LABORATORY EVALUATION OF IRON DEFICIENCY ANAEMIA

THE BLOOD

Erythrocytes

The degree of anaemia depends on the presenting circumstances. The anaemia may be mild if patient is being evaluated for underlying disease or unrelated disease. If anaemia is the presenting complaint, the blood haemoglobin level is usually 8gm/dl or lower.

The mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) values are reduced in the usual patients, and the mean corpuscular haemoglobin concentration (MCHC) is reduced in long standing or severe disease. The degree of change in red cell indices is related in part to the duration and in part to the severity of anaemia. In mild iron deficiency anaemia of short duration, the indices may be normal.

Anisocytosis is an important early sign in iron deficiency. The red cell distribution width (RDW) as determined by the Coulter Model s Plus, averaged 16.3% in iron deficiency compared with the normal range of 13.4 ± 1.2%. An increased RDW appears to be 90 to 100% sensitive for iron deficiency but only 50-70% specific.

Both percentage and the absolute number of reticulocyte tend to be normal or slightly increased, but rarely are they reduced.
The important finding in blood smear is exaggeration of their central pallor. The more severe the anaemia, the greater the degree of this change and the more numerous the corpuscles affected. In extreme grades of hypochromic anaemia, most of the erythrocytes are mere rings. Microcytes and a moderate no of poikilocytes are also found. In almost all instances, however, a variable number of well-filled erythrocytes are present, and some macrocytes, often polychromatophilic can be identified.

**Leukocytes and Platelets**

The total leukocyte count is usually normal in number, but absolute granulocytopenia may occur in long standing cases. A fresh haemorrhage of large volume may cause neutrophilic leukocytosis, even with the appearance of an occasional myelocyte. In anaemia due to hookworm eosinophilia is common.

The platelet count usually increases to about twice the normal level, and values return to normal after therapy. In some patients with severe or long-standing IDA have mild thrombocytopenia, possibly because of complicating factors such as folate deficiency or splenic sequestration.

**BONE MARROW**

The bone marrow finding in iron deficiency anaemia is characterized by mild to moderate erythroid hyperplasia. Cellularity of marrow is moderately increased. Dyserythropoiesis as karyorrhexis and nuclear budding may also be found occasionally. The individual normoblast appear small and may have scanty cytoplasm, often with irregular ragged border called micronormoblast. When the therapy is given, initially erythroid hyperplasia occurs leading to hypercellular marrow, but as the haemoglobin comes to normal, the cellularity of the marrow becomes normal too.

**IRON METABOLISM**

*Serum iron and iron binding capacity*

In iron deficiency anaemia serum iron decreases and iron binding capacity is usually increased. There is considerable variability in the values of serum iron. The
variability results from both technical and physiological factors. Precision of the method in a single laboratory is about 1 to 5%, but interlaboratory precision is poorer. Part of technical problem relates to contamination of glassware and reagents with iron. The values in an individual can vary from 10 to 40% within single day or from day to day, because the total amount of iron usually found in plasma (about 3 mg) is small compared to the amount flowing through the plasma from storage sites to marrow (20 to 30 mg/day). So serum iron is highly sensitive to small changes in marrow iron uptake or storage iron out flow.

The transferrin saturation is calculated with the following formula.

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

When the transferrin saturation value falls below 16%, the rate of erythrocyte production is limited. Values below 16% are noted in association with both iron deficiency and anaemia of chronic disorders, but considerable overlap exists between these two conditions. Values less than 5% is almost certainly due to iron deficiency anaemia.

EVALUATION OF IRON STORES

Biopsy

Iron stores can be estimated by sampling one of the two principal storage depots, the bone marrow or the liver. Aspiration biopsy of bone marrow is usually preferred, as it is safer. In bone marrow aspiration, hemosiderin appears as golden-yellow refractive granules, which when stained by Prussian blue method, renders hemosiderin blue. This marrow hemosiderin stores can be graded from 0 to 6+. Normal marrow is graded 1+ to 3+.

In liver biopsy, even gross inspection of Prussian blue-stained specimen can provide a reliable estimate of iron stores. The grading is done from 1 to 4+.
Serum ferritin

Determination of serum ferritin concentration is the method of choice for evaluating iron stores. Ferritin is an intracellular iron storage protein, but some traces are also secreted in plasma. The serum protein is glycosylated and contains little or no iron. That is why it is different from intracellular protein. Serum ferritin is formed on endoplasmic reticulum, whereas storage ferritin is formed on free ribosomes. In most clinical circumstances, the serum ferritin concentration is proportional to total body iron stores. In adults with serum ferritin level between 15 and 500 µg/L, each 1µg/L corresponds to about 8 to 10 mg of storage iron. It also corresponds to about 120 to 140µg/Kg body weight \(^{70,71}\). During pregnancy, serum ferritin is a reliable indicator of the iron status in the first trimester and becomes less reliable after 20th week due to physiological dilution of plasma \(^{72}\).

The tiny quantity of ferritin in plasma is measured by three types of assays: immunoradiometric assays (IRMA), radioimmunoassays (RIA), and enzyme-linked immunoabsorbent assays (ELISA) \(^{73}\). Another method proposed by Jean Pinter et al is semiquantitative ferritin measurement based on a modification of a two-site enzyme linked immunoassay and it requires only 2 drops of whole blood and a total incubation time of 90 minutes \(^{74}\).

Erythrocyte Zinc Protoporphyrin

Under normal circumstances, red cell precursors synthesize slightly more protoporphyrin than is needed for heme synthesis. The excess is called free erythrocyte protoporphyrin (FEP) and remains in cell through out its life span. When iron is not available for heme synthesis protoporphyrin combines with zinc to form zinc protoporphyrin. The amount of erythrocyte zinc protoporphyrin (EZP) increases in iron deficiency anaemia and is one of the most sensitive laboratory findings. As the EZP remains within the cell through out its lifespan, the value remains elevated even after the treatment. The value of EZP is increased in anaemia of chronic disease and lead poisoning. Wong SS et al found that Zinc protoporphyrin has a relatively high degree of diagnostic efficiency better than iron and ferritin for hospitalized patients \(^{75}\).
Erythrocyte protoporphyrin (EP), is the most simple, inexpensive, and sensitive means of detecting the patient with iron deficiency in a population with a low prevalence of lead poisoning\textsuperscript{76}. The optimal cut off limit for EP test appears to be 35\(\mu\)g/dl of whole blood. At this level, 88\% of the subjects with low serum ferritin can be detected\textsuperscript{77}.

**Transferrin receptor**

Transferrin receptors (sTfR) are disulphide-linked transmembrane proteins that facilitate the entry of transferrin bound iron into cells. A soluble truncated form of protein can be detected in plasma. The assay of sTfR is done by ELISA procedure. The values in normal subjects were 2.8 to 8.5 mg/dl\textsuperscript{78-80}. By sandwich enzyme immunoassay based on polygonal antibody, its reference range is 1.11 to 1.97 mg/L\textsuperscript{81}. In iron deficient subjects the values are increased. In one study, the mean value in iron deficiency was 13.9 mg/L compared with 5.4 mg/L in normal control\textsuperscript{79}. STfR values do not increase in the anaemia of chronic disease. Therefore, this estimation may be useful in distinguishing iron deficiency anaemia and anaemia of chronic disease, when serum ferritin levels are not definitive. Serum TfR levels vary with total mass of red cell precursors. So, the value is increased in haemolytic anaemia, thalassaemia and polycythemia and decreased in hypoplastic anaemia and renal failure.

Study done by Remacha AF et al showed that the best cut-off point of sTfR between IDA and anaemia of chronic disease was 4.7 mg /l. Applying this cut off point of sTfR, sensitivity was 92\% and specificity was 81\%. Using the ratio of sTfR x 100/serum ferritin, the best cut off point was 8 (specificity 100\%)\textsuperscript{82}.

Serum transferrin receptor is easily measured by ELISA methods. Unlike ferritin, the concentration of serum transferrin receptors is unaffected in inflammatory diseases and infections\textsuperscript{83}. 