Iron Studies

Dr Roshitha de Silva
Department of Pathology
Faculty of Medicine
University of Kelaniya
Objectives

- Iron homeostasis
- Serum iron
- TIBC
- TSAT
- Serum ferritin
- IDA
- Haemachromatosis
Body iron metabolism

• Normal intake of iron 10-20 mg/d
• About 5-10% is absorbed
• Iron balance is regulated by gut absorption
• Limited capacity to alter the rate of iron excretion
Rate of absorption

- Rate of erythropoiesis
- State of iron stores
- Contents of diet
  - Vit C, red meat
  - Antacids, ppi, calcium, eggs, cocoa, coffee, tea, Phytates
- Chemical state of iron ($\text{Fe}^{3+} < \text{Fe}^{2+}$)
Absorption at the cellular level
Transport, Storage

• Transported to plasma $\rightarrow$ transferrin $\rightarrow$ taken up by cells
  • Combine with haem $\rightarrow$ Hb
  • Stored as ferritin (or haemosiderin)
Iron Distribution

- Haemoglobin
- Storage iron
  - Ferritin
  - Haemosiderin
- Tissue iron
- Myoglobin
Excretion

• Cells desquamated (skin/intestine)
• Menstruation/pregnancy
• Iron in faeces is exogenous
• Urine excretion is negligible
Lab assessment of iron status

Serum iron
Serum ferritin
TIBC
Transferrin saturation
Serum iron

- Varies widely
- Diurnal variation (higher values in the morning)
- Menstrual cycle
- Pregnancy
Serum iron

• Levels change (without changes in iron stores) in
  – Acute infections ↓
  – Trauma ↓
  – Chronic inflammatory disorders (RA) ↓
  – Malignant diseases ↓
Analysis

• Iron is released from transferrin by lowering the pH
• Reduced from Fe$^{3+}$ to Fe$^{2+}$
• Complexed with a chromogen
  – Bathophenanthroline
  – Ferrozine
• Measurement of absorption
Analysis

• Addition of acid to serum
  – Release iron from transferrin
  – Reduced from Fe\(^{3+}\) to Fe\(^{2+}\)
  – Precipitates serum proteins

• Interference
  – Haemolysis
  – Copper - thiourea
Transferrin

- Transferrin is the carrier protein which binds iron in serum.
- Binds iron very tightly but reversibly (apoT when not bound to iron)
- About 30% of the binding sites are occupied
- Increases in iron deficiency
- Decreases in
  - acute phase response
  - chronic liver disease
Transferrin Cycle
Albumin

α₁-Antitrypsin
α₁-Acid Glycoprotein

α₂-Macroglobulin
Ceruloplasmin
Haptoglobins
α-Lipoproteins

Hemopexin
Transferrin
Plasminogen
β-Lipoprotein

C₃

Fibrinogen
Immunoglobulins
C Reactive protein
TIBC

- Indirect measure of transferrin
- Ability of transferrin to bind iron
- The maximum amount of iron that T can bind
Serum Iron and Iron Binding Capacity

Total Iron Binding Capacity (TIBC) = Unsaturated Iron Binding Capacity (UIBC) + Serum Iron (S.I.)
TIBC

• Analysis
  – Add sufficient Fe$^{3+}$ to saturate iron binding sites
  – Then excess Fe$^{3+}$ is removed (MgCO$_3$)
  – Measure iron
# TIBC results interpretation

<table>
<thead>
<tr>
<th>Condition</th>
<th>Serum iron</th>
<th>Transferrin &amp; TIBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency anaemia</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Anaemia of chronic disease</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Pregnancy, OCP</td>
<td>Normal</td>
<td>High</td>
</tr>
</tbody>
</table>
Transferrin saturation

• (Serum iron/TIBC) x 100
• less than 20% - iron deficiency
• more than 50% - iron overload

• In inflammation??
Serum Transferrin Receptor

- Immature red cells in BM got lot of TR on their membrane.
- The number of TR ↑ses in iron def and ↓ses in iron OL
- This is seen in serum TR
- But it reflects the amount of erythropoietic activity rather than the iron status
Serum ferritin

• Consist of protein shell and an iron core
• Found in all cells in the body-hepatocytes, macs in BM
• Very little amount is in the serum. This amount is proportional to the total body stored iron
Serum ferritin

• Closely related to body iron stores
• Low level indicates depleted iron stores
  – Sensitive and specific indicator of iron deficiency
• High serum ferritin
  – 10% - iron overload
  – 90% - Chronic alcohol consumption, metabolic syndrome, obesity, diabetes, malignancy, infection and inflammatory conditions
  – normal STFR rules out iron overload
Ferritin analysis

• Immunoassay
Ferroportin

- Transmembrane iron transporter
- Exports iron into the plasma from the duodenum, macrophages and hepatocytes
- Iron is then bound to transferrin
Hepcidin

• Hepcidin, produced in hepatocytes, is the key regulator of iron homeostasis
• It is a negative regulator of iron release from macrophages (red cell processing and iron recovery, iron store), hepatocytes (iron store) and enterocytes (iron absorption)
Action of Hepcidin

1. Elevated storage iron
2. Liver synthesizes hepcidin
3. Feeds back to the GIT
4. Prevent iron absorption
Figure 1 - Mechanism of hepcidin-mediated cellular iron regulation

Low hepcidin

Iron uptake
Iron-exporting cells (duodenal enterocytes, macrophages, hepatocytes)
Ferritin
Fpn
Fe
Iron release into plasma

High hepcidin

Iron uptake
Iron-exporting cells (duodenal enterocytes, macrophages, hepatocytes)
Ferritin
Fpn
Fe
Hepcidin

Fpn = ferroportina (adaptado de Ganz ³).
Anaemia of Chronic Disease

- Seen in chronic infection, chronic inflammation and malignancy
- Hb values typically 9.5-10.5 g/dL

<table>
<thead>
<tr>
<th></th>
<th>IDA</th>
<th>ACD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum iron</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>TIBC</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>TSAT</td>
<td>Very low</td>
<td>Low</td>
</tr>
<tr>
<td>Ferritin</td>
<td>Low</td>
<td>High/Normal</td>
</tr>
<tr>
<td>ESR</td>
<td>Normal</td>
<td>High</td>
</tr>
<tr>
<td>CRP</td>
<td>Normal</td>
<td>High</td>
</tr>
</tbody>
</table>
Case 1

- 30 year old female presented with general symptoms of anaemia and her lab results are as follows:
  - Haemoglobin: 10 g/L
  - Serum Iron: 15 µmol/L (5-25µmol/L)
  - TRF: 3.4 g/L (2.0-3.5g/L)
  - T SAT: 15 % (5-35%)
  - Serum Ferritin: 7 µg/L (20-200µg/L)
Case 2

- A 47 year old farmer presents with tiredness. His iron studies show the following abnormalities:
  - Serum Iron: 40 μmol/L (5-30 μmol/L)
  - TRF: 2.2 g/L (2.0-3.2 g/L)
  - T SAT: 73% (10-45%)
  - Serum Ferritin: 128 μg/L (30-500 μg/L)
Case 3

• A 57 year-old man who is a chronic alcoholic presents to his GP with tiredness. His LFTs show few abnormalities and his iron studies were also abnormal:

  • T.protein  67 g/L  (63-80g/L)
  • Albumin  38 g/L  (34-45g/L)
  • ALT  100 U/L  (5-40 U/L)
  • AST  67 U/L  (10-40 U/L)
  • GGT  344 U/L  (5-50 U/L)
  • ALP  228 U/L  (35-110 U/L)
Case 3 cont.

- Serum Iron  10 μmol/L (5-30 μmol/L)
- TRF  2.3 g/L (2.0-3.2 g/L)
- T SAT  25 % (10-45 %)
- Serum Ferritin  711 μg/L (30-500 μg/L)
Single best answer question 1

• Which condition is associated with the lowest percent saturation of transferrin?
  A. Haemochromatosis
  B. Anaemia of chronic disease
  C. Iron deficiency anaemia
  D. Non-iron deficiency anaemia
  E. Pregnancy
Single best answer question 2

• Which condition is most often associated with a high serum iron level?
  A. Nephrosis
  B. Chronic infection
  C. Polycythaemia vera
  D. Noniron deficiency anaemia
  E. Chronic inflammation
Single best answer question 3

- Which of the following is likely to occur first in iron deficiency anaemia?
  A. Decreased serum iron
  B. Increased TIBC
  C. Decreased serum ferritin
  D. Increased transferrin
  E. Decreased MCV
MCQ

• Which statement regarding iron studies is/are true or false?
  A. Ferritin is a beta globulin
  B. Serum iron levels begin to fall before the body stores become depleted
  C. A normal level of serum ferritin rule out iron deficiency
  D. The commonest cause of high serum ferritin is iron overload
  E. TIBC depends on serum iron and serum transferrin levels
Thank You