Special Communication

CLINICAL MEASUREMENT OF IRON STATUS

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Iron balance in man depends on three factors—the iron requirement for production of hemoglobin, the iron losses from physiological and pathological processes, and the amount of iron ingested and absorbed from the intestine. Alteration in iron balance may sometimes lead to a considerable change in the total amount of iron in the body with subsequent iron deficiency or overload. The optimal amount of iron is present when the synthesis of physiologically active compounds is not limited. Ferritin and hemosiderin are reserve compounds synthesized when the amount of iron present in the body is in excess of active metabolic requirements. In iron overload these compounds are present in increased amounts. In iron-depletion all the iron containing compounds of the body may be reduced in amount although they do not all become affected at the same time.

The parameter of iron status chosen for study in any given clinical situation will depend on the precise objective and whether iron-overload or iron deficiency is suspected. Iron status is best determined by measuring the amount present in the three main iron containing compartments of the body:—

(*a*) Storage iron
(*b*) Transport iron
(*c*) Erythrocyte iron

A. Iron Storage

Under normal conditions, about a quarter of the iron in the body is in a storage form. Iron storage may be defined as iron available for mobilization to satisfy increased body requirements for the synthesis of metabolically active compounds. Iron stores are principally a reserve that can be drawn upon when the need arises. In states of negative iron balance it serves as a buffer against the depletion of hemoglobin and iron enzymes. When iron deficiency develops it is the first fraction of the body's complement to be depleted and after acute blood loss it is rapidly mobilized to satisfy the needs of the erythroid marrow.

Iron storage is composed of ferritin and hemosiderin and is found mainly in the liver, spleen and bone marrow. Smaller amounts of ferritin may be found in a large number of tissues where it appears following excessive iron intake in relation to the cells metabolic needs (Arora et al., 1970). Ferritin can act as both short and medium term reserve from which iron is readily utilized into the transferrin bound plasma pool, or for intracellular haem synthesis. Hemosiderin seems to be less metabolically active and acts as a longer term reserve.

There are two basic patterns of iron-storage in the body. Under normal circumstances most of the visible iron is found in reticuloendothelial cells which largely derive from the breakdown of red cells and hemoglobin. In addition small amounts are present in parenchymal cells which appear to derive their iron from that bound to transferrin. Storage uptake and release are closely matched to the need of the bone marrow. Assessment of the level of iron stores has been found to be of major importance in defining the iron-status of individual patients.

1. *Mobilization by Phlebotomy*

The most direct method of measuring iron stores is to determine the amount available for hemoglobin formation following repeated phlebotomy. After repeated venesection the subjects are unable to maintain their hemoglobin levels and at this point it is assumed that available iron stores have been depleted. The amount of iron removed in the form of blood is then calculated. This method is only suitable for use in exceptional circumstances, due to drawback that in patients with greatly increased iron stores there may be difficulty in mobilizing all the relatively insoluble hemosiderin in reticuloendothelial cells and iron deficiency anemia may occur at the same time that hemosiderin aggregates are still visible in the bone marrow.

2. *Tissue Biopsy*

The liver and bone marrow are the most important storage sites and the amount of iron present in these organs can be estimated either chemically or histologically. This is done most conveniently by examining particles of bone marrow histologically in order to judge the amounts of stainable iron present in reticulum cells. Absence of iron in bone marrow smears and sections can be used as a criterion for iron deficiency and has the advantage of a high intrinsic validity, that is, it is a direct measure of storage of iron content.

The chemical estimation of non haem iron in human bone marrow aspirates has been described by Trubowitz et al. (1970) and the use of needle biopsy samples of liver for iron estimation has been found useful in diagnosis of overload (Barry and Sherlock, 1971).

It has been suggested that the liver and the marrow have an equal capacity to store iron under normal circumstances (Gale et al., 1963).

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Other studies have shown a greater scatter of results although the overall relationship still holds (Weinfeld 1964). There is a good overall correlation between the histological grading of the amount of iron in borne marrow and the chemical determination of marrow iron (Weinfeld 1964) but there is an appreciable degree of overlap between the individual grades.

Determination of iron concentration storage in 4000 specimens of liver obtained at necropsy in 18 different countries showed a positive correlation between the liver iron concentration and age in some African Bantu males but no such correlation was found in other groups (Charlton et al., 1970). In all countries iron concentration in females under the age of 40 years were lower than in males but over the age of 50 years iron storage levels in women were similar to those found in men. In the United States there is a significant increase in mean liver iron concentration with age in women but not in men (Sturgeon and Shoden, 1971). In an effort to identify the population groups that might be exposed most directly to the effects of iron deficiency, the percentage in each group with liver iron concentration lower than 50 ug per gram was determined. The lowest iron stores were found in male Indians living in New Delhi, of which 30% had virtually none (WHO 1968).

3. *Serum Ferritin*

With the recent development of a sensitive immunoradiometric assay, ferritin has been shown to be present in normal serum (Addison et al., 1972; Miles et al., 1974). The mean ferritin concentration in normal men was found to be 69.2 ug/L and in normal women 34.8 ug/L, the difference reflecting the differences in iron storage levels. In healthy adults the concentration of ferritin in serum is directly related to the available storage of iron in the body (Jacobs and Worwood, 1975).

The advantages of radioimmunometric assay are its quantitative and reproducible results together with its increased sensitivity in detecting low iron-stores as compared with the histochemical method. In addition, serum ferritin measurements may enable individuals with microcytic anemia secondary to infection, or hemoglobinopathy to be differentiated from those with iron deficiency.

4. *Chelation Methods*

Iron stores can be assessed indirectly by measuring iron excretion following the ingestion of an iron chelating agent. Diethylenetriamine pentaacetic acid (DTPA) and desferrioxamine, were introduced in 1961 and either can now be used to estimate iron stores with useful precision.

The simple chelation tests provide no more than a good semi quantitative measure of body iron stores as the amount of iron appearing in the urine does not depend solely on the quantity available for chelation. The correlation between chelate- induced iron excretion in the urine and body iron stores is most useful when stores are increased.

DTPA—chelatable iron correlates closely with liver iron concentration in hemochromatosis and other iron loading disorders, and appears to be generally reliable as a quantitative measure of iron (Bary et al., 1970; Barry and Sherlock, 1971).

B. Transport Iron

Most of the iron entering the plasma is derived from the breakdown of hemoglobin in the reticuloendothelial system, and the major portion leaving the plasma is directed to the bone marrow for hemoglobin synthesis. In addition iron released from stores, tissue enzymes and the gut also contributes to the plasma iron pool ,although to a much lesser degree. The iron entering the blood stream is rapidly oxidized, to the ferric (Fe+++) state and is then incorported into the specific iron binding protein "Transferrin" or "Siderophilin".

Normally the amount of transferrin present in the plasma is sufficient to bind 250-400 ug Fe per 100 ml. It is usually 20-40% saturated with iron giving a plasma iron concentration in the range between 80 ug per 100 ml. The range of serum iron concentration differs in the two sexes with an average of about 125 ug per 100 ml in men and 100 ug per 100 ml in women. The average serum iron concentration at birth was 193 ug per 100 ml in infants (Sturgeon 1954) and this fell rapidly during the first few hours of life and then rose to 141 ug per 100 ml after 11 days. By 4-10 months of age there was a return to hypoferremia and thereafter a slow rise to adult levels at about the age of 2 years. The plasma iron concentration tends to decrease with age in women but this may be related to iron deficiency (Jacobs et al., 1969). There is normally a marked diurnal variation in plasma iron concentration, the highest values being found in the morning and the lowest values in the latter part of the day. It is thought to be due to variations in the release of iron from the reticuloendothelial system (Funk 1970).

The serum total iron binding capacity in adults varies between about 250 and 400 ug per 100 ml and has been found to be slightly higher in women than in men (Jacobs et al., 1969), probably because of the higher incidence of iron deficiency in women .There is no diurnal variation. At birth, the level in infants is above the normal adult range but it falls rapidly during the first day of life to about 50 ug per 100 ml rising to about 250 ug per 100 ml by the end of the first week of life (Sturgeon 1954).

In clinical practice the serum iron concentration is measured but this alone is of limited value in indicating iron status and the total binding capacity is usually estimated at the same time so that the percentage saturation of transferrin can be calculated. This is the most useful parameter of iron transport status.

The percentage of saturation of transferrin is the expression of great significance in defining the adequacy with which iron is being supplied to the erythroid marrow. Iron deficiency erythropoiesis occurred, when the transferrin saturation reduced to below 16%, from whatever cause, and that the iron supply to the bone marrow correlated better with saturation of transferrin than it did with the plasma iron concentration.

In iron deficiency anemia transferrin saturation is usually below 15%. Serum iron concentration and transferrin saturation may be increased in a number of conditions associated with decreased erythropoiesis (as in aplastic anemias or acute leukemia), hemolytic anemia or ineffective erythropoiesis (as in pernicious anemia) or with iron overload (as in hemosiderosis or hemochromatosis).

An abnormally high total iron binding capacity is a characteristic feature of iron deficiency, even though anemia may be absent. A decrease in plasma transferrin has been noted in depletion of plasma proteins through impaired synthesis or loss as in kwashiorkor, nephrosis, chronic liver diseases and in subjects with malignant disease. The other major cause of a reduced traneferrin level is infection. Reduced levels have been reported in hemolytic anemia, cooly's anemia and pernicious anemia.

C. Erythrocyte Iron

An adequate supply of iron for erythropoiesis results in the delivery of normal erythroctyes to the peripheral blood. A failure in supply may result in diminished numbers of red cells with a subnormal hemoglobin content and an abnormal morphology.

1. *Examination of the Peripheral Blood*

The characteristic changes in the blood film and red cell indices are time related and a function of the severity of anemia, which is a late stage in the process of negative iron balance. The morphological changes of hypochromia and microcytosis do not appear until hemoglobin has fallen below 12g%. The MCHC is useful measure of red cell hypochromia, although it has recently been shown that in hypochromic anemias there is a disproportionate trapping of plasma during hematocrit estimations which leads to an over estimation of the packed cell volume and suggest that the iron RCHC observal in iron deficiency states is partly artefectual at least where anemia is mild (England et al., 1972). Since accurate automatic counting methods have become available, it has generally been noted that the characteristic low MCHC, previously obtained by manual method in iron deficiency anemia is not seen. A very accurate estimation of the mean corpuscular volume (MCV) is however, possible by these methods and this is likely to become the more valuable of the absolute indices in iron deficiency anemia.

2. *Erythrocyte Protoporphyrin*

It has been known for some time that the concentration of red cell protoporphyrin provides a measure of the adequacy of iron-supply to erythrocyte precursors. This assay may have advantage over other parameters in current use, such as per cent saturation of transferrin. In states of iron-deficiency lack of iron results in an accumulation of free protoporphyrin within the red cells (Langer et al., 1972), and its concentration may be increased before the onest of anemia.

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