REVIEW ARTICLE

INDICATORS FOR ASSESSMENT OF ANEMIA AND IRON DEFICIENCY IN COMMUNITY

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Summary

The review discusses problem of nutritional anemia, particularly iron deficiency with or without anemia. Critical analysis continues to recommend cyanmethemoglobin method for estimation of hemoglobin; filter paper technique is suited for field surveys. Recently introduced HemoCue method for hemoglobin estimation is practical. However, variability in hemoglobin estimation as compared to cyanmethemoglobin need be evaluated in selected laboratories of the country to derive some form of conversion factor. The red cell indices especially mean corpuscular volume (MCV), percentage mature hypochromic erythrocytes (HyPom), red cell distribution width (RDW) and reticulocyte changes- hemoglobin concentration(CHr) and size(Ret Y), in institutions with modern automated analyzer are of interest as these are more sensitive indices than the hemoglobin level.

Serum iron, total iron binding capacity and transferrin saturation are not good indicators of iron status. Serum ferritin represents body iron stores; in infection free situation, it is an ideal indicator for diagnosis of iron deficiency and response to iron therapy in community. Recent studies suggest estimation of serum transferrin receptors in addition to serum ferritin in diagnosis of iron deficiency. Bone marrow iron assessment is useful in individual patient; it is not affected by infection. For country like ours, with high prevalence and severity of anemia, hemoglobin estimation, peripheral smear to exclude malaria and response to iron therapy remain important time tested tools to diagnose iron deficiency.

Keywords : Iron deficiency, hemoglobin, serum ferritin, transferrin receptors, red cell indices.

In India prevalence of anemia at all ages remains very high e.g. 84.9-86% pregnant women in rural areas are affected and 9.3-9.9% have severe anemia (1,2). These studies observed similar prevalence rates for adolescent girls and lactating women. Kapur et al (2002) in an urban slum with ICDS program for 2 decades observed 64% children between 9-36 months of age as anemic with 7.8% having severe anemia (3). Eighty eight percent of these children had low serum ferritin. Besides iron deficiency, folic acid in pregnancy and vitamin B12 deficiencies in early childhood are also common (4). Data from US NHANES survey III 1988- 94 showed iron deficiency prevalence of 6- 18% in children 12-35 months of age. It was observed that anemia is a poor predictor of iron deficiency. The positive predictive value of hemoglobin < 11.0 g/dl was 29 % (95%, CI 20-38%) and sensitivity was 30% (CI 20-40%). If hemoglobin cut off was 10.7 g/dl, the positive predictive value became 38% (CI 24-52%), but lowered the sensitivity to 15% (CI 7- 22%). Most children with iron deficiency are necessarily are not anemic (5). Recent studies show that sensitivity and specificity of diagnosing iron deficiency can be improved by assessing transferrin iron saturation, mean corpuscular hemoglobin concentration, and erythrocyte - protoporphyrin, percentage of hypochromic mature erythrocytes (HyPom) or reticulocyte hemoglobin concentration (CHr). However, these changes are indistinguishable to those in chronic disease anemia. The optimal diagnostic approach appears to measure the serum ferritin as an index of iron stores and the serum transferrin receptor as an index of tissue iron deficiency (6). The available approaches in diagnosing iron deficiency are discussed in this review.

Iron nutritional status : In normal individuals, 2/3 of total body iron is available for hemoglobin formation. The remaining 1/3 gets deposited as hemosiderin and ferritin. This can be mobilized when iron is needed in functionally active form. Normal women for pregnancy need around 1000 mg of iron. Iron stores in healthy full term can meet the infants iron requirements until 4-6 months of age. Infant increases body weight and blood volume by 3 times with increase in total body iron by two times, by the first birthday. However preterm and low birth weight babies having poor iron stores, become deficient earlier (within first 2 months of age). In maternal iron deficiency there is proportionately less iron transfer to fetus (7,8). Nature tries to compensate by secreting more iron in breast milk of the iron deficient mothers (9). Intrauterine and early life iron deficiency reduces brain iron content and alters neurotransmitters irreversibly, affecting mental functions (10).

Iron deficiency : is defined as a condition in which there are no mobilizing iron stores, causing a compromised supply of iron to tissues, including the red cells. It varies greatly with each stage of the life cycle, in general growing children (up to 2 yr of age), pregnant and lactating women and adolescents (second growth spurt) are the most vulnerable. In iron deficiency, hemosiderin and ferritin (iron stores) decrease; supply of iron to the transport protein (apotransferritin) gets compromised, resulting in decrease in transferrin saturation and increase in transferrin receptors.

Stages in iron deficiency (11) : First stage- depletion as storage iron is observed as fall in serum ferritin (reflects low iron in liver/spleen/bone marrow). There is generally absence of stainable iron in bone marrow during rapid growth period. Second stage- decrease in transport iron, results in low serum iron, increased total iron binding capacity and decrease in transferrin saturation. Both these stages are preanemic (Latent iron deficiency). Third stage- there is significant fall in supply of transport iron, restricting hemoglobin synthesis with increase in erythrocyte- porphyrin. Microcytosis appears, accompanied with hemoglobin fall to fulfill the laboratory definition of anemia. Iron deficiency as a steady state - as mild deficiency develops very gradually, and is almost in equilibrium due to conditions lasting for months or years. The laboratory values for serum ferritin and transferrin saturation are in normal limits, but such patients

respond to iron therapy.

Iron overload syndrome: or hemochromatosis (primary or secondary), affects liver, pancreas, heart, endocrine glands and skin. Serum ferritin and transferrin saturation are raised, while erythrocyte-protoporphyrin, MCV and hemoglobin remain in normal range.

Methods of assessing iron status

WHO/CDC (12) expert consultation recommended addition of transferrin receptor in addition to hemoglobin and serum ferritin for assessment of iron status in places where infection is common. In situations with high prevalence of anemia a) clinical assessment, b) hemoglobin estimation and c) response to iron supplementation may suffice. Detailed investigations will be required only in those who do not respond to iron supplementation.

1) Clinical assessment: in moderate to severe anemia (11,13) -i) significant pallor of eyelids (difficult in children due to frequent conjunctivitis), tongue, nail beds and palms (pale palm creases suggest severe anemia; ii) fatigue, low exercise capacity (mild anemia can produce decreased exercise tolerance); iii) fissures at the corner of the mouth suggest anemia; iv) nails, show pallor, flatness, softness to feel and later become spoon shaped (koilnychia). It is rare in children < 6 yr of age, as hemoglobin is sacrificed to maintain tissue growth; v) children develop irritability, pica for ice/mud/coal/substances containing lead; vi) findings of congestive failure indicate severe anemia, hemoglobin below 5.0g/dl; vii) iron deficiency affects mental functions (10) i.e. attention span, alertness and learning, and viii) deficiencies of vitamin B12 and folic acid are associated with psycho-neurological changes and pigmentation of mucous membranes and distal parts of the body. These clinical features are of diagnostic assistance for the individual patient with severe anemia to seek advice from a medical expert. However, clinical features fail to assess degree of anemia and therapeutic response. Thus hemoglobin estimation is the criterion. Anemia (as measured by hemoglobin or hematocrit estimations) is not specific of iron deficiency; however it is the commonest deficiency in India, thus a presumptive diagnosis can be made. Normal hemoglobin and hematocrit distribution varies with age, gender, at different stages of pregnancy and with altitude and smoking. Given the wide range of hemoglobin concentrations in the population as a whole, an individual can have a substantial decrease in hemoglobin with the value still in the normal range.

2) Hemoglobin and hematocrit: can be used to assess anemia in community where prevalence is of public health significance. The problem arises in capillary blood collection, the technician should be very careful. To obtain the best possible skin puncture sample, it is important to warm the extremity in order to facilitate a free flow of blood and to avoid any squeezing of the finger. In community surveys discrepancy up to 0.5 g/dl between capillary and venous blood is acceptable. Collections of venous blood give accuracy and allow study of other parameters, if needed.

Hemoglobin estimation (14-19)

a) **Copper sulphate method-** used in blood banks, discriminates donor's hemoglobin content > 10g/dl.
b) **Sahli's method (acid-hematin) -** developed color is unstable, begins to fade almost immediately after it reaches its peak. It does not estimate fetal hemoglobin in infants.
c) **Oxyhemoglobin method -** Though simplest and quickest, does not have a stable HbO2 standard. It is also not satisfactory in presence of methemoglobin, sulphemoglobin etc.
d) **Alkali-hematin method -** It gives true estimate of total hemoglobin, except the resistant hemoglobins i. e. fetal and Bart's hemoglobins require heating in a water bath for 4 min. Method is cumbersome and less accurate than the oxyhemoglobin and cyanmethemoglobin methods.
e) **Cyanmethemoglobin method -** is the best time tested method for estimating the hemoglobin concentration quantitatively. Blood is diluted in a solution containing potassium cyanide and potassium ferricyanide. Hemoglobin, in blood is found in variety of forms including oxyhemoglobin, carboxyhemoglobin, methemoglobin, fetal hemoglobin and other minor components are converted to cyanmethemoglobin (HiCN). Sulphemoglobin is not converted, but is rarely present in significant amount. Standard is internationally developed and is stable for several yr. In field surveys blood is delivered on to No 1 Whatman filter paper (1.5 x 1.5 cm square), dried and transported. Drabkin's solution in screw capped tubes can be taken to the field; once the blood is mixed some loss of fluid will not alter the result. This method is reproducible and suited for field hemoglobin estimation. It is also possible to use battery operated colorimeters for hemoglobin estimation in field. Main errors in measurement arise from i) dilution, ii) sample turbidity (incomplete lysis of blood cells), lipids, and raised leucocyte count. In automated method the precision is less than 1%.
f) **HemoCue system- hemoglobin estimations:** India had large field survey experience in NFHS II (1998-1999) (18), it is suited for rapid field surveys, no dilution required, equipment is portable; results are available within 45 sec and read directly. Comparison with cyanmethemoglobin method showed variability at different hemoglobin levels. Thus no simple conversion factor is applicable. It is not possible to compare data on prevalence and severity of anemia as earlier studies used cyanmethemoglobin method. Taking cyanmethemoglobin as gold standard the sensitivity of hemoCue method was 0.75 and specificity 1.0, corresponding hemoglobin

values with both the methods fell within ± 2 SD with correlation coefficient of r = 0.922. These two methods showed the magnitude of difference as - 1.19 g/dl (C I: - 1.40- 0.98), thus suggesting an overestimate of hemoCue values by 10-15 % (correction factor being 0.389 + 0.831 Hb- hemoCue) (20). There is need to validate hemoCue method in 2-3 laboratories of our country, as it has field applicability, and overcomes dilution, collection, transportation and sample turbidity etc. This method is costlier as compared to cyanmethemoglobin method (for equipment as well as cuvettes).

g) **Hematocrit (microhematocrit)/ packed cell volume:** It is an acceptable and recommended method for anemia determination. Widely used for simplicity and availability. Equipment can be transported, needs constant voltage for operation. Blood is collected in anticoagulant treated capillary tube and spun in a portable microhematocrit centrifuge; plasma trapping is around 1-3% (15). Trapping of plasma is more if red cells are abnormal i.e. sickle cell, microcytosis, macrocytosis and spherocytes. Individuals with shock or dehydration may have risen or normal hematocrit due to hemoconcentration. Automated analyzer do not depend on centrifugation technique but calculate by direct measurement of RBC number and red cell volume (precision cv <1%).

3) Peripheral smear (15,19): In a well spread, fixed and stained peripheral smear; see whole film under low magnification, later selected areas be examined under oil emersion. In India anemic patient's peripheral smear on careful examination can be of importance to detect malarial parasite. Microcytic hypochromic red cells suggest iron deficiency. Macrocytic red cells with large hypersegmented neutrophils, suggest megaloblastic anemia (vitamin B12 or folic acid deficiencies). Marked leucopenia and thrombocytopenia are seen in aplastic anemias. Toxic granulations in neutrophils are indicative of infection. Immature leucocytes are observed in leukemias.

4) Response to iron therapy (21): Rise in hemoglobin (by 1.0 g/dl or hematocrit by 3% in 1-2 months), in an individual on iron therapy indicates iron deficiency anemia.

5) Red blood cells count and indices:

i **Red cell count-** Both erythrocytes and leucocytes are counted in whole blood. As the number of red cells is more than 500 times than the leucocytes the error introduced is negligible. In automated analyzer the observed precision is <1 % (cv) as compared to manual method with value of >11% (cv).

ii. **Mean corpuscular volume (MCV) -** is highly reproducible, less subjective to sampling error in finger prick, as dilution by the fluid does not influence the cell volume. It is a useful red cell index providing insights into path physiology of red cell disorders (classification of anemia). MCV is directly measured on the automated analyzer (by dividing the summation of red cell volumes by the erythrocyte count; cv <1%). But can be calculated from the erythrocyte count and the hematocrit (cv around 10%). MCV is higher at birth, decreased rapidly during first 6 months of life. Decreased MCV is observed in iron deficiency, thalassemia, infection and chronic diseases. Disproportionately low MCV with normal hemoglobin suggest thalassemia minor (22). Increased MCV is observed in megaloblastic anemia and liver disorders. Mentzer index- the ratio of MCV to RBC count in million; values < 13% is in thalassemia minor in 85%; while > 13% in 85% chances in iron deficiency (23). In contrast, MCH and MCHC do not add much significant clinical information.

iii. **Red cell distribution width (RDW):** Quantifies the red cell volume heterogeneity estimated by the more modern analyzers and reflect the range of red cell size measured with in a sample. It is useful in characterizing the microcytic anemias: iron deficiency- (high RDW, normal to low MCV) and uncomplicated heterozygous thalassemia (normal RDW, low MCV). Value > 15% proved highly sensitive (71 to 100%) but relatively non- specific (50%) for iron deficiency.

iv. **Percentage hypochromic mature RBC (hyPom),** can be measured on new automated analyzer (24).

v. **Reticulocyte:** a) Reticulocyte hemoglobin content (CHr) decrease is an early sensitive indicator of iron deficiency erythropoeisis. It is also reliable in assessment of iron therapy response (25). b) Presently automated hematology analyzer has automated reticulocyte counting and size (Ret Y) measurement as part of the testing. Ret Y is a sensitive indicator of iron deficiency (26). It correlates closely to sTfR, has highest overall sensitivity and specificity of the panel of tests used in differential diagnosis of iron deficiency vs chronic disease anemia.

6) Spun hematocrit (14): When erythrocytes are centrifuged (sediment by slow speed), the supernatant in iron deficiency patient is extremely pale as compared to its normal straw color. This is useful in differentiating iron deficiency from thalassemia syndromes as serum appears darker (with RDW extremely high > 20%).

7) Serum iron levels (range 50-150 ug/dl) (14): Over 3 years of age has marked diurnal variation, being higher by 30% in the morning as compared to the night. Value < 30ug/dl in the morning sample after 8 hr fast, suggests iron deficiency. The absolute value for total iron binding capacity (TIBC) is mostly increased in iron deficiency and decreased in anemia of chronic disorders.

$$\text{Transferrin saturation} = \frac{\text{serum iron} \times 100}{\text{TIBC}}$$

Transferrin saturation value (normal 20-45%) is more consistently helpful than either value alone.

In both children and adults value < 5% is diagnostic of iron deficiency, while <16% is suggestive of iron deficiency or for anemia of chronic disorders. However it does not reflect iron stores, is related to efficiency of moving iron out of iron processing cells (reticuloendothelial macrophages, hepatocytes or absorptive erythrocytes) and erythron. Non iron deficiency anemia with infection/inflammation may have transferrin saturation <16% rarely <10% but has low TIBC of 200ug/dl. The lower serum iron and TIBC are associated with decreased production of transferrin (B- globulin).

8) Soluble transferrin receptor (sTfR): It is a normal proteolytic cleavage product of transferrin receptor derived from erythroid precursor cells. An increase in serum transferrin receptors is a sensitive response during the early development of iron deficiency. Serum transferrin receptor levels increase progressively as the supply of iron to the tissues become deficient. The advantages are that it does not get affected by infection or inflammatory process; does not vary with age, sex or pregnancy. However, serum transferrin receptor level may be elevated in ineffective erythropoiesis (thalassemia syndrome) (27).

9) TfR-index: Ratio of sTfR to the log of serum ferritin; values > 1.5 suggest iron deficiency alone or in combination with inflammatory conditions. Value < 1.5 is characteristic of anemia of chronic diseases. TfR index is also sensitive enough to detect iron deficiency, before iron deficient erythropoiesis is clinically apparent. Both sTfR and TfR index are decreased in iron overload (28, 29).

10) Erythrocyte protoporphyrin (EPP) (14): In deficiency EPP increases moderately as there is less iron available to bind to protoporphyrin for conversion to heme. However does not differentiate from chronic inflammatory anemia. EPP shows marked increase in lead poisoning. Lead directly inhibits ferrochelatase the last enzyme in heme biosynthesis. In contrast, in thalassemia less globin chain synthesis, leads to commensurate decrease in protoporphrin synthesis (EPP). Studies in African children showed significant ethnic differences in sTfR and zn-PP(zinc protoporphyrin) levels suggesting separate cut off (30).

11) Iron stores: a) serum ferritin concentration gets altered independently of change in body iron burden. It predicts hepatic iron content to 95%. Unlike serum iron levels, serum ferritin value is not affected by recent iron therapy. However, it is also an acute phase reactant protein that is elevated in response to infection. Therefore, only an unequivocal low ferritin level in CRP negative (< 5 mg/L) patient can be used to make the diagnosis of iron deficiency. In India prevalent infections and protein energy malnutrition limit its use. Conditions like ascorbic acid deficiency, fever, infection/inflammation (chronic inflammation like rheumatoid arthritis), acute or chronic hepatic damage (release of intracellular ferritin), hemolysis and ineffective erythropoiesis (thalassemia major) increase the ferritin levels. The sensitivity of serum ferritin in diagnosis of iron deficiency was 80%, compared to 57% for HyPom and CHr, respectively (69% for both the RBC indices) and 26% to hemoglobin; taking TfR-index as the gold standard. Serum ferritin in relation to iron stores is depicted in Table 1.

Table 1: Serum ferritin in relation to iron stores

	Serum Ferritin in ug/L in children < 5 years	Serum Ferritin in ug/L in children > 5 years
Depleted iron stores	<12	<15
Depleted iron stores in presence of infection	<30	<30
Severe risk of iron overload	Adult male >200 and female >150	

High values of serum ferritin at birth indicate abundant iron stores. Infant, young children and pregnant women (diminishes in late pregnancy, even when bone marrow iron is present) usually have serum ferritin values near or in the range reflective of depletion; however a low level does not imply functional iron deficiency. Further it is of limited value in diagnosis of iron overload and reliance on this modality alone may be faulty.

12) Bone marrow iron (14) remains the gold standard for diagnosis of iron status. In iron deficiency, marrow hemosiderin is absent; in anemia of chronic disorders iron is always present. In early childhood it may not be reliable as bone marrow stainable iron generally not seen due to rapid growth. Iron stores are greatly increased in thalassemia major and sideroblastic anemia.

13) Hepatic iron (32) is of value but single needle biopsy may not be of help. This parameter is used in animal experiments to produce iron depletion (33). It is useful in diagnosis of iron overload.

Diagnosis of iron deficiency in pregnancy

- Hemoglobin level of <11.0g/dl in 1st and 3rd trimester and <10.5g/dl in 2nd trimester is recommended as cut off for anemia, by the American Centre for Disease Control and Prevention.
- MCV is raised in pregnancy (whether iron deficiency or not).
- Serum ferritin falls in 2nd and 3rd trimesters with or without iron deficiency.
- TIBC and serum iron- have sensitivity in pregnancy.
- EPP - fluctuates less in pregnancy.
- Serum transferrin receptor test is sensitive and is not affected in pregnancy.

Conclusion

To summarize in India we have situation of wide spread anemia and infection at all ages. Anemia is most likely due to iron and/or folate deficiencies.

Vitamin B12 deficiency is also common in younger age group. Hemoglobin estimation, peripheral smear and therapeutic response to iron are good time tested tools and should be used first in diagnosis of iron deficiency. In view of high prevalence of anemia to detect iron deficiency community level screening is recommended.

A. Universal Screening:

- Clinical assessment
- Hemoglobin estimation and peripheral smear to exclude hem parasite(s)
- Clinical response to iron administration
- Stool examination- to exclude worm infestation and 'Hem occult' blood test.

B. Selective screening for an individual: To confirm diagnosis of iron deficiency additional tests suggested are serum ferritin (with negative CRP) and tranferrin receptor (calculate TfR index) (27,28).

The subject of iron deficiency and search for ideal tool in diagnosis has continued for over sixty years (34), for a simple and reliable test to diagnose early iron deficiency. The estimation of iron and ferritin in saliva need further exploration (31,35). The newer indices RDW, HyPom, CHr and Ret Y need studies in Indian population.

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