron studies (32): It includes serum iron, total iron binding capacity (TIBC), transferrin saturation (SI)

Serum Iron: It's major drawback is **diurnal variation after 3 years of age**. Normal serum iron level varies considerably. It has a diurnal variation with a peak in the morning and trough in the evening. Hence if **morning sample shows a serum iron < 30 µg/dl, it is suggestive of IDA**. Serum iron concentration may also be affected by chronic infection, malignancies and chemotherapy

as well as iron medication. **Values below 40 mcg/dl (<12 mcg/dl in young children) are considered diagnostic** of iron deficiency (in absence of infection or other disorders which affect iron metabolism).

Total Iron Binding Capacity (T.I.B.C.) & Transferrin Saturation (T.S.): TIBC is the measure of plasma transferrin, which is free, not bound to iron. **The normal value of TIBC is 250 to 350 mcg/dl.** Since **serum iron is about 100 mcg/dl, normally** only one-third of transferrin is utilized to bind iron, giving a **normal transferrin saturation of 33%.**

An increase in TIBC is indicative of IDA. In iron deficiency states, TIBC is increased (> 350 mcg/dl) and transferrin saturation is reduced to below 16% (<12% for children) whereas it is normal in anemia of chronic infection.

TS: It is the ratio of above two values and is consistent and hence is a very useful test for IDA.

$$\text{TS} = \frac{\text{Sr. Iron}}{\text{TIBC}} \times 100$$

It is important to realise that while performing iron studies one should collect fasting sample (non-lipemic). All forms of iron supplements should be stopped for 48-72 hours before collection.

Free Erythrocyte Protoporphyrin (FEP) and Protoporphyrin: Heme (P:H) ratio: Deficiency of iron results in the accumulation of free erythrocyte protoporphyrin (FEP) the precursor of heme, in red blood cells when it has insufficient iron to combine with to form heme. It is measured by a Hemofluorocytometer. The blood can be conveniently tested by putting a drop of blood on a cover slip/ glass slide and reading the result directly. Normal values of FEP are 30-40 mcg/dl RBC and P:H ratio 16 (+5.3). Elevation of FEP mainly EZP (Erythrocyte zinc porphyrin level is an early & sensitive indicator of iron deficiency. FEP is markedly elevated in lead poisoning and in IDA it is moderately raised EZP is also elevated in chronic lead poisoning and sideroblastic anemia. This is not used regularly due to the cost of the machine and problems of standardization. Advantage of FEP is that unlike serum iron studies, FEP values are not altered immediately after iron therapy.

Both FEP and P: H ratio is elevated in iron deficiency. FEP values above 70 mcg/dl RBC and of P:H ratio above 32 is thought to represent iron deficiency. In uncomplicated iron deficiency anemia, red cell FEP levels may range from 100 to 1000 µg/dl. Free Erythrocyte Protoporphyrin (FEP) and Protoporphyrin: heme (P:H) ratio -FEP. Both are elevated in iron deficiency.

Table: Confirmatory Tests for I.D.A.

Age in years	Serum ferritin (ng/dl)	Transferrin saturation (%)	RBC free erythrocyte protoporphyrin (µg/dl)
0.5-4	< 10	< 12	> 80
5-10	< 10	< 14	> 70
11-14	< 10	< 16	> 70
> 15	< 12	< 16	> 70

TESTS FOR THE STORAGE COMPARTMENT:

The **serum ferritin** is a sensitive laboratory index of iron status. Serum ferritin is increased in infections, inflammation, liver disease, parasitic infestations, enteric infections and even upper respiratory tract infections. It is estimated that each ng/ml of serum ferritin is equivalent to 8-10 mg of storage iron. A level of < 10 micro gm/L in children and < 12 micro g/L in adults is suggestive of iron depletion but gives no information about its magnitude. Ferritin levels are estimated by radioimmuno assay (RIA), or ELISA Techniques.

One major limitation of serum ferritin is that its level is increased in chronic disorders e.g. chronic infection and inflammation, malignancies, chronic liver disorders. In presence of any of these, a coexisting iron deficiency anemia can be missed. Serum ferritin though decreased in IDA (< 15 ug/dl), it may be increased when associated with infection including cold & URTI or with liver diseases.

Soluble Transferrin Receptor (STfR):

Transferrin receptors (TfR) facilitate the entry of transferrin bound iron into cells by a process of endocytosis. STfR increases with enhanced red cell production but iron deficiency is the only disorder in which there is increased serum receptor combined with a low level of red cell production. In iron deficiency anemia, transferrin receptors are increased due to an increased turnover associated with ineffective erythropoiesis. Unlike the serum ferritin, which only identifies iron deficiency, STfR measures its severity. STfR is based on ELISA assay and requires only a few microliters of plasma or serum. The normal reference value 2.8 to 8.5 mg/L. Values above 9 mg/L are considered abnormal.

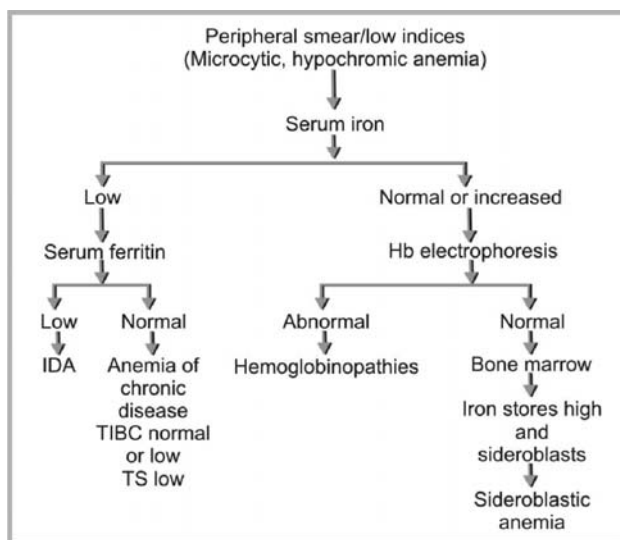
Bone marrow examination

“Bone marrow aspiration is not recommended for the diagnosis of IDA, as there are simpler, non-invasive and relatively inexpensive tests, which diagnose IDA reasonably well.” **Bone marrow iron staining, though a gold standard, is very painful, expensive and cumbersome to perform.** However, Bone marrow when done shows increased cellularity with micronormoblastic erythroid hyperplasia though the normoblasts may be smaller than normal (micronormoblasts). Normally 10% and more normoblasts will have iron granules in the cytoplasm. These iron granules are in the form of hemosiderin. On staining with Prussian blue (Perl's reaction), there is little or no stainable iron seen.

Routine stool examination and urine examination must be done to rule out associated helminthiasis, occult blood in stool and RBC, pus cells and hematuria in urine. **Mantoux test is useful in ruling out primary complex and tuberculosis.** Reticulocyte hemoglobin content may become a new method for diagnosis of IDA and automated hematology instruments may make it possible. **Molecular genetics of iron deficiency**-Human transferrin gene show many types of polymorphism and it has been reported that human transferrin G2775 mutation is a risk factor for iron deficiency.

"Response to therapy, Therapeutic Test"- In uncomplicated IDA, administration of iron shows a predictable reticulocytosis and a rise in hemoglobin. Hb concentration remains the most dominant predictor of response to therapy in uncomplicated iron deficiency.

Approach to Anemia Hypochromic Microcytic Type



Other causes of hypochromic microcytic anemia -

- Thalassemia minor
- lead poisoning
- associated infection
- sideroblastic anemia
- wrong diagnosis

Treatment of iron deficiency anemia

Basic principles of management include

- Correction of anemia
- Treatment of underlying cause

Treatment of iron deficiency anemia depends upon the severity and associated complications.

- Those with very severe anemia and/or congestive heart failure, with a Hb < 5 g/dl require hospitalization.
- Oral iron therapy with monitoring is adequate for those without evidence of congestive heart failure.
- Packed red cell transfusion is required for those in congestive heart failure irrespective of the level of hemoglobin. A small dose of packed red cells (5 ml/kg) should be slowly administered over 2 to 3 hours to avoid volume overload.
- A small dose of furesomide may be administered before transfusion.
- Blood transfusion is required only in most severe cases with Hb concentration <3 g/dl.

Medicinal Iron Therapy

Oral iron therapy is the ideal treatment for IDA. It is safe, economical & as effective as parenteral therapy. For infants and children, the recommended therapeutic dose is 3 to 5 mg of elemental iron

per kg body weight per day. Higher doses are unnecessary & may increase side effects, reducing patient compliance. Although the desired Hb level is usually reached in 2 months, iron therapy should continue for another 2 to 3 months to build up iron stores.

National Nutritional Anemia Control Programme (NNACP) recommends two tablets of iron folate tablet per day (each tablet containing 100 mg of elemental iron and 500 mcg of folic acid) for a minimum of 100 days⁽³⁹⁾. Although the desired Hb level is usually reached in 2 months, iron therapy should continue for another 2 months to build up iron stores to 250-300 mg or the serum ferritin level to 30 mcg/l.

Iron Preparations

All dietary iron has to be reduced to ferrous form to enter the mucosal cells. Bivalent iron salts like ferrous sulfate, fumarate (33% elemental iron) gluconate have been preferred over ferric salt preparations. Other ferrous salts include lactate ferrous succinate, glycine sulfate, glutamate, citrate, tartrate and pyrophosphate. These compounds, in addition to being more expensive, offer no advantages over ferrous fumarate, gluconate or sulfate.

Usually ferrous preparations should be taken on empty stomach as their absorption is affected by food. Uncoated & sugarcoated tablets are cheapest & disintegrate in stomach. However, they are quickly oxidised on exposure to humid conditions & hence are less stable. Enteric coated tablets are more expensive, disintegrate only partially in gastric acidity. However, side-effects are minimal, therefore compliance is better. However Enteric-coated tablets may not disintegrate easily in stomach.

Iron in the form of hemoglobin has little advantage over other iron preparations, and fails to provide the daily therapeutic requirement in the recommended doses. A novel approach currently under study is 'sprinkler' which contains microencapsulated FeSO_4 or microencapsulated ferrous fumarate. These can be sprinkled on any complementary food at the table given by caregiver. Iron being encapsulated does not change the color and the taste of the food.

Combination of other nutrients-

Ascorbic acid in the dose of 100 mg /15 mg elemental iron enhances absorption by 30%. But it is expensive and increases side-effects. Folic acid can be combined with iron at negligible extra cost. Vitamin B₁₂ may need to be given to non-responders or those with evidence of megaloblastic anemia. There is however no rationale in combining iron with multitude of vitamins, minerals and other hematinics in public health programs.

Newer Iron Preparations:

Iron polymaltose complex (IPC) and Carbonyl iron are newer oral preparations of iron which have shown variable efficacy in different studies. Iron Sucrose and iron gluconate are newer intravenous preparations available today, but there is limited experience with these compounds, though they are reported to have lesser side effects compared to iron dextran.

Table- Iron Amount and Percentage Iron Absorption

Preparation	Elemental iron		Absorption
	(mg) per tablet	(mg) per tablet	
Ferrous Fumarate	200	66	33
Ferrous Gluconate	300	36	12
Ferrous Sulfate (7H ₂ O)	300	60	20
Ferrous Sulfate, (anhydrous)	200	74	37
Ferrous sulfate, (1H ₂) (exsiccated)	200	60	30

Side effects

Intolerance to oral iron is basically related to the amount of iron in the gut. Usual side effects are **nausea, vomiting, constipation, diarrhea and abdominal discomfort**. Difficulty of sustaining motivation for two to three months in subjects who do not perceive themselves to be ill is a great challenge. Patients discontinue therapy as soon as they feel better and/or experience discomfort with the medications. Non-compliance is stated to be more often due to poor counseling and lack of motivation. Iron taken with a meal is better tolerated, though absorption is reduced. The required amount may also be given in two divided doses, which will reduce side effects. In our experience dose given at bedtime 1-2 hours before sleep is better tolerated (reduced gut motility improves absorption and reduces side effects) & has better compliance.

A positive response to therapy can be defined as a

- Daily increase in hemoglobin concentration of 0.1 g/dl (0.3 to 1% rise in hematocrit) from the fourth day onwards.
- Reticulocytes increase within 3 to 5 days and reach a maximum at 5-10 days;
- Reticulocyte counts being 8-10 % in severe anemia.
- The maximum rate of recovery from severe anemia in a child may be 0.25 to 0.4 g/dl per day increase in Hb or a 1% per day rise in hematocrit, which is more rapid than is anticipated in the adult.

Daily Versus Weekly Supplementation

Iron absorption from GI tract depends upon iron content in the mucosal cells. More the absorption less the iron content. Iron administration every 3rd day (intestinal mucosal turn over time in rat is 3-4 days) was more efficient in iron deficient rats than when administered daily.^(35,37,40)

In humans, the intestinal mucosal turnover time is 5-6 days and serves as the basis for the weekly preventive supplementation regimen; one of the major problems with daily supplementation regimen is that supplements must be taken for a long period of time for achieving desired improvement in iron status. With daily administration of iron, gastrointestinal complaints were more common in

majority of the studies and were rare with weekly dose. A recent meta-analysis on utility of weekly iron supplementation concludes that this modality provides therapeutic and prophylactic benefit. The effect on hemoglobin is only marginally lower than daily supplementation. In the public health scenario, weekly supplementation has the advantage of being offered under supervised conditions. On the basis of a recent multicentric study in India, national consultation has now recommended that adolescent girls on attaining menarche should consume weekly dosage of one IFA tablet containing 100 mg elemental iron and 500-mcg folic acid accompanied by appropriate dietary consumption.

Parenteral Iron Therapy

There is no evidence that the rate of Hemoglobin response is different in oral or parenteral therapy.

Parenteral route should be used only in definite indications:

- Severe intolerance to oral iron
- Anemia not responding to oral iron.
- Poor compliance
- Gastrointestinal bleeding aggravated by oral iron therapy.
- Bleeding more than the increase in hemoglobin with oral iron
- Pre-operative in urgent surgeries
- Malabsorption syndromes (proven)

Iron dextran complex is most commonly used preparation. Newer preparations include iron gluconate, iron sucrose etc. But experience with these compounds is limited.

Adverse Reactions- Apart from severe anaphylactic reaction and death, other common reactions include fever, vomiting, abdominal cramps, pain in abdomen, and skin rash at local site (when given IM), arthralgia, myalgia, and a serum sickness like picture.

Total dose needed can be given including replenishing of stores, calculated by the following formula:

Iron (mg) = weight (Kg.) X Hb. Deficit (g/dl) X 80/100 X 3.4 X 1.5 or Weight (Kg.) Hb. Deficit (gm./dl) X 4.

Response to Therapy

Iron absorption, is maximum during the initial phase of therapy and declines from 14% in the 1st week to 7% in the 4th week to 2% after 4 months. A positive response to therapy can be defined as a daily increase in hemoglobin concentration of 0.1 g/dl (0.3 or 1% rise in hematocrit) from the fourth day onwards. Approximately, 2 months are required to achieve a normal Hb level. Reticulocytes increase within 3 to 5 days and reach a maximum at 5-10 days, reticulocyte counts being 8-10% in severe anemia. The maximum rate of recovery from severe anemia in a child may be 0.25 to 0.4

g/dl per day increase in Hb or a 1% per day rise in hematocrit which is more rapid than is anticipated in the adult.

Table: Response to iron therapy in iron deficiency anemia

12-24 h	Replacement of iron enzymes; subjective improvement; decreased irritability; Increased appetite
36-48 h	Initial bone marrow response; erythroid hyperplasia
48-72 h	Reticulocytosis, peaking at 5-7 days
4-30 days	Increase in Hb level
1-3 mo	Repletion of stores

Causes for poor response to oral iron therapy

- Poor compliance
- Inadequate dose
- Associated infection
- Occult hemorrhage

Strategies for prevention of iron deficiency anemia

Basic approach to prevention of IDA includes

1) Education and associated measures to increase dietary iron intake

Dietary modification

Exclusive breastfeeding is recommended till the age of 4 to 6 months. Premature babies and growing babies should be given oral iron supplementation after the age of 2 months. Avoid bottle feeding. Introduction of iron containing weaning foods like cereals, vegetables, fish, egg, minced mutton, chicken should be started after the age of 6 months. An infant taking 600-650 ml of breast milk daily ingests approximately 0.3 mg. of iron/day. However, the bioavailability of this iron is quite high, as much as 0.15 mg of iron per day is absorbed which is sufficient for an exclusively breast fed baby. From 6 months of age the iron requirement increases markedly and hence the iron from breast milk alone is no longer sufficient.

Older children: encourage diet containing iron like sprouted cereals, consumption of green and leafy vegetables, non-vegetarian food like mutton, chicken, fish, egg and liver preparations. Encouraging the **timely introduction of iron containing weaning food** is an important step in prevention of anemia in early infancy and childhood. Introduction of iron containing food after the age of 4-6 months is the most important step in prevention of anemia of infancy. Staple food may be fortified with iron.

Supplementation of medicinal iron. In developing countries like India, prophylactic supplementation of oral tablets of iron (**20 mg iron + folic acid 10 µg**) **once a day every year for 100 days has been** recommended by National Nutritional Anemia Control Program

Other health measures -

Helminth control (Deworming), prevention of malaria is essential. Salt and food fortification particularly with salt (1 mg of iron/gm of salt) is one of the effective ways to control IDA. Commercially prepared iron rich weaning foods though available in developed countries, are very expensive and beyond the reach of the majority of the families and so not recommended. **Home made weaning foods** rich in iron and vitamin C (such as cooked vegetables, raw fruits) are not difficult to prepare at home and hence parents should be taught how to prepare mashed vegetables, citrus fruit juices, egg preparation, minced mutton etc. and motivated to introduce these to the infants in early life after 6 months of age.

Cooking of the food in iron pots may increase the iron content of a meal several fold. This is especially true for soups containing vegetables. Frying in iron pans does not increase the food's iron content. In developing countries where meat intake is low, vit. C (ascorbic acid) is the single most important enhancer of iron absorption. Adding as little as 50 mg of ascorbic acid to a meal, will double the iron absorption (an orange or lemon, or cabbage 100 g or 200 gm. of amaranth will provide sufficient amount of Vit. C).

Food based intervention

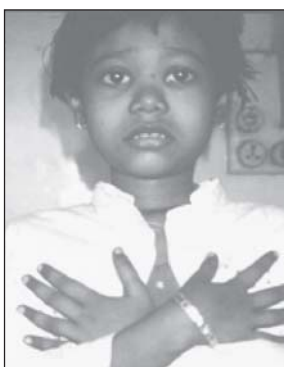
Fortification of suitable food vehicles with absorbable forms of iron is a highly desirable approach in controlling iron deficiency. It is possible to fortify a staple food that is consumed in significant quantity regularly by most people. Another approach is to fortify a widely consumed condiment - fish sauce, curry powder, salt, sugar and have all been successfully fortified with iron.

Ferrous fumarate, ferrous gluconate, lactate and ferrous sulphate have been extensively used for the fortification of wheat flour, bread and other bakery products, corn-soya-milk preparation (CSM) salt, sugar, fish sauce, rice, etc. The combined use of ferric orthophosphate and sodium acid sulfate or ferrous sulphate, orthophosphoric acid in the fortification of table salt has recently been reported to produce acceptable long term bioavailability with only slight discolouration. This gives additional 10-15 mg of iron to adult per day. Iron salt EDTA (ethylene diamine tetra acetate) has been successfully used to fortify sugar / wheat flour in Gambia (13 mg of iron / 100 mg sugar).

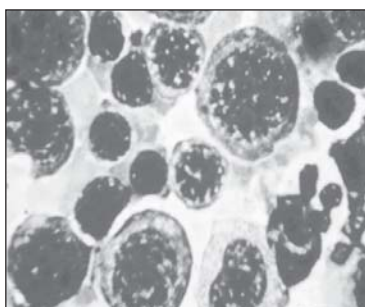
Basic approach in prevention of IDA should include education and associated measures to increase the dietary intake of iron, dietary modification to enhance the iron absorption, fortification of food articles, in addition to control the infection and worm infestation. Supplementation with medicinal iron is key to success which can be achieved by daily or intermittent (Bi weekly/Weekly) administration of oral iron to target group. Reduction of nutritional anemia should receive top priority through proper planning by using better utilization of existing health infrastructure.

MEGALOBLASTIC ANEMIAS

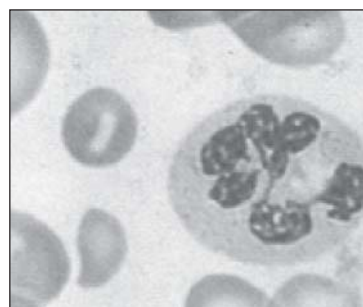
Megaloblastic anemia in children is mostly nutritional and could be due to deficient B₁₂ and folate in the diet.



Adolescent with
Megaloblastic Anemia



Bone marrow of
Megaloblastic Anemia



P.S. of Child with
Megaloblastic Anemia showing
hypersegmented neutrophils

Causes of nutritional megaloblastic anemia

- Decreased intake: Commonly seen with vegetarian diets as it is destroyed by heating and cooking.
- Exclusively breastfed infants of mothers with deficiency.
- Children fed with goat's milk particularly in villages, as goat's milk is deficient in folic acid.
- Malabsorption syndrome and chronic diarrhea, failure to secrete intrinsic factors.
- Chronic intestinal diseases like Crohn's disease, celiac disease, regional ileitis.
- Drugs like phenytoin and pyrimethamine and those containing drug combinations.
- Increased demand during rapid growth, (during infancy, in premature babies, adolescents), recovery from PEM and chronic hemolytic anemia (thalassemia, sickle cell anemia and Spherocytosis).

Clinical features ⁽⁴³⁻⁴⁹⁾

Common symptoms include pallor, failure to grow, irritability, apathy, fatigability, restlessness and lethargy. Older children manifest with sore red tongue, atrophy of papillae, angular stomatitis, glossitis and diarrhea. Hyperpigmentation of knuckles, hands and around the lips is diagnostic. Persistent chronic progressive deficiency may lead to mental apathy, failure to thrive, regression of milestones and tremors. Vitamin B₁₂ deficiency may lead to neurological abnormalities or degeneration and demyelination of peripheral nerve leading to peripheral neuropathy, involvement of selected column in the spinal cord and defective cerebral function. Folate deficiency does not cause neuropathy

Investigations

High index of suspicion and proper evaluation of peripheral smear are helpful in early diagnosis. Peripheral smear shows macrocytosis (large RBCs) and hyper segmented Neutrophils (usually more than 5 lobes seen in more than 3 percent of neutrophils). RBC indices show increased MCV (> 95m³). It may be associated with Leukopenia and thrombocytopenia and rarely purpura. Bone marrow examination confirms the diagnosis and there are increased megaloblasts in bone marrow aspiration smear. If facilities are available, estimation of B₁₂ and folic acid should be done.

Treatment (46,48,51)

- Requirement of folate is 100 to 200 μg daily. Therapeutic dose is 1 to 5 mg of folate/daily for 2 to 3 weeks for therapeutic response and for replenishment of the stores. Encourage folate containing diet, fresh leafy vegetables, legumes, nuts and meat. Goat's milk is deficient in folate.
- Dose of vitamin B₁₂ is 100 to 200 μg given IM on alternate days or oral dose of 100 μg /day for 2 to 3 weeks
- Treatment with folate alone can produce hematologic response in B12 deficiency, but does not correct the neurological damage caused by vitamin B₁₂ deficiency. Thus all megaloblastic anemias should be treated with adequate doses of both folate and vitamin B₁₂.
- Anemia unresponsive to folate or B₁₂ may be caused by certain metabolic disorders or anti-metabolic drugs.

THALASSEMIA SYNDROMES

Thalassemia syndromes are a heterogeneous group of single gene disorders, inherited in an autosomal recessive manner, prevalent in certain parts of the world posing a major health problem.⁵³

Thalassemia Syndromes refers to a group of blood disorders characterized by defects in the synthesis of one or more of globin chains that forms Hb tetramers. They result in reduced or complete absence of production of one or more of the globin polypeptide chains of the hemoglobin molecule leading to, imbalance in α & non- α chains of Hemoglobin. In case of thalassemia, there is reduced or absent production of β chains, leading to excess α chains.

Excess α chains that have no complementary non- α chains with which to pair, form insoluble inclusions that precipitate on red cell membrane and damage it, leading to premature destruction of RBC in bone marrow (Ineffective Erythropoiesis) and in peripheral circulation, particularly in reticulo-endothelial system of spleen. (Extra vascular hemolysis).

Due to reduced production of adult Hb in postnatal life, the normal switch mechanism leading to reduction in γ chain synthesis does not occur. This leads to higher fetal Hb (Hb F) in post natal life.

Severe Thalassemia usually becomes manifest in the first year of life when the fetal hemoglobin ($\alpha_2\gamma_2$) starts declining and adult hemoglobin cannot be stably formed.

The precise mechanism controlling the switch from fetal to adult hemoglobin is not fully understood (54-57). The high affinity of Hb F for oxygen leads to tissue hypoxia, which in turn stimulates erythropoietin secretion leading to both characteristic hemolytic facies with fronto-parietal and occipital bossing, malar prominence and malocclusion of teeth. Other complications that occur are distortion of ribs and vertebrae, and pathological fracture of bones, splenomegaly and its complications (Hypersplenism), Hepatomegaly, Gallstones and chronic leg ulcers, etc.

The clinical syndromes associated with Thalassemia, arise from the combined consequences of

- Inadequate hemoglobin production
- Unbalanced accumulation of one type of globin chain, leading to precipitation.

The former causes anemia with hypochromia and microcytosis, where as the latter leads to ineffective erythropoiesis and haemolysis.

Classification

Thalassemias are classified depending on, which globin chain is defective (alpha), (beta), (gamma), (delta, etc.)

Accordingly, they are designated as -

α Thalassemia	α chain is involved.
β Thalassemia	α chain is involved
$\beta\delta$ Thalassemia	β, δ , chain are involved

Double Heterozygous states such as Sickle cell Thalassemia, Hb E Thalassemia, Hb D Thalassemia etc

Classification of α Thalassemia :^(2,3,4)

The gene for α chain is duplicated on chromosome 16 with each diploid human cell containing four copies of the α gene.

Table : Classification of α Thalassemia Syndromes

Syndrome	Clinical Features	Hemoglobin pattern	Genes affected
Silent carrier	No anemia, normal red cells at birth	1-2% Hb Bart's (γ_4)	1
α Thalassemia trait	Mild anemia hypochromic microcytic red cells at birth	5-10% Hb Bart's (γ_4)	2
HbH disease	Moderate anemia, hypochromic, microcytic red cells	5-30% HbH (β_4)	3
Hydrops fetalis	Death in utero caused by severe anemia	Mainly Hb Bart's, small amounts of HbH	4

β Thalassemia is the most common genetic variant, associated with Thalassemia syndromes in populations from the Mediterranean basin, Indian subcontinent and South-East Asia. However population migration these days has, no longer, restricted the gene frequency to above tropical areas in which it was first observed, and hence is seen all over the world.⁽⁵³⁻⁵⁵⁾



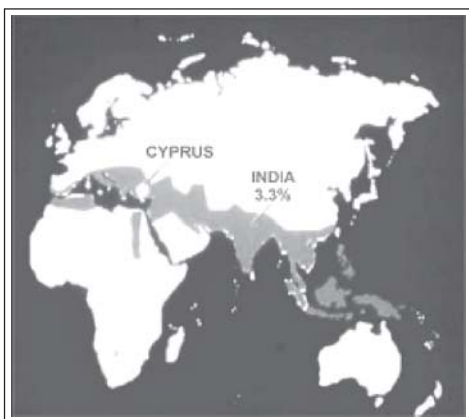
Thomas Cooley and Lee⁽⁵³⁻⁵⁵⁾. Detroit pediatricians first described in 1925, a series of cases of severe anemia occurring in Italian children with hepatosplenomegaly, growth retardation, discoloration of skin, and of the sclera, with peculiar bone changes in children during the Transactions of "American Pediatric Society" and it was also called as Cooley's anemia.

The term 'THALASSEMIA' was first used in 1932, by Whipple and Bradford. The word was taken from the Greek language which means "sea" (anemia around the sea). As it was first described around Mediterranean countries, it was also called as, "Mediterranean anemia". However, it was soon realized that it also occurs in south East Asia, Indian subcontinent, and Middle East, and not only around Mediterranean regions.

The First case reported in 1932 in India is by Dr. Mukherjee from Calcutta (India)
Dr. Sukumaran from Mumbai did pioneering work in the field of diagnosis of thalassemia syndromes in India.

Epidemiology

Thalassemia incidence: World Scenario-



All over the world there are more than 200 million - (1.5% of world population) carriers of Thalassemia gene, and in South East Asia, there are 40 million carriers of this gene (50% of these are in India alone i.e. 20 million).

The thalassemia belt stretches across African continent, Mediterranean regions, Middle East, Indian subcontinent, South east Asia, Thailand, Cambodia, Laos, Vietnam, Malaysia, Singapore, Southern China. The observation that the prevalence of Thalassemia and Falciparum Malaria was similar, suggested the hypothesis that nature developed genetic mutation to overcome mortality and morbidity of malaria¹⁰⁻¹³ which is same as malaria belt¹⁻³.

The Mean prevalence of the carrier status in India is 3.3% (ranging from 1 to 17% in various communities). If a line is drawn between Mumbai (Bombay) & Kolkata (Calcutta.) on the Map India, the region above the line has an incidence of 3-17 %, where as region below the line has incidence of less than 3% .

It is estimated that every year approximately 100,000 children with thalassemia major are born all over the world. With the birth rate of 22.8 per 1000 in India, **it is estimated that there are about 9,000-10,000 cases being added every year and there are around 65,000 - 67,000 Beta Thalassemia patients in our country.** The prevalence of Thalassemia incidence varies in different communities, religions and ethnics groups.

A higher frequency is noted in **certain communities** such as

- Sindhis, Punjabis, from North,
- Migrants from Pakistan to north India such as Khatri's and Khukrejas,
- Bhanushalis, Lohanas, Kutchis, Baniyas from Gujarat,
- Neo-Buddhists, Mahars, Chamars, Buddhists, Kolis, Agri's, Kunbi's from Maharashtra,
- Reddi's, Gouda and Lingayats, Kurgs from Andhra & Karnataka etc.
- Goud Saraswats from Goa.
- Certain Communities from Muslims & Christians.

The β globin genes are represented one on each chromosome 11. The β -thalassemias also include four clinical syndromes of increasing severity:

The former two result from a **single gene defect (heterozygous)** whereas the latter two from **both genes affected (homozygous)**.

Table: Classification of β -Thalassemia

Syndrome	Clinical Features	Hemoglobin Pattern	β -globin genes
Heterozygous state			
Silent carrier	No anemia, normal	Normal	1
Thalassemia trait microcytic red cells	Mild anemia, hypochromic	Elevated HbA2	1
Homozygous state			
Thal. Intermedia	Moderate, requires Some transfusion	HbF elevated	2
Thalassemia Major	Severe, transfusion Dependent	HbF elevated	2

β -thalassemia is common in our country.

Molecular genetics

More than 200 mutations are described all over the world. The 5 Common mutations responsible for more than 90% of thalassemia mutations in our country include 619 bp deletion, IVS 1 - 5 (G - C), IVS 1 - 1 (G - T), FS 8/9 (+ G), FS 41/42 (- CTTT).

Recently, some more rarer mutations have been described. These include Codon 4/5 and 6 (ACT CCT GAG - ACA TCT TAG), Codon 47/48 (+ ATCT), Codon 55 (+ A), IVS 2 - 837 (T - G), Codon 88 (+ T), Codon 5 (- CT) IVS 1 - 110 (G - A), Codon 15 (TGG - TAG).

Clinical manifestations of β thalassemia

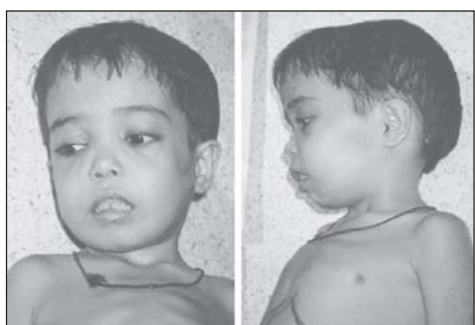
Children with Thalassemia major are generally diagnosed between 6 months and 18 months of life with pallor, failure to thrive, irritability, intercurrent infections and hepatosplenomegaly.

If undiagnosed and untreated, more than 90% do not survive beyond 3 to 4 years of age. **In India, in many places children born with Thalassemia major, die undiagnosed, or due to lack of ideal treatment.**

The spectrum of clinical manifestations of β thalassemia varies widely.

At one end of the spectrum is "Serious Homozygous form (Thalassemia Major) that presents in early infancy (6 - 18 months) with progressive pallor, hepatosplenomegaly, and bony changes and if untreated, is invariably fatal during first few years of life. Untreated or irregularly treated children develop significant hemolytic faces including frontoparietal bossing with a hot-cross-bun appearance of the skull (caput quadratum with "hair-on-end" appearance on X-ray skull), depressed bridge of nose, malar prominences and malocclusion of teeth with protrusion and malocclusion of maxillary teeth.

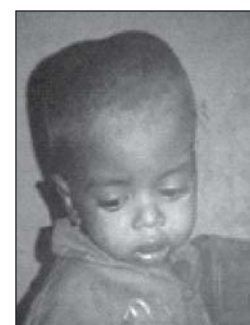
Whereas at the other end of the spectrum is a Heterozygous form (Thalassemia minor) in which the patient can lead a practically normal life except for mild persistent anemia, not responding to hematinics. They have a perfectly normal life span.



Thalassemia major
appearance



Thalassemia Intermedia



Hot-cross-bun
Caputquadratum

In between these two extremes are forms with varying degrees of clinical manifestations of anemia, splenohepatomegaly and bony changes who maintain their life fairly comfortably and are not dependent on blood transfusions for their survival and are called thalassemia-intermedia (they are also homozygous)."

Investigations

CBC is frequently sufficient to suspect the diagnosis of Thalassemia Major. Peripheral blood Smear is diagnostic with characteristic bizarre picture of red cells:

Complete Blood Count (CBC)

A CBC reveals generally severe anemia, a high leucocyte count (due to immature myeloid cells as well as nucleated red cells - also known as a "leucoerythroblastic reaction"). The red cell indices reveal a severe hypochromia with microcytosis. Often in our patients due to delayed diagnosis, there is significant macrocytosis due to relative folate depletion.

Peripheral Blood Smear (PBS) Examination

The peripheral smear shows a striking and characteristic bizarre picture with hypochromic, microcytic as well as macrocytic red cells, anisopoikilocytosis, target cells, polychromasia (more common in thalassemia intermedia), basophilic stippling, nucleated red cells and sometimes immature myeloid cells.

Reticulocyte count.

Reticulocyte count is generally low to normal in thalassemia major, whereas in thalassemia intermedia, it is increased to 3 to 6 %. The reason for a low reticulocyte count in thalassemia major is significant ineffective erythropoiesis preventing the precursor red cells from maturing to reticulocyte stage to be thrown into peripheral blood. In thalassemia intermedia, since the ineffective erythropoiesis is milder, the reticulocytes are increased in peripheral blood due to the anemia.

Thalassemia minor

The CBC in thalassemia trait is associated with high red cell count relative to hemoglobin concentration and hematocrit, resulting in a marked fall in Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH) as well as Mean Cell Hemoglobin Concentration (MCHC).

The red cell distribution width (RDW)

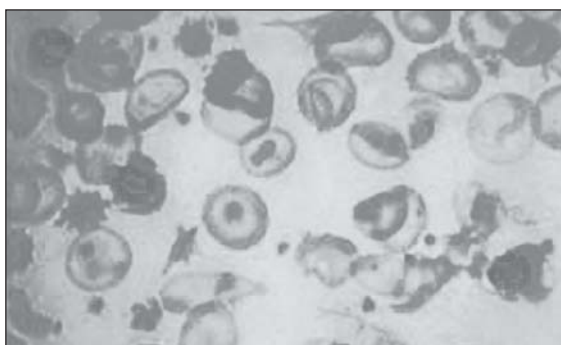
RDW Values - RDW is the coefficient of variation of red cell volume distribution. It is the objective documentation of subjective anisocytosis. It is significantly high suggesting a very high degree of anisocytosis. RDW is normal in thalassemia trait as well as in anemia of chronic infection, while it is increased in Iron deficiency anemia (IDA) Normal range of RDW : 11.5% to 14.5%.

Naked-Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT):

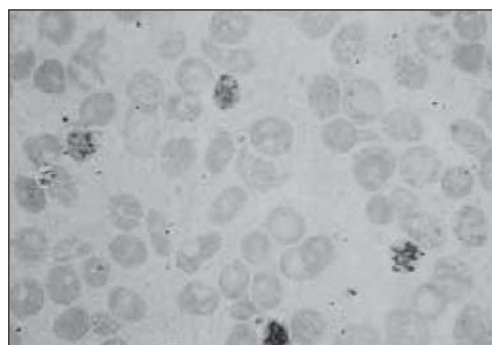
Many investigators have studied Naked-Eye Single Tube Red cell Osmotic Fragility Test (NESTROFT) as a screening modality in β thalassemia trait. The test has a high sensitivity of 95 percent, but its poor precision, interobserver variability and low specificity has precluded it from becoming a robust test.

Radiological findings include widening of medulla due to bone marrow hyperplasia, thinning of the cortex, and trabeculations are seen in long bones, meta-carpals and metatarsals. X-ray- Skull AP and lateral views show " **Hair on End** appearance.

Hemoglobin Electrophoresis is diagnostic of thalassemia syndromes. **Fetal hemoglobin is increased in the patient and HbA2 is over 3.4 percent in both parents (Thalassemia minor).**



Target cells & Normoblasts on Peripheral Blood Smear of Thal. Major



Increased Reticulocyte count



X-ray - Skull Lateral "Hairs on End appearance" Copyright Authors



Osteoporosis in the Long Bones.

Iron studies-increased serum iron, reduced total iron binding capacity and increased transferrin saturation as well as increased ferritin levels are seen.

Bone marrow examination is an invasive and painful test and not indicated for the diagnosis of thalassemia major. If done, it shows normoblastic erythroid hyperplasia with excessive iron on iron staining with Prussian blue.

Quantitation of various hemoglobins

Separation of hemoglobins either by electrophoretic mobility or chromatographic separation is the confirmatory investigation for diagnosis of thalassemia syndromes.

Quantitation of various hemoglobins can be done by the following methods : Isoelectric Focussing, Microcolumn Chromatography, High Performance Liquid Chromatography, Both anion and cation - exchange HPLC, Cellulose Acetate Electrophoresis, Paper Electrophoresis

Micro column chromatography and HPLC, by automated machines are now becoming increasingly popular due to the ease of performing the test, less time consumption and greater reliability and reproducibility. It has thus become the gold standard for diagnosis of thalassemia syndromes and other hemoglobinopathies. It generates graphs depicting various abnormal and normal hemoglobins with quantification. Hb A2 value of > 9 percent indicates the presence of a co-eluting abnormal hemoglobin such as Hb E, Hb D Iran and Hb Lepore.

For a reliable diagnosis of Thalassemia, it is advisable to correlate clinical profile and ethnicity of the individual with various laboratory investigations to confirm the diagnosis. Hemoglobin electrophoresis is the confirmatory test for diagnosis of most cases of thalassemia syndromes. However, a complete blood count and examination of the peripheral smear provide very vital information and are an important primary screen in thalassemia syndromes.

Management of a Thalassemic child

Management of thalassemia involves a multidisciplinary therapeutic team approach and should be preferably done at a comprehensive thalassemia center with outdoor transfusion facilities.

The team should consist of the following :

- Pediatric hematologist
- Pediatrician
- Dedicated Nurses
- Transfusion Medicine Specialist
- Physiotherapist
- Endocrinologist
- Psychologist
- Social worker

Comprehensive management includes the following

- Confirmation of diagnosis.
- Correction of the anemia with repeated RBC transfusions.
- Removal of iron with iron chelating agents.
- Treatment of complications.
- Cure of the disease by bone marrow transplantation.
- Pharmacological methods to increase gamma chain synthesis.
- Gene replacement therapy.
- Prevention of the disease by antenatal diagnosis and genetic counseling.

Transfusion therapy

Transfusion therapy in thalassemia has two major goals:

- To prevent anemia
- To suppress endogenous erythropoiesis to avoid ineffective erythropoiesis.

Indications for transfusion therapy

Blood transfusion is mandatory for all children with thalassemia major and for those children with thalassemia intermedia who cannot maintain Hb above 7 g/dl or who show evidence of growth retardation and severe bony changes. Regular blood transfusions are presently the mainstay of treatment of thalassemia major.

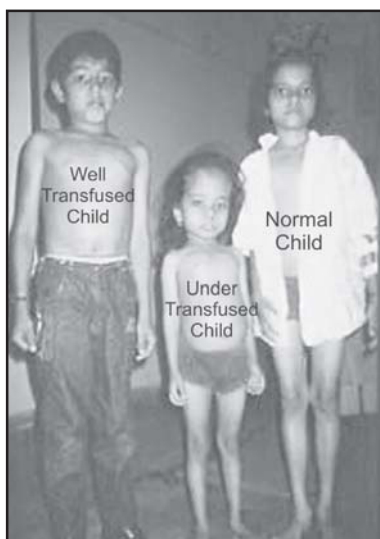
The objectives of transfusion therapy are to Maintain Hb above 9.5 to 10 g/dl. (Moderate transfusion regimen). The current recommendation is to maintain the mean Hb level of 12 g/dl and transfuse the child at level of 9 to 10.5 g/dl.

In 1980, Propper and colleagues introduced a further improvised regimen called supertransfusion, and maintained a pretransfusion hemoglobin of above 12 gms%. However this did not prove significantly superior to hypertransfusions and was given up. Hypertransfusion remains the most accepted regimen in most parts of the world. However, in Europe, a yet newer regimen termed the "moderate transfusion regimen" has been adopted and recommended by the Thalassemia International Federation. In this regimen, pretransfusion hemoglobin is maintained between 9 and 10.5 gms%

Various Transfusion Regimens (Progress in Transfusion Therapy)

Year of Regimen	Transfusion Regimen	Pre-transfusion Hb
1960s	Palliative	Hb to 8.5 gm% (Wolman et al)
1970s	Hyper transfusion	Hb 10 - 12 gm% (Piomelli and workers)
1980	Super transfusion	Hb > 14 gm% (Propper and colleagues)
2005	Moderate transfusion	Hb 9 to 10.5 gms% (European Regimen)

Effect of Blood Transfusion on Growth & Development



Post-transfusion level of Hb should not rise above 15 to 16 g/dl.

This will prevent excessive erythropoiesis, thus avoiding expansion of the bone marrow and precluding early features like marrow hyperplasia leading to bony abnormalities, hepatosplenomegaly and increased gastrointestinal absorption. Prevention of chronic hypoxia, which in turn promotes normal growth and development is important. Whenever possible, it is important to know the complete genotype of the red cells to prevent red cell alloimmunization following repeated transfusion. However, this is not feasible in India and the alternative to this is Coomb's cross-match for each transfusion to prevent alloimmunization.

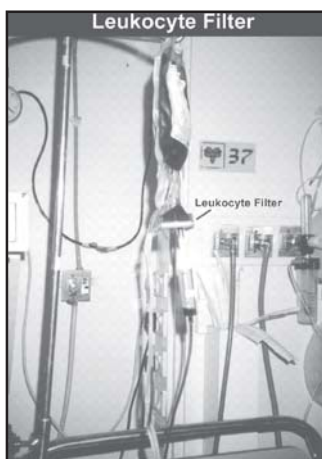
The most ideal way to transfuse thalassemics is using group and type specific packed red cells that are compatible by direct antiglobulin test.

The hematocrit should be standardized to 65 to 75%. This maintains the desired viscosity as well as aids in calculating the yearly requirement in a given patient. It is ideal to use leucodepleted red cells done in the blood bank. However, wherever, this is not feasible, using filters (Leucocyte) at bedside is another alternative; however, this is not affordable to most of our patients. The affordable alternative to this is use of triple saline washed red cells. The red cells should be fresh, not more than 4 to 5 days old to maintain adequate levels of 2,3-DPG. Various other methods of leucodepletion are available, including use of frozen red cells (highly expensive and impractical), filtration in the blood bank, use of apheresis etc.

Amount and rate of transfusions :

Approximately 180 ml/kg of red cells are required to be transfused per year in non-splenectomized, non-sensitized patients to maintain the hemoglobin above 10 gms%, whereas splenectomized patients require 133 ml/kg per year. Even without hypersplenism, the requirement is 30% higher in non-splenectomized patients. These red cells should be transfused at the rate of 10 to 15 ml/kg (3 to 4 ml/kg/hour) every 2 to 4 weeks to maintain the hemoglobin above 10 gms%. Patients with cardiac decompensation should be given red cells at the rate of not more than 1 to 2 ml/kg/hour.

Transfusion transmitted diseases like malaria, syphilis, hepatitis B, hepatitis C, cytomegalovirus, and HIV infection can occur. Therefore it is mandatory to screen blood for HIV, HBV, HCV and malaria by sensitive tests. Tests for p24 antigen and DNA PCR to detect HIV infection in the window period are expensive and impractical for our nation at present.



All thalassemic children who are negative for the hepatitis B surface antigen and antibody, should receive hepatitis B vaccine in 4 doses at 0, 1, 2 and 12 months intramuscularly.

Outdoor transfusion services - In the past, a thalassemic child had to be admitted for blood transfusion alongside other sick children of the ward. With the advent of outdoor transfusion centers, transfusion can be well planned causing minimal psychological trauma to the child and parents as transfusion is given in a cordial compliant surrounding with other thalassemic children.



Initiation of transfusion therapy :

Before embarking on a lifelong transfusion therapy, it is preferable to establish the diagnosis firmly with DNA analysis. This would help to know the severity of thalassemia as well as would help in prenatal diagnosis for future pregnancies. One can ascertain the diagnosis of thalassemia intermedia by observing the rate of fall of hemoglobin without transfusions. If the hemoglobin drops to below 7 gms% without transfusion, in absence of any concurrent illness, it is imperative to put the child on a regular transfusion program. If the child maintains hemoglobin above 7 gms%, the diagnosis of thalassemia intermedia has to be considered.

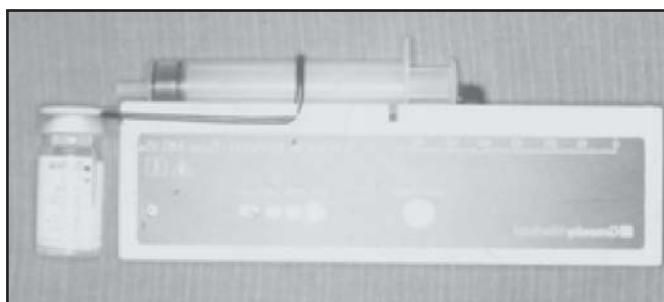
Whenever possible, it is equally important to know the complete genotype of the red cells to prevent red cell alloimmunization following repeated transfusions. However, this is not feasible in India and the alternative to this is Coomb's cross-match for each transfusion to prevent alloimmunization.

Iron overload and chelation therapy

A major problem encountered in the management of thalassemia is iron overload. Regular red cell transfusions to maintain hemoglobin as well as increased iron absorption from GI tract due to ineffective erythropoiesis and consequent low hemoglobin in irregularly transfused children is responsible for iron overload. The goal of iron chelation is to reduce the iron overload and subsequently maintain ferritin levels below 1000 ng/ml.

The standard available chelators used are:

Desferrioxamine (Desferal DFO): Desferrioxamine (DFO) is the gold standard therapy and is as yet the most effective and safe iron chelator. The dose is 20 to 40 mg/kg/day given subcutaneously over 8 to 10 hr for 6 nights a week with the help of subcutaneous desferal infusion pump.



Subcutaneous Desferal Infusion pump

Intravenous desferal

It can be given particularly in those with very high iron overload through port-a-caths (central line). However, it is not easy to maintain the central catheter and infections are extremely common.

High dose desferal (3 to 9 g/day) can be given in severe hemosiderosis to prevent/reverse cardiac toxicity of iron overload. It is useful adjunct to subcutaneous infusion. Close monitoring for adverse reaction is required. Though, desferrioxamine is the gold standard in the management of iron overload in thalassemia major, it has not become popular particularly in the developing countries and is being used in only 10 to 15 % of thalassemics in our country due to its high cost.

The need for continuous subcutaneous injection over 6 to 8 hours with the desferal pump has resulted in poor compliance particularly in the adolescent groups who often revolt against the use of this pump. Hence, many of the thalassemic children develop complications related to iron overload and may require to be given high dose intravenous desferal through the port or central line. This again is beyond the reach of many children in developing countries.

Toxicity of deferral (desferrioxamine):

Minimal or no tachyphylaxis has been observed. When given parenterally there may be liberation of histamine leading to bradycardia, hypo/hypertension, rigors, headache, photophobia, feeling cold and hot, etc. When given subcutaneously local pain, indurations, irritability and redness may occur. Visual abnormality may occur in 4 to 10 percent of patients and includes decreased acuity of vision, peripheral field vision defects, defective dark adaptation, thinning of retinal vessels, retinal stippling and abnormal visual evoked responses and cataract. High frequency sensory-neural hearing loss has been reported in 4 to 38 percent of patients. As the auditory and visual toxicities are reversible, yearly slit-lamp examination and audiometry are mandatory to detect them early and if found, desferal should be stopped. Delayed linear growth has also been reported in children below three years of age with desferal. These may be accompanied by mild skeletal abnormalities such as short trunk, sternal protrusion and genu valgum.

Role of vitamin C: Ascorbic acid deficiency increases insoluble iron (hemosiderin). Vitamin C helps in conversion of hemosiderin into ferritin, from which iron can be chelated. High doses of vitamin C can lead to increased free radical liberation and lipid peroxidation, resulting in tissue damage and rapid cardiac decompensation and even death. Addition of 100 mg of Vitamin C daily,

prior to DFO therapy increases iron excretion. Sixty percent of DFO chelated iron is excreted in urine and 40 percent in stool.

Newer chelating agents

Over the last 20 years, more than 500 oral chelating compounds have been tried all over the world in search of an ideal chelating agent which can be effective, cheap, safe and can be given orally. Among the various drugs under trial, few have completed animals studies, a few are being tried in human volunteers.

Deferiprone: One of the drugs which has been approved in some countries across the world is Dimethyl-hydroxy pyridone (1,2 dimethyl-3-hydroxy Pyrid-4-one (L1), developed in Hider's laboratory-London, now generically named as Deferiprone and available in India under the brand name of Kelfer. It mobilizes iron from transferrin, ferritin and hemosiderin. It has undergone extensive trials in USA, UK, Canada, India and various other centers.

Dose: 75 to 100 mg/kg body weight/day in three to four divided doses. Results show that it is 70 to 100 percent as effective as desferrioxamine. There has been no evidence of ear or eye toxicity. Urinary excretion of Ca, Cu, Mn, Mg does not get affected. Kidney and liver parameters did not show any alteration. Some children can have GI symptoms like nausea, vomiting, pain in abdomen and diarrhea. Twenty to thirty percent children develop arthropathy, which is reversible after reducing the dose or on stopping it. However, if the drug is not discontinued in time or its dosage reduced, it may lead to destruction of joint cartilage and irreversible damage to the joint. ANA, anti ds-DNA, antihistone antibodies have been reported positive in a few cases, suggesting a drug-induced lupus. Absolute neutropenia and thrombocytopenia also have been reported in occasional cases.

Physical examination of the joints and complete blood count including platelet count must be done regularly when child is on deferiprone (L1) therapy. With recent advances in chelation therapy especially with the availability of oral chelating drugs like deferiprone (L1) compliance has remarkably improved. The cost of therapy has reduced considerably and hence even in the developing countries many children are able to get chelator therapy.

To bring down the cost, to improve the compliance and efficacy of the chelation therapy and reduce the side effects, combination therapies have been tried.

Oral Deferiprone (L1) 75 mg/kg/day for 4 to 5 days in a week and DFO 30 to 40 mg/kg day subcutaneously with the help of subcutaneous infusion pump over 6-8 hours on weekends (2 days) has been shown to be more efficacious in reducing both liver and heart iron. This is based on the principle of shuttle hypothesis of one chelator mobilizing the iron from stores in tissues and the other helping in excretion from the blood stream.

Some of the newer drugs found to be a effective and safe for chelation of iron are:

ICL 670, Exjade of these, the most promising is ICL 670, which is a novel chelating agent and belongs to tridentate thiazole. This drug has been found to be effective in the dose of 10 mg BD or 20 mg OD orally. Since it has a half-life of 11 to 16 hours, it can be given once a day.

This drug has shown excellent preclinical activity and safety in animals and human subjects. Oral **ICL 670 is five times as effective as subcutaneous DFO and 10 times more potent than deferiprone**. Iron excretion is predominantly fecal. It excretes iron from both reticuloendothelial cells as well as parenchyma cells of various organs and chelated iron excreted by liver through the bile. It also has the ability to prevent myocardial cell iron uptake, remove the iron directly from myocardial cells and exchange the iron with DFO. Only side effects reported include mild abdominal pain, gastrointestinal discomfort, constipation, skin rash. No changes in auditory, visual (ocular) or cardiac functions were observed. Co-administration of ICL 670 with Inj DFO has synergic effect and helps in reducing dose of both the drugs thus improving the compliance and cost of the treatment **Shuttle effect is also seen with this combination, as ICL 670 acts as intracellular chelator and DFO as extra cellular**. It does not chelate zinc or copper.

Gene manipulation

HbF gene restimulation using pharmacologic drugs has helped to reduce the precipitation of unpaired Hb chains. Various drugs such as 5-Azacytidine, Hydroxyurea, Butyrate derivatives have been tried and found to be successful in limited situations, especially in thalassemia intermedia. Recent literature suggests the use of combination of erythropoietin and hydroxyurea in thalassemia major to increase expression of HbF gene and improve the hemoglobin thereby reducing the need for transfusions.

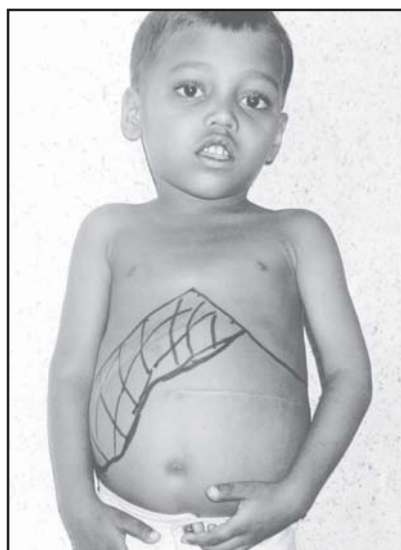
Splenectomy

With the advent of hyper and super-transfusion therapy, splenomegaly and hypersplenism have become a rarity. Hence splenectomy is usually not needed in these patients. If the child has already developed splenomegaly and signs of hypersplenism are present, and is above 5 years of age, splenectomy should be considered.

Indications for splenectomy in thalassemia include an increase in the yearly requirement of packed cells more than double the basal requirement, i.e. packed cell 230 to 250 ml/kg/year or more or decrease in WBC and platelet counts is a relatively late manifestation of hypersplenism. All children needing splenectomy should receive Pneumococcal vaccine, H. influenza type b vaccine, and meningococcal vaccine 6 to 8 weeks prior to surgery. In endemic areas, prophylactic antimalarial treatment maybe given to prevent malaria.

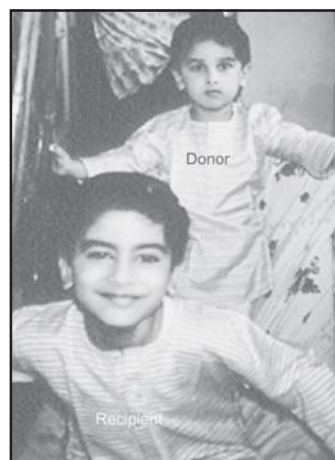
Prophylactic penicillin therapy must be continued life-long.

- Episodes of suspected infection should be treated promptly and newer wide spectrum antibiotics maybe empirically started to prevent septicemia and other complications (if necessary these children should be hospitalized). Blood culture and sensitivity of antibiotics must be performed to guide treatment.



Bone marrow transplantation

A ray of hope for permanent cure and better future for children with genetic disorders has emerged with the rapid advancement in the techniques and the success of bone marrow transplantation. The credit of first bone marrow transplantation in thalassemia major goes to E Donald Thomas who performed this procedure in 18 months old thalassemic child in 1982, using HLA matched older sister as donor. This child was cured of thalassemia. The first BMT in India in thalassemia was successfully done by Dr. M. Chandy at Christian Medical College, Vellore.



The principles of bone marrow transplantation in thalassemia are:

- To destroy and prevent regeneration of defective stem cells.
- Sufficient immune suppression for good engraftment of normal marrow.
- To infuse stem cells with normal gene for β globin chains.
- To prevent GVHD with high dose therapy of busulphan, cyclophosphamide, total body irradiation and other modalities.

All over the world over more than 1000 transplantations for thalassemia major have been done, with a 70 to 80 percent cure rate.

The **three most important adverse prognostic factors** for survival and event-free survival are (Lucarelli et al)

- Presence of Hepatomegaly (liver more than 2 cm below costal margin)
- Portal fibrosis
- Irregular chelation

Patients are classified as different classes as below to prognosticate the outcome of bone marrow transplantation:

- Class I : None of the above factors
- Class II : One or two factors
- Class III : All of the above

For those in Class I, the success rate is around 93 % and with all three factors present, the success rate drops to as low as 60%.

Bone marrow transplantation is most successful in patients who are young, properly transfused, and well-chelated and clinically well preserved without Hepatomegaly. The cost of BMT in India is around 5-8 lacs and is being done at Christian Medical College, Vellore, Tata Memorial Hospital, Parel, Mumbai, and AIIMS in Delhi and many other centers in the country.

Endocrine evaluation and growth monitoring

Iron overload is responsible for dysfunction of many of endocrine glands like thyroid, parathyroid pituitary gland, gonads and pancreas leading to hypothyroidism, altered calcium metabolism, stunted growth, delayed puberty and Diabetes. Clinically they may not manifest initially and hence, regular evaluation is necessary. Glucose Tolerance Test, Thyroid function tests, Serum Calcium and Phosphorus should be evaluated from 5 years age onwards annually. Ten years onwards, one must monitor the height velocity, perform the investigations for cardiac complications (2D-ECHO), consider pertinent hormone assays and do a DEXA scan for bone density.

Cardiac complications

Cardiac failure and arrhythmias are the main cause of mortality in thalassemic children. Holter monitoring, 2D-Echo, stress test, assist in evaluation, but do not quantitate cardiac function. T2 weighted Cardiac MRI is the only method of assessing severity of cardiac iron overload. Cardiac complications seen in thalassemic children are evidence of overt cardiomyopathy, abnormalities such as left atrial dilatation, aortic root dilatation, reduced left ventricular internal dimension in systole and diastole. Ejection fraction is decreased and abnormality in ECG may be present.

SICKLE CELL DISEASE

Sickle cell disease is a group of inherited disorders with abnormalities in Hb synthesis characterized by production of hemoglobin S. (Hbs) The term *sickle cell disease* is used generically to refer to all of the sickling syndromes.

Herrick first described sickle cell disease in 1910 in a West Indian patient. It is an inherited autosomal recessive disorder. Hb S, forms the sickle shape, imparted to deoxygenated red cells, is responsible for a spectrum of disorders that vary with respect to degree of anemia, frequency of crises, extent of organ injury, and duration of survival.

Sickle cell trait has its highest prevalence in areas hyperendemic for malaria. This mutation is thought to have originated in areas of the world where malaria was common, since people with sickle trait do not get malaria. The sickle trait actually offers some selective protection from the parasite that causes malaria. Selective removal of sickled cells from the circulation probably reduces the degree of parasitemia and substantially limits the infectious process.

The highest prevalence of Hb S is in tropical Africa and among blacks in countries. It occurs with lower frequency in the Mediterranean basin, Saudi Arabia, and parts of India. In the United States, Latin America, and the Caribbean, approximately 8% of blacks carry the sickle gene.

Pathophysiology

Sickle cell anemia occurs due to mutation of β globin gene situated in short arm of chromosome 11. Ingram in 1956 demonstrated that in **sickle** mutation, thymine substitutes for adenine in the sixth codon of the β gene(GAG'!GTG), thereby encoding valine instead of glutamic acid in the sixth position of the β chain.

This minor change in structure is responsible for significant changes in molecular stability and solubility of Hb S. Hemoglobin is normally present in soluble form in red blood cell corpuscles. "Sol" form of Hb, changes to "Gel" form when Hb S is deoxygenated. Deoxygenation of hemoglobin S results in the polymerization of hemoglobin S. Normal hemoglobin cells are smooth, round, and flexible, biconcave shape so that they can travel through the vessels/capillaries in our bodies easily. Whereas sickle cell hemoglobin cells are stiff and sticky, and form into the shape of a sickle, or the letter "C". When they lose their oxygen, they become more fragile. The polymerization of hemoglobin S, results in distortion of the shape of the red blood cell and a marked decrease in the deformability of the red blood cell. Repeated sickling and unsickling, results in fixation of membrane in sickled configuration leading to irreversible sickle cell (IRC) and hemolysis.

Sickle cells tend to cluster together; the cluster causes a blockage and stops the movement of healthy, normal oxygen-carrying blood. These rigid sickle cells are responsible for the "vaso-occlusive" phenomenon; this blockage is what causes the painful and damaging complications of sickle cell disease. The spleen also suffers damage from the sickled cells blocking healthy oxygen carrying cells. (Auto-splenectomy). Without a normal functioning spleen, these individuals are more at risk for infections. Infants and young children are at risk for life-threatening infections. Sickle cells only live for less number of days-about 15 days, while normal RBC's can live up to 120 days, thus leading to persistent anemia.

There are five major mutations of the sickle cell gene. In Africa, we find four of the major sickle haplotypes which are associated with different geographical areas: "Senegal" -in Atlantic West Africa, "Benin" in central West Africa, "Bantu" (also known as CAR) is in central Africa, African haplotype is known as "Cameroon." In India and parts of Saudi Arabia, the African haplotypes are not seen, but a unique "Arabian-Indian" haplotype is found. Patients with this haplotype have mild disease and elevated levels of fetal hemoglobin.

In heterozygous state, red cells contain both normal adult Hb (Hb A) and the variant HbS. Heterozygotes rarely have clinical manifestations of disease. **If both parents are trait**, there is a one in four, or 25 percent, chance with each pregnancy, for a child to be born with sickle cell disease. This means that there is a three in four chance, or 75 percent chance for the child to not have sickle cell disease. There is also a 50 percent chance that a child will be born with sickle cell trait, like the parents they are at risk for passing the gene on to their children.

Additionally, disease may result from the combination of two variant hemoglobins (Double Heterozygous) and an interacting Thalassemia gene. These doubly heterozygous states are designated by both aberrant gene products, such as Hb SC disease or Hb S/- β thalassemia.

Sickle Cell Syndromes

There are three common sickle cell syndromes:

- **Homozygous sickle cell disease (also known as Hemoglobin SS)**
- **Sickle-Beta Thalassemia** (double heterozygous)
- **Hemoglobin SC disease.** (double heterozygous)

Clinical features:-

Clinical features have great variation in the manifestations of sickle cell disease.

Anemia Repeated cycles of deoxygenation and sickling irreversibly damages the red cell membranes and results in hemolysis. Bone marrow increases red cell production but is unable to compensate for the rate of hemolysis. This results in moderate-to-severe anemia. Children exhibit few clinical manifestations of anemia, since they readily adjust by increasing heart rate and stroke volume. However, they have decreased stamina, which may be noted on the playground or when participating in physical education class. Pallor may be evident. Jaundice may be manifested as scleral icterus and/or urobilinogen excretion and evidence of increased bilirubin production.

Splenic sequestration : Splenic sequestration in children < 6 years old is often preceded by infection. Although hypofunctional at an early age, the spleen has an anatomic structure that responds to certain stimuli, usually febrile illnesses. It suddenly becomes enlarged and traps blood cells. Hemoglobin levels dramatically drop from baseline values and the reticulocyte count is elevated. The platelet count often is slightly decreased; hypovolemic shock occurs if a large volume of blood is trapped or sequestered. For anemic crisis with **splenic sequestration, early red cell transfusions** are given, since the process can progress rapidly to shock. **Do not allow hemoglobin to rise above 10 g/dL**, since the spleen may disgorge trapped cells, which can create a relative polycythemia and increased blood viscosity. Children with a single sequestration event frequently have recurrences. **Surgical splenectomy or a short-term transfusion regimen** has been suggested for this complication. Penicillin prophylaxis significantly reduces the incidence of **S pneumonia** infection and is believed to decrease the mortality rate.

Aplastic crisis also leads to anemic crisis. Most commonly, it is caused by infections with parvovirus, which results in the rapid onset of severe anemia. This usually is due to suppression of erythroid production by the marrow, and causes the hemoglobin level to fall precipitously. Hemoglobin values are much lower than usual for the patient. Reticulocyte count is very low, usually under 0.5% and often 0%. Platelet and white blood cell counts usually are normal. **Transfusion is required in an aplastic crisis if the anemia is symptomatic** (e.g. dyspnea, signs of hypovolemia). Since aplastic crises are self-limited, it may be possible to avoid transfusion if the child is stable and can be adequately observed. Folic acid commonly is prescribed, (1 mg/d) to prevent development of megaloblastic anemia due to increased folate requirements caused by hemolysis.

Pain

Pain, resulting from vascular occlusion and ischemia, is the most common feature of sickle cell disease and can affect any body part. Bone pain often is due to bone marrow infarction. Since it tends to involve bones with the most bone marrow activity and since marrow activity changes with age, certain patterns are predictable.

During the first 18 months of life, the metatarsals and metacarpals can be involved, presenting as **Dactylitis or Hand-Foot syndrome**. As the child grows older, pain often involves the long bones of the extremities, sites that retain marrow activity during childhood. As marrow activity recedes further during adolescence, pain involves the vertebral bodies, especially in the lumbar region.

Although the above patterns describe commonly encountered presentations, any area with blood supply and sensory nerves can be affected. **Abdominal pain** can result from referred pain from other sites or intra-abdominal solid organ or soft tissue infarction.

Triggers: Since deoxygenated hemoglobin S becomes semisolid, the most likely physiologic trigger is **hypoxemia**. This may occur from chest syndrome or with other respiratory complications. Dehydration can precipitate pain since acidosis results in a shift of the oxygen dissociation curve (Bohr effect), causing hemoglobin to desaturate more readily. **Hemoconcentration** is a common mechanism, as is a **lowered body temperature**, probably as the result of peripheral vasoconstriction. Proper clothing and avoidance of exposure ensures normal core temperature.

Prevention:-

Family counseling is useful to avoid the above-mentioned triggers whenever possible. **Hydroxyurea** may decrease frequency and severity of pain episodes. **Chronic transfusion therapy** designed to maintain hemoglobin S below 30% could prevent pain episodes almost completely. Potential complications make this an impractical long-term approach.



Hand Foot Syndrome- Dactylitis in a case of Sickle Cell Anemia

Osteomyelitis must be considered when fever accompanies localized bone pain. Osteomyelitis most commonly is due to *Staphylococcus aureus* and *Salmonella* species. Provide antibiotic coverage for both until an organism is isolated. In view of the frequency with which *S pneumoniae* is the responsible organism for meningitis, vancomycin is suggested initially for all cases. Antibiotic coverage can be modified once sensitivities are known.

Stroke - While it is unusual for children to have stroke, approximately 11% of patients with sickle cell anemia have strokes before they reach the age of 20 years. Hemiparesis is the usual presentation. Other deficits may be found, depending on the location of the infarct. 70-90 % children have repeat episode within 36 months. Convulsions frequently are associated with stroke. "Silent" central nervous system damage with cognitive impairment is observed in children. Main stay of treatment is maintaining on chronic transfusion so as to keep HbS level below 30%. Unless chronic transfusion therapy is provided, 70-90% of children who have a single stroke have subsequent events. Acute stroke may require urgent exchange transfusion. All acute neurological symptoms require investigation.

Acute chest syndrome-Results due to vasoocclusion of pulmonary vessels leading to infarction and pulmonary sequestration. It is seen in 40 percent of all patients and is more common in children; more severe in adults. It is an important cause of mortality and morbidity and requires parenteral antibiotic therapy.

Priapism Priapism is persistent painful penile erection. May last for few minutes to several hours. It usually subsides spontaneously. Severe cases cause erectile dysfunction and impotency.

Skin Ulcers



Skin Ulcers

Skin ulcers are relatively infrequent. Nonetheless, when skin ulcers occur, the problems are very serious. The most common site of skin ulcers is over the lateral malleoli. Treatment should be conservative.

Rest, elevation, and dry dressings with antimicrobial ointments are the best approach to this problem.

Cholecystitis

40% of adolescents with sickle cell anemia will have gallstones due to chronic hemolysis. Cholelithiasis is common in children who are affected. **Appropriate medical and supportive care must be provided.**

Febrile Illness.

Since it is impossible to distinguish bacteremia in its early stage from minor febrile illnesses, all fevers must be evaluated carefully and treated vigorously. The standard approach is hospitalization and parenteral antibiotic administration until blood cultures are negative or the child shows clinical improvement. Organisms that pose the greatest danger are encapsulated respiratory bacteria, particularly *Streptococcus pneumoniae*. The mortality rate of such infections has been reported to be 10-30%.

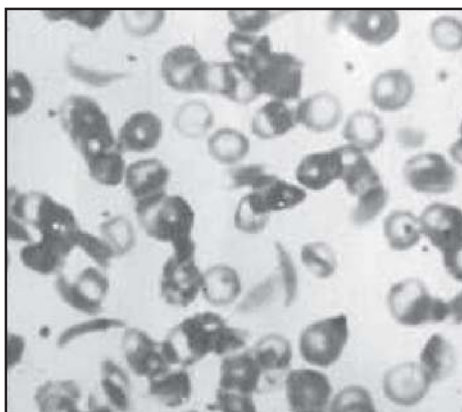
Lab Findings in Sickle cell anemia

Increased reticulocyte counts (5-15%) are seen. Total leucocyte count is increased in range of 12,000-20,000 /cmm. MCV is normal but MCHC may be increased. If severe anemia is present then peripheral smear may have nucleated RBC's, target cells, poikilocytes, hypochromasia, sickled red cells and Howell jolly bodies.

It is Confirmed by Hemoglobin electrophoresis or HPLC. Bone marrow is hyperplastic with erythroid predominance. **(Only required to confirm Aplastic crisis)**

Radiological abnormalities are common more often in vertebrae (Beaking of vertebrae), mild

expansion of marrow spaces, osteoporosis, sclerosis of long bones and femoral head, renal concentrating ability is also decreased.



Peripheral smear in a case of Sickle Cell Anemia

Diagnosis can be confirmed by Sickle solubility test and High performance liquid chromatography. Additional studies may be done as and when required including imaging studies to evaluate the location and extent of the lesion. CT scan is done after the hemoglobin S concentration is reduced below 30%. MRI is preferable. Ultrasonography can be used to visualize stones and detect signs of thickening gall bladder walls or ductal inflammation, indicating possible cholecystitis.

RED CELL MEMBRANE DISORDERS (SPHEROCYTOSIS, ELLIPTOCYTOSIS, OVALOCYTOSIS.)

The membrane of the red blood cell (RBC) consists of lipid bilayer and proteins over the outer surface of the cell. Specialized interactions occur between specific membrane proteins or lipids, or both, to maintain the stability of the membrane. These proteins are necessary to maintain the normal biconcave shape of an erythrocyte. A deficiency of any of the components of this membrane can lead to distorted red cell morphology, increased breakdown of red cells and anemia, i.e., **intracorpuseular (intrinsic) hemolytic anemia** and reduced red cell life span. Normal RBC survival span is 110-120 days (half life, 55-60 days)

Hereditary or acquired defects in structural components of the red cell membrane can result in decreased survival and increased destruction of RBCs leading to variable degree of anemia and include- Hereditary spherocytosis(HS), Hereditary elliptocytosis, Hereditary stomatocytosis

HEREDITARY SPHEROCYTOSIS (HS) ⁽⁹⁶⁻¹⁰⁰⁾

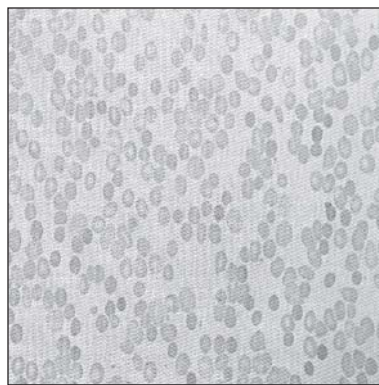
It is the commonest red cell membrane defect and is especially common in people of North European or Japanese descent. The prevalence may be as high as 1/5,000. It is a genetically-transmitted, as **Autosomal Dominant trait**, although sometimes the mode of inheritance can be **recessive**, and an estimated 25% of cases are due to **spontaneous mutation**.

It is characterized by the production of red blood cells that are sphere-shaped rather than donut-shaped, and therefore more prone to Hemolysis. A patient has a 50% chance of passing the disorder onto his/her offspring.

Spherocytosis has wide spectrum of symptoms & a varied clinical presentation, ranging from asymptomatic to severe hemolysis. The degree of anemia, jaundice and splenomegaly are extremely variable and may be absent, mild, moderate, or severe to the point of threatening life. **At one end of the spectrum** clinical symptoms can begin as early as in the intrauterine period to as late as adulthood and may be detected at any age. Hydrops fetalis with death in utero can occur in the most severe cases & jaundice needing phototherapy, exchange transfusion may be seen in neonatal period. **The other end of the spectrum** is silent disease detected on investigation, at the age of 80 years as a grand child has spherocytosis. **In-between** are range of symptoms that include – pain in abdomen, recurrent jaundice, gallstone - (Pigmentary gallstones appear starting from 4-5 years of age.) Hemolytic facies - less marked than in Thalassemia major, aplastic crisis, hemolytic crisis etc. In infancy and childhood the presentation is variable. Some children present with **pallor, acholuric jaundice, icterus, exercise intolerance, and progressive splenomegaly**.



Mother with Spherocytosis
Holding Newborn with
Jaundice & Spherocytosis.



Smear showing Spherocytes



Child with spherocytosis
with Anemia & jaundice

The diagnosis of hereditary spherocytosis is established by a combination of clinical examination, detailed history including family history, and laboratory tests. A **positive family history** is seen in upto 75% of patients. The red blood cells appear spherical and **lack the central pallor**. Peripheral blood smear shows **microspherocytes** and polychromasia. The percentage of microspherocytes usually correlates with the severity of hereditary spherocytosis. The mean corpuscular volume is normal, and the **mean corpuscular haemoglobin concentration is raised (36-38 g/dl)**. The

red cell distribution width (RDW) is increased. Evidence of **hemolysis** is in the form of **reticulocytosis (3-15%), decreased haptoglobin, indirect hyperbilirubinemia.** **Ultrasonic detection of gallstones** may be seen. **Coomb's test** is negative. **Increased red cell osmotic fragility** is seen. Spherocytes lyse in higher concentrations of saline than normal red cells. This feature gets accentuated when RBCs are deprived of glucose for 24 hours at 37°C (**Incubated osmotic fragility test**). However, this test is not specific for hereditary spherocytosis, but may be positive in hereditary elliptocytosis.

Complications of Hereditary spherocytosis include growth retardation, folate deficiency, hemolytic crisis, aplastic crisis due to parvovirus infections- erythroblastopenic crisis, chronic leg ulcers, gallstones and hemochromatosis.

Treatment

A regular follow up must be done once a child is diagnosed to have HS. An annual visit to the physician is recommended even in the absence of symptoms for clinical examination including a general assessment, measurement of spleen/Liver size, growth, and exercise tolerance. Regular Transfusion dependence is unusual, repeated transfusion must be avoided. Folic acid supplementation is needed to meet the increased bone marrow requirements. An ultra sonogram of abdomen at regular intervals is needed to look for gallstones, starting from the age of 5 years & estimation for iron load-Ferritin level can be done during follow up visits in the presence of chronic anemia. Erythropoietin may be of benefit in reducing or avoiding transfusion, and can usually be stopped by the age of 9 months. Splenectomy is needed in moderate and severe cases. Indications for splenectomy are persistent/Severe anemia, reticulocytosis > 10%, repeated hypoplastic or aplastic crisis or faltering growth

Splenectomy should be deferred till six years. Two to three weeks prior to splenectomy children should be vaccinated against Pneumococcal, Meningococcal, Hemophilus influenza B infections and Hepatitis B at the onset of diagnosis.

Following Splenectomy the children should receive **prophylaxis with penicillin** (age < 5y: 125 mg BD, age above 5y: 250 mg BD).

Hereditary elliptocytosis

The prevalence of hereditary elliptocytosis in the United States is not greater than **2.5 to 5 per 10,000**. However, in **West Africa** and **Southeast Asia**, where malaria is endemic, it may affect greater than 30 percent of the population. Treatment is needed only if there is chronic hemolysis. Folic acid supplementation (1mg/day) is needed. Splenectomy may be indicated if the haemoglobin is below 10 g/dl or there is reticulocytosis

APLASTIC ANEMIA (AA) IN CHILDREN ⁽¹³⁰⁻¹⁴⁸⁾

Aplastic anemia (A.A.) is a rare but potentially fatal, hematological disorder and is characterized by a reduction in the effective production of one or any of mature erythrocytes, granulocytes, & platelets by the bone marrow that leads to peripheral blood Pancytopenia. It is characterized by hypocellular bone marrow, in the absence of an abnormal infiltrate & with no increase in reticulin.

There is an absence or marked reduction of Haemopoietic cells in the bone marrow and an increase in fat cells.

AA results from the failure of normal haemopoiesis resulting in pancytopenia (i.e. Aplastic Anemia) or mono or bicytopenia (Eg. agranulocytosis)

Aplastic Anemia was first described in 1888 by Ehrlich in a young Pregnant Woman who had explosive fatal illness characterized by severe anemia, bleeding into skin, retina, and high fever. At autopsy, the bone marrow was found to have been completely replaced by fat. The term aplastic anemia was first used by **Chauffard in 1904. The term *aplastic anemia* was apparently introduced by Vaquez and Aubertin in discussions of the society of the Hospital of Paris in 1904.** The word “**aplasia**” is derived from the Greek Verb, “to create, give shape,” and thus emphasizes the functional abnormality in blood cell production

The disorder may be **Acquired**, or **Inherited (genetic, but not necessarily expressed at birth)**, or **Congenital, (present at birth) or any combination of these variants.**

Aplastic anemia (AA) may be classified as

- **Idiopathic**
- **Secondary**

Idiopathic- In about 50% of these cases, the etiology is unknown (Idiopathic AA).

Secondary – Acquired, Inevitable, Inherited / Congenital, Malignant

Miscellaneous- Diffuse eosinophilic fasciitis, pregnancy

Acquired AA - Acquired AA results from the failure of normal haemopoiesis. It is an Idiosyncratic **reaction** with unpredictable recovery & severity that may follow exposure to a drug or virus or radiation. **Inevitable AA** follows exposure to cytotoxic drugs or radiation. Predictable, dose-related reversible marrow suppression like benzene, radiation, etc. can cause this disease. Susceptibility is presumably genetic. Some drugs e.g. chloramphenicol, have both dose-related and idiosyncratic effect, rarely one following the other.

Drugs and chemicals which may cause marrow aplasia

Antibacterial	Chloramphenicol more often oral than Intravenous Streptomycin; Penicillin, Methicillin, Sulphonamid, Quinacrine
Antimalarial	Mepacrine, Quinacrine, Chloroquine, Mefloquin, Trimethoprim Pyrimethamine
Antihistamine	Chlorpheniramine, Cimetidine, Ranitidine,
Anticonvulsant,	Phenytoin or hydantoins, Mesantoin, tridione, Carbamazepine
Tranquillizer	Promazine, Chlorpromazine,
Anti-inflammatory	Indomethacin, Diclophen, Gold, Phenylbutazone, Napraxone
Antipurines	Azathioprine

Anti diabetic	Chlorpropamide, Tolbutamide
Anti thyroid	Propylthiouracil, Methimazole
Solvents	Benzol, Whitespirit (benzene, naphtha, Carbon tetra Chloride)
Kerosene,	Glue (sniffing), Hairdye
Antidepressant	Phenothiazines, Dothiepin
Insecticides	DDT, Linadane, Chlordane, Organophosphates Benzene & it' derivative
Other drugs	Allopurinol, Quinidine, Penicillamine, Acetazolamine, Amphotericin, Aspirin

Viral infection. Infection like Post-Hepatitis- Parvovirus, EB virus, CMV, HIV, etc Viral infection is probably an under diagnosed cause. The destructive effect of virus may be due to direct action or an immune effect such as T-lymphocyte mediated suppression. **The best known association is with viral hepatitis- usually “non-A,B,C.”** Hypoplasia is usually severe, though the preceding hepatitis may not be, and manifests at 9-10 weeks after onset of hepatitis. Hypoplasia occurs in isolated instances of infection with **EBV, HIV, Varicella, CMV, Dengue-type viruses, Measles, Mumps and Parvovirus.** Transient hypoplasia may occur in **rickettsial infection, e.g. Q fever and Mycobacterium infection**

Congenital/Inherited Inherited case may or may not be present at birth (congenital).- Fanconi's anemia, Dyskeratosis congenita, Reticular dysgenesis, Shwachman-Diamond syndrome, Miscellaneous e.g. Familial aplastic anemia, Monosomy 7, Down's syndrome

Malignant- Malignant AA presents particularly as acute lymphoblastic leukemia in childhood, which may present as aplastic anemia.

Severity of aplastic anemia-

The severity of aplastic anemia was **classified by Cammita or Co-workers.** Specific blood criteria for defining AA are that the peripheral blood must show at least **two of the following three Criteria:**

Severe aplastic anemia

Hemoglobin < 10 g/dl Platelets < 50 x 10⁹/l Neutrophils < 1.5 x 10⁹

along with the presence of either of marrow criteria:

Marrow criteria:

- **Severe hypocellularity (<25%)**
- **Moderate cellularity (<50%) with < 30% of Hematopoietic cells.**

There are established criteria for the severity of AA. Severe AA has been defined by **the International AA Study Group in 1976.**

Of those patients who die, the majority will die during the first 4 months and/or during the first two years (approximately 60%).

Very severe aplastic anemia is further defined by a granulocyte count less 200/ μ l. The prognosis for patients with moderate AA is considerably of pancytopenia or transfusion-related hemosiderosis. Overall age and level of granulocytopenia are the two most important prognostic determinants.

Clinical Presentation

Symptoms and signs depend on cell line involved. **Thrombocytopenia** will lead to bleeding manifestations especially skin bleeds, mucosal bleeds, hematuria and rarely intracranial hemorrhage. **Neutropenia** will lead to infection and PUO, with or without localization of infection. **Anemia** appears last and if severe will lead to fatigue, breathlessness, puffiness, oedema of feet and CCF. One should look for evidence of etiological factors like hepatitis, history of drug intake. Presence of hepatomegaly, lymphadenopathy, bone pains etc. usually rules out aplastic anemia and suggest more sinister disease like leukemia.

Aplastic anemia-Child with Bleeding Manifestation.

Bone marrow aspiration smear.



Pancytopenia without leukemic cells in the blood is most likely due to aplasia, but diagnosis requires aspirate and trephine biopsy of marrow. Pancytopenia is usually severe (Hemoglobin to about 3 g/dl). In about 40% of cases **erythrocytes are macrocytic.** There will be **anemia with normal RDW with normocytic, normochromic RBCs,** occasional **Macrocytosis.** Reticulocytes are usually decreased, but occasionally are inexplicably excessive for the anemia. Iron study may show iron overload. Stressed erythropoiesis is evident in form of **raised HbF and i-antigen** in some patients.

Lymphocytes are normal to decreased. **Platelet size is not increased (increased in immune thrombocytopenias).** There is presence of **leucopenia with decreased ANC,** as also a **decreased platelet count with normal MPV.**

It is mandatory to do bone marrow trephine biopsy as well as aspiration to diagnose AA. It shows **hypo cellular marrow with empty spicules, increased fats spaces,** and increased lymphocytes/plasma cells etc.

Chromosomal studies should be done **to rule out Fanconi's anemia** which will show chromosomal breaks.

MANAGEMENT OF APLASTIC ANEMIA

Besides various forms of 'definitive therapy', availability and access to long term adequate 'supportive care' decides the outcomes.

The options available for treatment are

Bone marrow transplantation (BMT)

Immuno-modulation

Anti-lymphocyte globulin (ALG)

Anti-thymocyte globulin (ATG)

Cyclosporine-A (CA)

Methylprednisolone (MP)

Androgens

Hematopoietic growth factors

Supportive therapy:

Antibiotics, Platelet support, Blood support and management of iron overload.

BONE MARROW TRANSPLANTATION

It is an effective modality of treatment for SAA. However, problem areas include:

Donor availability, full house HLA matched family member, almost always a sibling, is needed as a donor. Success rate from HLA identical but unrelated donor or transplant across HLA barriers from blood relatives is very low. Unfortunately HLA-matched sibling donors are not available for large number of patients.

Age: Elderly patients have high incidence of graft versus host disease. Hence BMT is often deferred after the age of 40-45 years.

Transfusion history : Heavily pre-transfused patients especially where blood products are used without leukocyte filters, suffer from high incidence of graft rejection.

Infection at time of transplant: This always increases the problem.

Thus, a good candidate for BMT should satisfy following criteria: untransfused, uninfected, young patient with HLA-matched sibling being available as a donor. Such patients have 60-70% chance of long term survival following BMT. Rest may succumb following BMT due to acute graft vs host disease (GVHD), Interstitial pneumonitis, other infections or Veno-occlusive disease (VOD).

IMMUNO-MODULATION

Anti Lymphocyte Globulin and Anti Thymocyte Globulin:

These are the two most popular immuno-modulatory agents effective in management of AA. ALG is used in higher dose i.e. 40 mg/kg/day for 5 days, while ATG is given at 15 mg/kg/day for 5 days. Duration of treatment varies from 5-10 days. Shorter intervals of treatment with antithymocyte globulin (ATG) (5-10 days) are probably as efficacious as longer therapy (28 days).



ATG can cause severe anaphylactic and allergic reactions. Anaphylaxis could be lethal, but fortunately it is rare. A skin test is useful and may indicate the need for desensitization. Other allergic reactions like fever, chills and urticaria are common. Serum sickness after about 10 days of therapy is also common. Serum sickness is described in about 47% of patients. 60 to 80 mg of Methyl Prednisolone daily is helpful in avoiding allergic reactions as well as serum sickness. It takes a few months for overt improvement to occur. Overall long-term survival after immunosuppressive therapy is comparable to BMT.

Cyclosporin-A: This drug in doses upto 12-15 mg/kg/day for 6-12 months, when combined with ATG/ALG, produces remission rates of about 70%. Nephrotoxicity can be dose limiting. All patients on Cyclosporin must receive *Pneumocystis carinii* prophylaxis. Doses are usually adjusted to achieve blood levels between 200-500 mcg/L. Cyclosporine levels are usually determined at 2 weeks intervals. Major adverse effects are reflected in liver toxicity. When transaminases are up, the clinician should stop therapy for 1-4 days and resume treatment with a lower dose. If this does not help, therapy should be stopped.

High dose corticosteroids are popular in Europe and probably effective in patients treated within few weeks of diagnosis, this therapy is reserved for occasional patients due to tremendous toxicity. The dose of methylprednisolone is 20-30/100 mg/kg for a week tapered over a month.

ANDROGENS Androgens have a mixed reputation. Probably dose makes the difference. Nandrolone Decanoate at 5 mg/kg/week IM for at least 3 months may be tried. The advantage of this preparation is freedom from hepatotoxicity.

HEMATOPOIETIC GROWTH FACTORS

Granulocyte-Colony Stimulating Factor and Granulocyte-Macrophage-Colony Stimulating Factor, when added to antibiotics are helpful in management of infected neutropenic patients. They may also be useful as a part of immunosuppressive regimen as they promote hematopoietic regeneration. Interleukin - 1 (IL-1) and interleukin-3 (IL-3) have shown no efficacy in aplastic anemia. This is still in experimental stages.

SUPPORTIVE THERAPY

Infections and antibiotics:

Selective gut decontamination with antibiotics, aggressive treatment with broad-spectrum antibiotics and/or amphotericin-B must be administered at the first sign of infection, Broad spectrum full dose parenteral antibiotic therapy is needed to control sepsis which is a major cause of mortality in aplastic anemia.

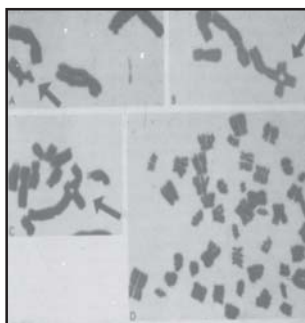
Bleeding and platelet transfusions: The convenient goal is to maintain platelet count over 10,000/cmm. The most feared complication is spontaneous intracranial hemorrhage, which can be fatal. **Aspirin must be avoided.** Coagulation defects due to antibiotics and vitamin-K deficiency must be corrected.

Maintaining hemoglobin up to 7-8g/dl (or even more if there is cardio-respiratory disease) is essential so as to permit average physical activity. Over a long period, this can lead to **iron overload** related organ toxicity and hence need for iron chelation. Leukocyte filters should also be used.

INHERITED/CONSTITUTIONAL-APLASTIC ANAEMIA (genetic syndromes with hypoplasia of bone marrow).

These genetic Syndromes may be associated with number of congenital abnormalities, especially of the bones, kidney and hearts and have various mode of inheritance.

Hematological manifestations may not be present at birth or early infancy and may not become manifest until the first year to even first decade of life and may manifest during adolescence or even during adult life and may initially present with single cytopenia and subsequently progress to pancytopenia.



Child with fanconi syndrome with bifid thumb/digital anomalies and mental subnormality.

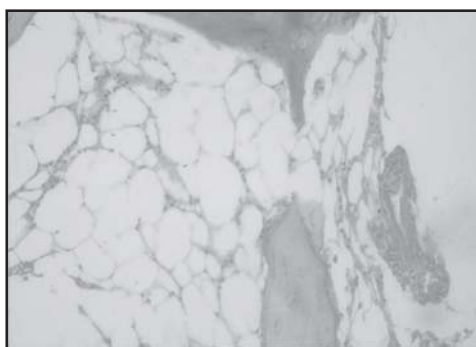
Chromosomal study showing Chromosomal breaks & condensation

Fanconi's anemia (FA)

It is the best recognised constitutional pancytopenia. A number of other infrequent genetic disorder also have been described. In 1927 Fanconi reported cases of three brothers who had pancytopenia with typical physical changes. Since then more than 700 cases of FA have been reported worldwide.

It is a type of inherited aplastic anemia, characterised by physical changes including generalized or perioral hyper pigmentation, cafe-au-lait spots, short stature, microcephaly, mental subnormalities, skeletal anomalies like absent or hypoplastic thumb, bifid or triphalangeal thumb, absent radius, hypogonadism, spinal anomalies, renal anomalies like ectopic kidney, double ureter, eye anomalies, deafness, ear malformations, GI anomalies, cardiopulmonary anomalies etc.

By definition diagnosis of FA requires presence of chromosomal changes; and both physical changes and anemia need not be present. **In fact more and more FA cases are diagnosed, who do not have aplastic anemia or typical physical changes, by studying chromosomal changes in family members of an index case of FA.** 15-30% of patients with FA are physically normal or have only short stature and or skin changes. The basic defects are in the poor DNA repairs leading to spontaneous or induced breaks in Chromosomes. Blood abnormalities are rare before 18 months and may not manifest till about 20 years. Average age at onset of pancytopenia is about 6 and a half years for boys and about 8 and a half years for girls. Thrombocytopenia is usually the first sign, and may be misdiagnosed as idiopathic if the association with somatic anomalies is not recognized. Granulocytopenia and then anemia follow, evolving over months to years.



Trephine biopsy showing
hypocellular marrow



IVP showing
ectopic kidney



Fanconi Anemia
with polydactyly

Mean age at diagnosis is 7 to 8 years with 4 present cases < 1 year old and 10 percent cases > 16 years of age. Male to female Ratio is 1.06: 1.0. Mode of Inheritance is Autosomal recessive type of inheritance with chances of FA occurring in Siblings and cousins. History of parental consanguinity may be present.

Investigations done in a case of suspected FA include CBC which reveals progressive anemia, macrocytosis, low reticulocyte count, changes of stressed erythropoiesis like increased HbF levels and presence of 'i' antigen. Gradually patient will progress to pancytopenia once aplasia of bone marrow sets in.

Bone marrow and trephine biopsy - In the presymptomatic period the marrow may appear normal or show hyperplasia

Diagnosis is most reliably made by quantitation of **chromosomal breaks** (cultured blood

lymphocytes) induced by the DNA cross-linking agent, diepoxybutane (DEB, Auerbach et al 1989). Abnormal fragility appears to be specific for Fanconi's anemia and is detectable from birth and before onset of cytopenias.

Prognosis & Therapy

Without therapy 80 percent FA Patients die at age of 16 years and 2 years following aplasia and most by 4 years following aplasia. Twenty five percent survive beyond third decade.

Bone marrow transplantation (BMT)

The only hope of long term survival is BMT for FA patients.

Due to inherent chromosomal instability, these patients are sensitive to radiation and chemotherapy used for conditioning. Accordingly low doses of cyclophosphamide should be used for better outcome. The success of BMT is 78% with conditioning regimen using 20 mg /kg of Cyclophosphamide over 4 days and 5 cGy of total **abdomino -lymphoid radiation** and 48% when cyclophosphamide is used in the dose of 50 mg/kg over 4 days.

FA patients are prone to develop malignancies later on. It includes **15-20% chances of leukemia** mainly **AML** and liver cancers mainly due to androgens. Rare cancers include **preleukemia, gynecological tumors, Wilm's tumor, meduloblastoma etc.**

Traditional therapy for those who can not undergo BMT is **steroids and androgens like Oxymethalone, Nandrolone**, alone or in combination and may take months to achieve maximum benefits. Mean age of Survival with Androgen in 17 years or 7 years following onset of aplasia which is little better than no Therapy. Immunotherapy with ATG/ALG or IV Pulse Methylprednisolone is helpful in less than 10 percent of patients. This proves that it is the seed which is in FA and not soil. **Other Measures like supportive care, use of blood products, prevention of HLA sensitization, treatment of infection, monitoring of Toxicities of drugs used etc should be as for acquired Aplastic Anemia.** Transient responses have been observed with Granulocyte colony stimulating factors (G-CSF), Erythropoietin, IL3, IL-6. Genetic counselling is important.

RED CELL ENZYMOPATHY

Defects of enzymes of glycolytic pathway, hexose monophosphate shunt or pentose phosphate pathway in red cells, lead to hemolytic anemia and are described as red cell enzymopathy or erythro-enzymopathy (EEP).

The most well known and commonly involved widely distributed EEP is the deficiency of G6PD, which is involved in the reaction of pentose phosphate pathway. **Deficiency of pyruvate kinase** and other enzymes of glycolytic pathway also result in hemolytic anemia but are less commonly seen as compared to G6PD deficiency. More than 500 million people are estimated to be involved (most of them mostly asymptomatic). **Though the distribution of G6PD deficiency is world wide, highest prevalence is observed in Mediterranean countries, Africa and Asia.**

In India, G6PD deficiency was first reported almost 40 years ago. Prevalence in India varies from 0-27 % in various castes, tribes and ethnic groups. The disorder is transmitted as x-linked recessive trait.

Malaria hypothesis

Incidence in prevalence of G6PD deficiency has led to the hypothesis that G6PD deficiency is a polymorphism that confers protection from *Falciparum malaria*. Malarial parasite grows less well in red cells deficient in G6PD. Decreased parasitemia has been documented in these individuals. 442 different variants of G6PD have been identified. From India, 13 different biochemically characterized variants have been reported.

The main role of pentose phosphate pathway is related to metabolism of glutathione (GSH) through production of reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) GSH is important for preservation of sulfhydryl group in many proteins including hemoglobin and to prevent the damage from oxidative radicals in general. It involves oxidation of glutathione (GSH) to GSSG. Thus GSH should be constantly available in the reduced form which is effected by GSH reductase through NADPH, the latter is provided by G6PD-thus the crucial role of G6PD in preventing oxidative damage. On account of G6PD deficiency, the red cells of these patients have limited capacity to regenerate GSH and the reserve gets depleted soon. Exhaustion of GSH allows oxidation of sulfhydryl group of hemoglobin (and other proteins) resulting in denaturation of hemoglobin. Coarse precipitates of hemoglobin lead to damage of red cell membrane and hemolysis.

The patients with G6PD deficiency can present clinically as acute hemolytic anemia (AHA), neonatal jaundice (NNJ) and chronic non-spherocytic hemolytic anemia (CNHSA).

Acute Hemolytic Anemia

A child with G6PD deficiency is clinically and hematologically normal most of the time. However, they may present with severe symptoms & clinical picture upon ingestion of fava beans (favism), during the course of infection, or after exposure to certain oxidative agents. After a lag period of hours the child may become irritable or lethargic. Associated nausea, vomiting, abdominal pain, diarrhea may be present.

Urine is discolored, dark, as red, brown, or black, and **resembles Coke or strong tea or port wine**. Urinary discoloration is on account of intravascular hemolysis.

As there is acute drop in Hemoglobin the child will be pale and tachycardic; may present with appearance of acute pallor, breathlessness, hypovolemic shock and features of frank congestive cardiac failure, following ingestion of drugs like aspirin. Jaundice may be present. Occasionally, backache and abdominal pain are observed. The spleen is usually moderately enlarged, and the liver may also be enlarged; either or both may be tender. Gall stones are not uncommon. Hemoglobinemia and hemoglobinuria may result in azotemia or acute renal failure.

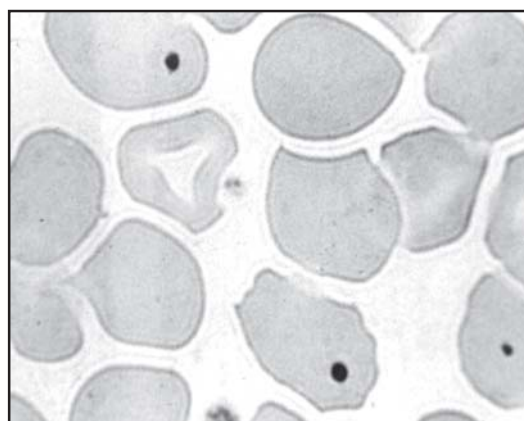
List of drugs known to cause hemolysis in G6PD deficient patients.

Ascorbic acid
Colchicine

Chloramphenicol
Cyproheptidine
Chloroquine
Chlorpheniramine
Cetirizine
Ciprofloxacin
Dapsone
Diphenhydramine
Dopamine
Doxorubicin
Furazolidone
Isoniazide
Loratadine
Menadiol sod. Sulfate
Menadione
Mepacrine
Nalidixic acid
Naphthelene
Niridazole
Nitrofurazone
Nitrofurantoin
Norfloxacin
Para amino-benzoic acid
Phenacetin
Phenytoin
Phenylbutazone
Phytomenadione
Primaquine
Probenecid
Proguanil
Pyrimethamine
Promethazine
Terfenadine



Child from Khoja Community with G6PD deficiency



PS showing Heinz Bodies

Laboratory findings during intravascular hemolysis and AHA include moderate to extremely severe anaemia which is **normocytic and normochromic**. There is often marked **anisocytosis** (reflected as a wide red cell size distribution on the electronic counter). There is also marked **poikilocytosis** with presence of distorted red cells “irregularly contracted” red cells, some of which can be spherocytes. The reticulocyte count is increased (Intense reticulocytosis).

The white blood cell count is usually moderately elevated, with a predominance of granulocytes. The platelete count may be normal, increased, or moderately decreased. The **unconjugated bilirubin level is elevated**, but “liver enzyme” levels are generally normal. The dark **urine**

tests strongly positive for blood (Hemoglobinurea)

G6PD Screening test-Fluorescent spot test and dichlorophenol indophenol (DPIP) decolorization method –Decolouration time prolonged.

Treatment of acute episode of intravascular hemolysis includes transfusion support for anemia and supportive care. Fluid therapy during such episodes is important for prevention and treatment of acute renal failure. Prevention of AHA centers around avoiding the known trigger drugs in these patients. During steady state, administration of folic acid is recommended as the requirement is increased due to increased red cell turnover. The most important complication that may require treatment is acute renal failure, which is exceedingly rare in children.

Neonatal Jaundice (NNJ)

Other than AHA, NNJ is a common manifestation of G6PD deficiency. Almost one third of male neonates have been described to have NNJ. Among neonates with severe jaundice requiring exchange transfusion, G6PD deficiency has accounted for a large number of cases. In a review of cases of kernicterus in world literature, G6PD deficiency was the cause in 13 out of 88 cases which is next in frequency to only Rh and ABO incompatibility. Prevention of NNJ is based on neonatal screening for enzyme defect and then taking precautions in cases with enzyme deficiency. Management of NNJ is like any other cause of unconjugated hyperbilirubinemia including phototherapy and exchange transfusion.

A novel approach of using a heme-oxygenase inhibitor tinmesoporphyrin (Sn-meso-porphyrin) has been found to be extremely useful in preventing the development of significant hyperbilirubinemia in G6PD deficient neonates

Chronic Non-spherocytic Hemolytic Anemia (CNSHA):

The term CNSHA in relation to deficiency of G6PD and other enzymes is used to describe chronic anemia with normal / near normal red cell morphology, particularly to differentiate it from hereditary spherocytosis.

CNSHA develops in a minority of cases with G6PD deficiency Clinical picture is quite variable. Unlike NNJ which can affect female children, CNSHA affects only male patients. Very few cases will need to be started on chronic transfusion therapy like patients with thalassemia and hemoglobinopathies. Splenectomy may be required due to large size, development of hypersplenism or to decrease the transfusion requirement.

Other erythroenzymopathies involved are

- Pyruvate kinase deficiency
- Hexokinase deficiency
- Glucose Phosphate Isomerase deficiency
- Phosphofructokinase deficiency
- Aldolase deficiency

- Triose phosphate Isomerase deficiency
- Glyceraldehyde-3- phosphate dehydrogenase deficiency
- PK deficiency
- Phosphoglycerate kinase deficiency,
- 2,3-Bisphospho-glycerate mutase deficiency
- Enolase deficiency
- Lactate dehydrogenase deficiency
- Glutathione Synthetase deficiency

In Indian population, the deficiency of these enzymes has been studied only sparingly. In contrast to G6PD deficiency, patients with deficiency of enzymes of glycolytic pathway usually have CNSHA with onset in neonatal period. Drug induced hemolytic anemia is not a common problem in them. Most cases benefit from splenectomy.

Effort has been made to simplify understanding “Anemia in children”, particularly etiology, clinical manifestations, practical approach to the diagnosis and range of management aspect of various diseases. Due to restricted number of pages and readers being of various specialities, care has been taken not to go in depth in any aspect of the disease.

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