Iron Deficiency Anaemia in Chronic Kidney Disease:
Information at Expert Level. Iain E Wittwer, TD, RN

Aims

1. To understand the normal metabolism of iron.
2. To understand how the body utilises absorbed iron and how Chronic Kidney Disease affects this.
3. To understand the effect of infection and inflammation and the rôle of hepcidin in the metabolism of iron.
4. To understand the effect of oxidative stress.
5. To understand how international anaemia guidelines have the potential to change clinical practice.

Introduction

It is accepted that people with Chronic Kidney Disease (CKD) will develop anaemia as their kidney functions declines. The cause of anaemia in this group of patients is multifactorial. These factors include:

- Damage to the tubulo-interstitial cells of the kidney, (responsible for erythropoietin production)
- Platelet dysfunction, which can precipitate bleeding in the gastrointestinal tract.
- Reduced erythrocyte survival, reduced by 30% to 60% in CKD
- Increased toxins production causing haemolysis of red blood cells.
- Malnutrition and deficiencies in iron, Vitamin B12 and folate.

Iron is an essential requirement in the life of all cells from microorganisms through to higher animals. Iron deficiency in people with CKD can be caused by poor absorption of oral iron and occult blood loss, along with the use of Erythropoiesis Stimulating Agent (ESA) increasing the amount of iron utilised during erythropoiesis. Iron deficiency has been shown to be the most common cause of anaemia worldwide. It is important to understand how the body normally metabolises iron and how this is affected in patients with CKD.

Normal Iron metabolism

Absorption of ingested iron will take place in the epithelial villi of the duodenum. The human diet will normally contain more iron than is required in either haem iron (Ferrous Fe2+) or non haem iron (Ferric Fe3+) with most dietary iron being in the latter form. Absorption of iron into the enterocyte is through the apical brush border, (the microvilli covered surface of the epithelial lining of the duodenum), and is in the form of Ferrous Fe2+. This is then transported into the enterocyte by the action of the specific transporter, divalent metal ion transporter 1 (DMT1). Before dietary iron in the Ferric Fe3+ form can be transported into the intestinal absorptive cells, called enterocytes, it needs to be changed into Ferrous Fe2+ form. To facilitate this change in form, a protein, duodenal cytochrome B (DcytB), is produced by the apical brush border to reduce ferric iron to its ferrous form. Once this has occurred, it is transported into the enterocyte by the action of DMT1.

Once absorbed into the enterocyte, iron will enter the iron pool. Utilisation of this iron will depend on the body’s iron requirement. Should the iron demand be low, it will remain within the enterocyte and be taken up by the iron storage protein, ferritin. After a few days, the enterocytes are sloughed off the duodenal villi and the iron sequestered by ferritin is lost. Where the iron demand is high, it will pass through the enterocyte basolateral membrane by the action of the iron export protein, ferroportin. It is thought that the iron oxidase, hephaestin, acts to assist ferroportin to allow iron to pass through the basolateral membrane and bind to transferrin as Ferric 3+.

Utilisation of iron

Once the iron has been absorbed it is bound to transferrin to be transported for utilisation by the body. It is then be used by the muscles and bone marrow and stored within the reticulo-endothelial macrophages and the liver parenchyme. The metabolism of iron is unique as there is no excretory mechanism in humans. 1mg to 2mg of elemental iron is absorbed daily for use within the body. The same quantity of iron is lost as is absorbed through the sloughing off the mucosal cells, desquamation and other forms of blood loss. Transferrin will carry 3mg of iron but erythropoiesis requires 20mg to 25mg to be delivered to the bone marrow. The same amount of iron is taken up by the macrophages by the process of phagocytosis (breakdown of old erythrocytes). Transferrin bound iron needs to be recycled up to seven times to maintain iron delivery during normal erythropoiesis. The breakdown of systemic iron is shown in Table 1.
Table 1: Breakdown of systemic iron

<table>
<thead>
<tr>
<th>Process</th>
<th>Systemic Iron</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron Intake</td>
<td>1 – 2mg daily through the duodenum</td>
</tr>
<tr>
<td>Iron transport</td>
<td>3mg bound to transferrin</td>
</tr>
<tr>
<td>Iron utilisation</td>
<td>300mg in the Bone Marrow 1800mg circulating in red blood cells 300mg in muscle as myoglobin</td>
</tr>
<tr>
<td>Iron storage</td>
<td>600mg in the reticulo-endothelial macrophages 1000mg in the liver parenchyme</td>
</tr>
<tr>
<td>Iron loss</td>
<td>1 - 2mg daily</td>
</tr>
<tr>
<td>Total body iron</td>
<td>3703mg in transport, being utilised and stored Daily intake is balanced by daily loss</td>
</tr>
</tbody>
</table>

Erythropoietin and iron are required in the formation of the red blood cells during erythropoiesis, which is driven by hypoxia with low haemoglobin (Hb) levels. Erythropoietin (EPO) is produced in the tubulo interstitial cells of the kidney. This stimulates the pluripotent stem cells within the bone marrow to produce the precursor cells for erythrocytes. The EPO will be taken up by the specific receptors on the Burst Forming Unit – Erythroid (BFU – E). Without the presence of EPO, the cells will suffer apoptosis or cell death. The stages of maturation from BFU-E to Colony Forming Unit – Erythroid (CFU-E) to proerythroblasts is dependent on EPO and will take up to 21 days. At the next stage of erythrocyte maturation, erythroblast, iron is taken up by the cell allowing progression to the reticulocyte stage of development. These two stages are iron dependent and take approximately 3 to 4 days. The final stage of erythropoiesis, which takes 2 days, is the erythrocyte or red blood cell.

Iron is necessary to transport oxygen to the muscles, where it forms myoglobin. There are non haematological uses for iron, described below:

- Improvement in physical performance and aerobic exercise tolerance
- Thermoregulation of core temperature which can be a consequence of a reduced ability to react cold related stress
- Improved cognitive function
- Improvement of restless leg syndrome
- Improved immune function

Limiting factors for iron utilisation in CKD

Normal iron metabolism is disturbed in CKD and may lead to iron deficiency for two reasons:

- In absolute iron deficiency, there is a major reduction of the total iron body content that will prevent erythropoiesis resulting in the development of anaemia.
- In functional iron deficiency, there are normal or raised iron stores but the release for use in erythropoiesis is blocked and anaemia develops as a result.

There are specific causes for the development of iron deficiency in people with CKD:

- A reduction in the amount of iron absorbed through the ferroportin channel, caused by the action of the active 25-amino-acid hepcidin. (Present in inflammatory states and infections).
- The blocking action of hepcidin, means that iron scavenged from old erythrocytes by the reticulo-endothelial macrophages is not released to transferrin for further use
- Transferrin levels can be anywhere between 33% to 50% less than normal levels, causing a reduction in the quantity of iron available for transport and utilisation by the bone marrow.
- Normal iron losses of 1mg to 2mg daily may increase as kidney function declines. A loss of between 10mg to 20mg daily may occur in patients receiving haemodialysis.

Infection and Inflammation and the Role of Hepcidin

The peptide hormone, hepcidin, initially noted in urine of patients with infections, was named for its liver origin and the antimicrobial properties exhibited by this hormone. This hormone is now recognised as a key regulator of iron metabolism. All localised and systemic infections will illicit an immune response to combat the invading pathogenic organism. In addition, any chronic condition, such as uraemia present in CKD, Diabetes and Heart Failure, will produce an inflammatory response. This causes a systemic acute phase immune response and cytokine release by the macrophages. During an immune response, macrophages produce cytokines such as interferon-α, interleukin – 6 (IL-6) and tumour necrosing factor-alpha (TNF-α). Kupfer cells in the liver will also release IL-6, which stimulate the hepatocytes within the liver to release hepcidin. The bio active hepcidin – 25 binds to the iron export protein, ferroportin. This results in the inhibition of the absorption of dietary iron. The ferroportin bound by hepcidin will be internalised within the enterocyte and is consequently degradated. Iron within the enterocyte is lost when duodenal villi are routinely sloughed off.

Raised hepcidin levels affect the storage and release of iron from other cells. To prevent iron utilisation by invading pathogens, circulating iron is diverted back in to the storage sites within the macrophages and liver. The action of cytokines and hepcidin block the release of iron from the reticulo-endothelial system (RES) and macrophages and can lead to functional iron deficiency. A reduction in the quantity of iron available to transferrin disrupts the process of erythropoiesis which may then inhibit the effect of ESA therapy in patients with CKD.

Diagram 1 demonstrates this process.
Iron and Oxidative Stress

Oxygen, whilst essential for life, has the ability to cause harm to the body\(^1\). Oxidative stress can occur where oxidant activity exceeds anti oxidant activity. Although iron is an essential requirement in the life of all cells it is tightly regulated\(^2\) due to its potential ability to create oxidative stress. Inorganic (non haem) iron, which has pro – oxidant characteristics, is known to be potentially highly toxic to the cells especially when it is not bound to transferrin. This is known as non transferrin bound iron (NTBI). Iron has the ability to lose and gain ions, which then changes its degree of oxidation. Ferric\(^{3+}\) is oxidised by superoxide into Ferrous\(^{2+}\) and this reaction can form the Reactive Oxygen Species (ROS) This oxidation process is the catalyst in the formation of the hydroxyl radicals (•OH) by the Fenton reaction\(^19\) which can lead to oxidative stress. These reactions, which occur at cellular level, are described below

- \(\text{Ferric}^{3+} (\text{Fe}^{3+}) + \text{Superoxide} (\cdot\text{O}_2^-) \rightarrow \text{Ferrous}^{2+} (\text{Fe}^{2+}) + \text{Oxygen} (\text{O}_2)\)

The next step is the Fenton Reaction,

- \(\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{Hydroxyl} (\text{OH}^-) + \text{Hydroxyl radical} (\cdot\text{OH})\)

The NTBI and Hydroxyl radicals, which may cause oxidative stress, have the ability to damage a wide variety of cellular molecules

- Lipids: Iron will cause the degradation of lipids caused by a chain reaction of lipid peroxidation. As cell membranes are phospholipid structures, they will be broken down and lead to cell death. Cholesterol oxidation can also lead to the formation of atherosclerosis\(^19,20\)
- DNA: the make - up of DNA may suffer damage related to the supplemental iron dose This can cause cross links in DNA protein and suffer single strand breaks and other problems\(^19,21\)

- The immune system: certain forms of oral iron medications are thought to have a detrimental effect on some immune cells. The effect of these oral supplements may lower the immune response and potentially be the cause of increased infections\(^19,22\)
- Gastric mucosa: NTBI will damage the intestinal mucosa causing ulceration and erosions\(^19\)

Although current IV iron formulations possess a protective carbohydrate “shell”, they can cause the generation of marked amounts of NTBI, otherwise known as free iron radicals, which can cause damage by oxidation\(^23\). It is possible that new iron preparations may reduce the amount of circulating NTBI, which may minimise the risks associated with oxidative stress outlined above.

Iron and ESA therapy – what the guidelines say

Iron is required for erythroblasts to mature during the process of erythropoiesis\(^1\). For ESA therapy to work effectively, adequate iron availability is essential. Lack of available iron is one of the main causes of ESA hypo responsiveness\(^3,24\). This problem can be caused by an absolute or functional iron deficiency\(^6,25\). National and International guidelines recommend ferritin, %TSAT and % hypochromic red cell levels to monitor iron stores in patients receiving ESA therapy\(^26,27\). Recent research\(^28\) led to the revision of the International Kidney Disease: Improving Global Outcomes (KDIGO) guidelines (2012)\(^27\), which recommend an increased use of iron supplementation. The treatment range now recommended by the experts as determined by blood results has been increased to a %TSAT of equal or greater than 30% and ferritin level of equal or greater than 500 µg/l (≤500 ng/ml)

It is possible there could be a greater use of iron supplementation in the treatment of renal anaemia following the revision of these International guidelines.

Alternative tests used to diagnose iron deficiency

The standard tests used in the diagnosis of iron deficiency anaemia are serum ferritin, %TSAT and % of hypochromic red cells, the validity of these tests are affected by inflammation and erythropoiesis. An ideal test for IDA should not be affected by these processes\(^14\). Other tests are available but less commonly used in standard practice. These include:

- Reticulocyte haemoglobin content (Chr): this measures the Hb content of the reticulocytes. It is a “snapshot” of the iron content available for erythropoiesis during the 1 to 2 day period that the reticulocyte matures into the erythrocyte\(^30,31\)
- Soluble transferrin receptor (sTfR): This is a transmembrane cellular protein, released by cells requiring iron. Levels of sTfR are increased in the plasma and serum where a lack of iron is present\(^25\). Whilst this marker is not affected by inflammation, sTfR may be affected by erythropoiesis stimulated by ESA thera-
The ratio between sTfR and ferritin in the sTfR–Ferritin Index may be a potentially more accurate marker for iron deficiency.

- Hepcidin levels: These will be raised where infection or inflammation is present and so may be an indicator of iron deficiency. Hepcidin-25 has been found in urine and an assay may be available. Immunoassays have been developed to test for serum hepcidin.

- Zinc protoporphyrin/haem ratio (ZPP/H): When the iron levels in the bone marrow are reduced, the take up of zinc rises. A high ZPP/H ratio will give a good indication of iron depletion in the bone marrow.

- Bone Marrow Aspiration: This is a definitive but invasive test which will show a total absence of iron from the bone marrow on staining. Whilst it is a “gold standard” test it would not be cost effective or necessary to perform this test on all patients.

A breakdown of normal values of the various tests used in the diagnosis of iron deficiency is shown in Table 2. Individual laboratories may vary slightly with their normal values for these tests, so these values should be used as a guide only.

Table 2 Alternative Tests used to diagnose Iron deficiency

<table>
<thead>
<tr>
<th>Laboratory Test</th>
<th>Normal value</th>
<th>Iron deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin</td>
<td>12 – 16g/dl (120 – 160g/L) women</td>
<td></td>
</tr>
<tr>
<td></td>
<td>13 – 17g/dl (130 – 170g/L) men</td>
<td></td>
</tr>
<tr>
<td>% hypochromic red cells</td>
<td>&lt;5%</td>
<td>&gt;6%‡</td>
</tr>
<tr>
<td>Chr</td>
<td>28 – 35pg</td>
<td>&lt;28pg</td>
</tr>
<tr>
<td>Ferritin</td>
<td>30 – 300ng/ml (65 – 670pmol/l)</td>
<td>&lt;15ng/ml but in CKD‡</td>
</tr>
<tr>
<td>Serum iron</td>
<td>9 -32µmol (50 - 180µg/dl)</td>
<td>&lt;100ng/ml</td>
</tr>
<tr>
<td>Transferrin</td>
<td>2 – 3.6g/l (200 – 300mg/dl)</td>
<td></td>
</tr>
<tr>
<td>TSAT%</td>
<td>20 – 50%</td>
<td>&lt;20%‡</td>
</tr>
<tr>
<td>sTfR</td>
<td>0.76 – 1.76mg/l (6.4 – 25.7nmol/l)</td>
<td></td>
</tr>
<tr>
<td>sTfR–Ferritin Index</td>
<td>&lt;1</td>
<td></td>
</tr>
</tbody>
</table>

Summary

Iron is essential for life but the metabolism needs to be tightly controlled as it is toxic to cells when not being utilised by haemoglobin and myoglobin or unbound to transport or storage proteins. Iron deficiency is a leading cause of anaemia throughout the world. Patients with CKD may become iron deficient as a result of disordered iron metabolism. This becomes more likely when they require ESA therapy or are receiving haemodialysis. A substantial number of people with CKD may experience an inflammatory response. Hepcidin will be released and further limit iron absorption. Where iron is not bound it can be released as NTBI and may lead to oxidative stress. The new KDIGO guidelines recommend an increased use of iron therapy and a more considered use of ESAs. These guidelines use ferritin, % transferrin saturation tests for iron deficiency, which may be affected by inflammatory factors. There are alternative tests available that may provide a diagnosis of iron deficiency but they are not widely used in practice.

Questions

1. How is iron metabolism affected in CKD?
2. How is the use of iron disordered in CKD?
3. What is the role of hepcidin in the development of iron deficiency in CKD?
4. Will iron therapy lead to oxidative stress?
5. Is there an alternative diagnostic test for iron deficiency available?
References

1. Neeta Baal O’Mara: Anaemia in Patients with Chronic Kidney Disease; Diabetes Spectrum; Volume21, Number1, November 1, 2008.


10. Saul Nurko: Anemia in chronic kidney disease: Causes, diagnosis and treatment; Cleveland Clinic Journal of Medicine, Volume 13, Number 3, March 2006.


