INTRODUCTION

Iron deficiency is defined as decreased total iron body content. Iron deficiency anemia (IDA) occurs when iron deficiency is severe enough to diminish erythropoiesis and leads to anemia. Iron deficiency is the most prevalent single deficiency state on a worldwide basis. It is attracts medical attention because it diminishes the capability of affected individuals to perform physical activity, and it diminishes both growth and learning in children ***(Harper et al., 2012).***

Iron equilibrium in the body is regulated carefully to ensure that sufficient iron is absorbed in order to compensate for body deficiency of iron, whereas body loss of iron quantitatively is as important as absorption in terms of maintaining iron equilibrium, it is a more passive process than absorption ***(Harper et al., 2012).*** When the body has sufficient iron to meet its needs (functional iron), the remainder is stored for later use in all cells, but mostly in the bone marrow, liver, and spleen. These stores are called ferritin complexes and are part of the human (and other animals) iron metabolism systems ***(Brady, 2007)***.

Anemia is one result of advanced-stage iron deficiency. Iron is vital for all living organisms because it is essential for multiple metabolic processes including oxygen transport, DNA syntheses, and electron transport. Iron deficiency has

deleterious effects on cell respiration, mitochondrial oxidative properties, and the electron transport chain ***(Ozcay et al., 2003).***

Increasing evidence has accumulated, indicating that a reduction in renal oxygen tensions also plays an important role in the progression of chronic kidney disease ***(Fine et al., 2001)***. The impact of hypoxia, respectively ischemia, on the progression of renal disease can be summarized through three main points. *First*, the peritubular capillary bed in the kidney, which provides the structural basis for adequate oxygen delivery to tubular cells, is a rather dynamic structure and that chronic diseases of the kidney are associated with a rapid decline in capillary density. *Second*, as a consequence of capillary loss and capillary hypoperfusion, tissue oxygen tensions usually decline in a diseased kidney. *Third*, low oxygen tensions may not only impair energy generation but act as a regulator of cellular functions and as a specific stimulus for the induction of certain genes. Altogether, these findings suggest that hypoxia is an important factor in the progression of kidney disease.

Most tubular segments have a very limited capacity for anaerobic energy generation and are thus dependent on oxygen to maintain active transtubular reabsorption of solutes, in particular sodium. The combination of limited tissue oxygen supply and high oxygen demand is considered the main reason for the susceptibility of the kidney to acute ischemic injury ***(Eckardt et al., 2005).***

Various renal biomarkers can be used to study human nephrotoxicity at an early stage ***(Lauwerys et al., 1992).*** Those that have proved most useful to define defects on various parts of the nephron include: the high molecular weight protein, albumin, for evaluating glomerular integrity. The brush-border membrane enzyme leucine-aminopeptidase (LAP) ***(Josch et al., 1967)*** and the lysosomal N-acetyl-β-d-glucosaminidase (NAG) ***(Thomas, 1969)*** were recommended as markers for investigating structural integrity of renal proximal tubules ***(El-safty et al., 2004).***

The fractional excretion of Sodium (FeNa) describes the fraction of the filtered load of sodium that is excreted in the final urine and is a reflection of tubular function. The functional reabsorption capacity of the distal segment of the nephron can be investigated by calculating the fractional excretion of urinary sodium ***(Koyner et al., 2010).***

Zinc and copper were recognized as the most important metals associated with enzymatic systems that have profound effect on human health**.** Excessive urinary losses of copper and zinc are associated with varios physiological and pathophysiological stresses, including diabetes mellitus ***(El-yazigi et al., 1993),*** surgery ***(Henzl et al., 1967),*** infection ***(Klaiman et al., 1981)*** and hepatic and renal diseases ***(Frommer, 1981 and El-safty et al., 2003).*** However, the impact of IDA on renal function has not been examined in depth.

AIM OF THE WORK

The present study was designed to assess renal functional and structural integrity in children with iron deficiency anemia to test the validity of a hypothesis that those children may develop glomerular & tubular dysfunction and also, examine the response to iron therapy on the kidney.

IRON DEFICIENCY ANEMIA

I- Definition:

Iron deficiency is the most prevalent nutritional deficiency and the most common cause of anemia. Iron deficiency anemia is characterized by a defect in hemoglobin synthesis, resulting in red blood cells that are abnormally small (microcytic) and contain a decreased amount of hemoglobin (hypochromic). The capacity of the blood to deliver oxygen to body cells and tissues is thus reduced ***(Provan, 1999)***.

According to the third National Health and Nutrition Examination Survey (NHANES III) data, iron deficiency, defined by two or more abnormal measurements (serum ferritin, transferrin saturation and/or erythrocyte protoporphyrin). Iron deficiency anemia, a more severe stage of iron deficiency (defined as a low hemoglobin in combination with iron deficiency ***(Looker et al., 1997)***

II- Iron metabolism:

Iron is one of the transitional metals which shares two important properties: the ability to exist in several oxidation states, and to form soluble complexes. These properties have made iron an important component of electron and oxygen transport system ***(Casanueva & Viteri, 2003).***

1) Iron absorption and regulation

A normal mixed diet contains about 15 -20 mg of iron, of which only 1-2 mg (10%) is normally absorbed ***(Siah et al., 2006).***

Iron is mainly absorbed in the duodenum and upper jejunum, its absorption is affected by many factors (Table 1) ***(Lozoff, 1998).***

Table (1): Factors affecting iron absorption

Favored by	**Reduced by**
Dietary factors ▪ Increased heme iron ▪ Increased animal food ▪ Ferrous iron salts ❖ Luminal factors ▪ Acidic pH (e.g. gastric HCL) ▪ Low molecular weight soluble chelates (e.g. vitamin C, sugars and amino acids) ❖ Mucosal factors ❖ Iron deficiency ❖ Increased erythropoiesis e.g. after hemorrhage ❖ Ineffective erythropoiesis ❖ Pregnancy ❖ Anoxia	❖ Dietary factors ▪ Decreased heme iron ▪ Decreased animal food ▪ Ferric iron salts ❖ Luminal factors ▪ Alkalis (e.g. pancreatic secretions) ▪ Insoluble iron complexes (e.g. phytates, phosphates , tannates and bran) ❖ Iron overload ❖ Decreased erythropiesis ❖ Acute or chronic inflammation

(Lozoff, 1998)

Iron found in foods is either in the form of heme iron 10% or non-heme iron (ionic) 90%: Heme iron is usually absorbed from the food more effectively than non heme iron

and is not affected by the factors promoting the absorption of inorganic iron ***(Lozoff, 1998)***.

In most foods, non-heme iron is in the ferric (Fe^{3+}) form and has to be reduced to the ferrous (Fe^{2+}) form by a ferric reductase enzyme before absorption can take place. So, absorption is enhanced by reducing substances, such as ascorbic acid, and by HCL in gastric juice ***(Munoz et al., 2009).***

The ferrous iron is transported across enterocyte cell membrane by the divalent metal transporter 1 (DMT1), The ferrous iron is either stored as intracellular ferritin or transported by a transporter protein, ferroportin, facilitated by the ferrioxidase activity of hephaestin, which return the iron to its Fe^{3+} state and thus promotes binding to transferrin, the predominant iron binding protein in the circulating plasma (figure1) ***(Heeney & Andrews, 2004).***

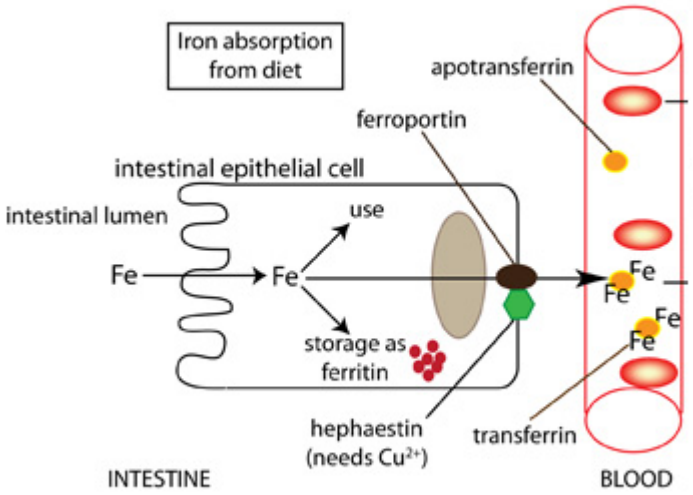

Figure (1): Iron absorption by mucosal cells of the duodenum and jejunum: The two pathways for iron absorption are illustrated one for heme and the other for non-heme iron ***(Munoz et al., 2009)***

Iron released into circulation binds to transferrin for transport to sites of cell usage and storage, the hepcidin-ferroportin mediated release of iron from enterocytes, macrophages, and hepatocytes is the critical determinant of iron homeostasis ***(Siah et al., 2006).***

Liver-derived hepcidin controls how much iron is absorbed from gut or released from storage sites according to the feedback between iron needs and absorption (figure2). Hepcidin expression occurs secondary to: increased iron stores, increased erythropoietic activity, decreased hemoglobin concentration and hypoxia ***(Fleming & Bacon, 2005).***

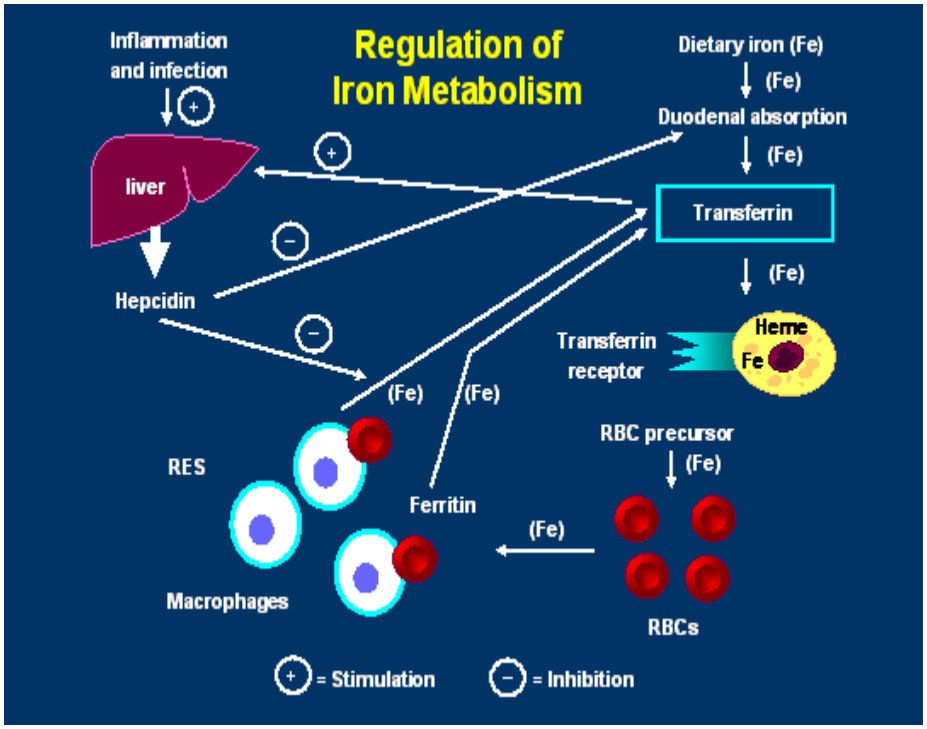

Figure (2): Regulation of iron metabolism ***(Warady et al., 2004).***

Nutritional iron is absorbed by intestine in small amounts as compared to the total iron pool present in various tissues and proteins. This is normally sufficient to compensate for equally small losses of iron. Exchange between tissues occurs via serum, where iron circulates bound to transferrin. However, as estimated from the normal 40% saturation of the iron binding capacity at any given time, only about 0.1% of the total iron is in the recycling pathway. Hence 99.9% of total iron is either incorporated in tissue specific proteins or stored in ferritin ***(Munoz et al., 2009).***

2) Iron transport:

There are four proteins of iron transport, storage and regulation: Transferrin, Transferrin receptors (TfRs), Ferritin and Iron-responsive element- binding protein (IRE-BP), also known as iron-regulatory factor (IRF) ***(Siah et al., 2006).***

Transferrin:

Transferrin is the iron transporting polypeptide in the plasma that is encoded by the TF gene. It is known as "apo-transferrin" when not bound to iron ***(Edwards, 1993)***.

Transferrin contains two specific high-affinity Fe^{3+} binding sites. The affinity of transferrin for Fe^{3+} is extremely high (10^{23} M^{-1} at pH 7.4), but decreases progressively with decreasing pH below neutrality. Transferrin binds iron very tightly but reversibly (figure 3). There is 50% dissociation at PH 4.8 and this completes at PH 4.5 ***(Dukovski et al., 2009).***

Transferrin concentration correlates with the total iron-binding capacity (TIBC) of serum. The absolute value for TIBC may be helpful in distinguishing between iron deficiency anemia and anemia of chronic disorders. The TIBC is increased in iron deficiency anemia and decreased in anemia of chronic disorders ***(Beutler et al., 2000)***.

Transferrin saturation is calculated from the following formula: Transferrin saturation (%) = Serum iron X 100 / TIBC. The normal value is 20-45% ***(Lee, 1999)***

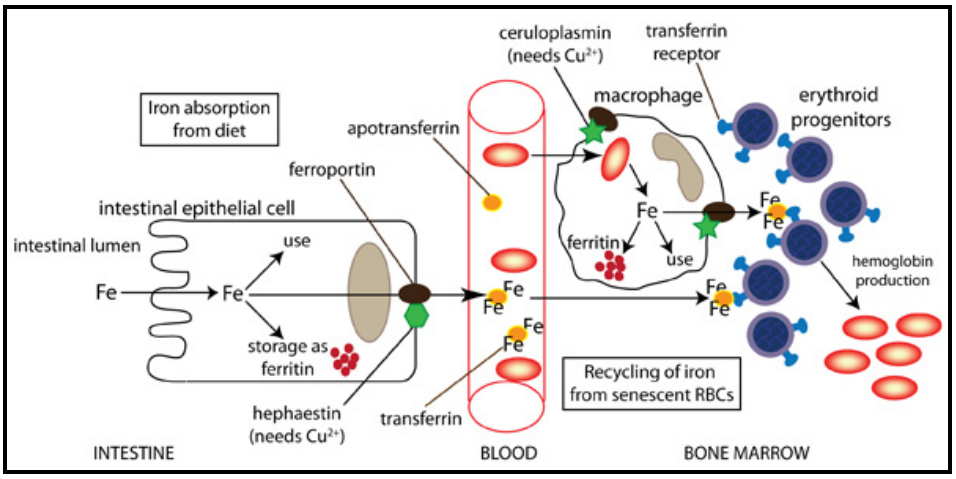

Figure (3): Role of transferrin in iron metabolism ***(Munoz et al., 2009)***.

Measurement of plasma transferrin levels is useful for the differential diagnosis of anemia and for monitoring its treatment. In iron deficiency anemia, the transferrin level is increased due to the increase in its synthesis, but the protein is less saturated with iron because plasma iron levels are low. On the other hand, if the anemia is due to failure to incorporate iron into erythrocytes, the trasferrin level is normal or low but the protein is highly saturated with iron. In iron overload, transferrin concentration is normal but saturation is >55% and may be as high as 90% ***(Koulaouzidis et al., 2009)***.

Transferrin Receptors (TfRs):

They are specific transmembrane glycoprotein dimmers composed of two identical subunits linked by single disulfide bond ***(Daniels et al., 2006).***

TfRs loaded with diferric iron is sequestered in endocytic vesicles and shuttled to intracellular endosomes (Figure 10) ***(Viroj, 2006).***

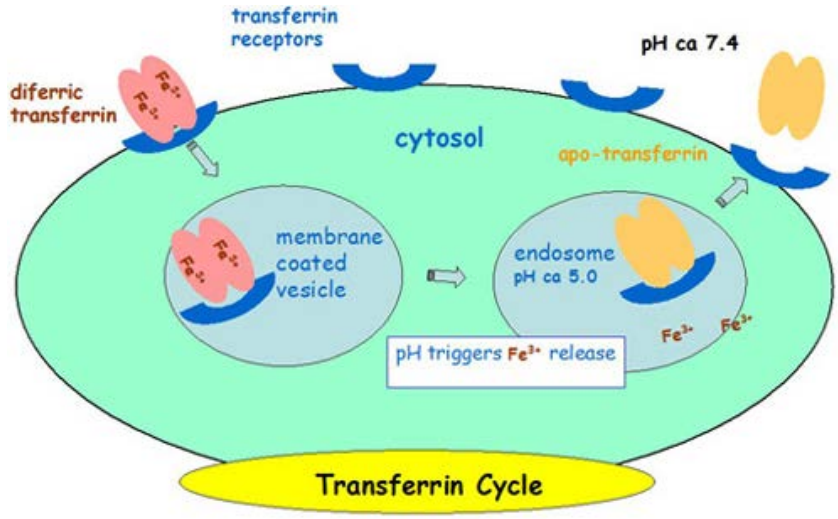

Figure (4): Transferrin -TFRs cycle ***(Viroj, 2006).***

Control of TfRs biosynthesis is a major mechanism for regulation of iron metabolism. Where in iron deficiency anemia the intracellular iron is low, as a result the synthesis of transferrin receptors is increased and ferritin is decreased, enabling the cell simultaneously to mobilize iron stores and compete for iron from the circulation. However, TFRs synthesis is suppressed by iron abundance ***(Viroj, 2006).***

Ferritin:

Iron in the storage compartment exists in two distinct forms: ferritin and hemosiderrin. Ferritin is a water-soluble major iron storage protein it consists of a spherical hollow protein called apoferritin and crystalline core that occupies the hollow interior of apoferritin ***(Fairbanks & Beutler, 2010).***

Ferritin makes iron available within cells while providing some protection from iron-induced oxidizing damage.

Hemosiderin, the other iron-storage compound, is found predominantly in the cells of the monocyte-macrophage system in the bone marrow, Kupffer cells in the liver, and the spleen ***(Cheepsunthorn et al., 2001) .***

Iron Responsive Element Binding Protein (IRE-BP):

The pivotal protein that allows iron to self regulates its intracellular availability is the IRE-BP. The IRE-BP is a transacting cytoplasmic RNA binding protein that regulates the expression of the mRNA and contains a cis-acting regulatory structure termed the IRE ***(Brittenham, 1995)***.

IRE-BR acts as a sensor of intracellular iron availability. In iron deficiency anemia, the decrease in the availability of iron within erythroid cells would increase the proportion of the IRE-BP in the high affinity state (without aconitase activity). Enhancing the binding between the IRE-BPs and iron

responsive elements which increase TFR-protein production but decrease in ferritin protein production. Therefore, more iron enters the cell, and less is sequestered from cellular access.

Conversely, an increase in the available cellular iron would increase the fraction of the IRE- BR in the low affinity (4Fe- 4S) state (with aconitase activity), decreasing binding between IRE-BP and iron-responsive elements and thus, decreasing TFR-protein production while increasing ferritin-protein production. So, less iron uptake and more is sequestered.

These balanced and opposing alterations in iron uptake and storage maintain consistent physiologic iron homeostasis within the developing erythoid cell ***(Lee & Herbert, 1999).***

III- Pathophysiology

Iron is an essential nutrient. It was involved in multiple critical body functions. The predominant use of iron is for the creation of heme groups that are incorporated into hemoglobin and myoglobin. Iron is additionally involved in the production of cytochromes and other enzymes ***(Wu et al., 2002; Segel et al., 2002)***.

The total body iron is maintained by a balance between absorption and body losses. Although the body only absorbs 1 mg daily to maintain equilibrium, the internal requirement for iron is greater (20-25 mg). An erythrocyte has a lifespan of 120

days so that 0.8% of red blood cells are destroyed and replaced each day. A man with 5 L of blood volume has 2.5 g of iron incorporated into the hemoglobin, with a daily turnover of 20 mg for hemoglobin synthesis and degradation and another 5 mg for other requirements (figure 4). Most of this iron passes through the plasma for reutilization. Iron in excess of these requirements is deposited in body stores as ferritin or hemosiderin ***(Harper & Conrad, 2012)*.**

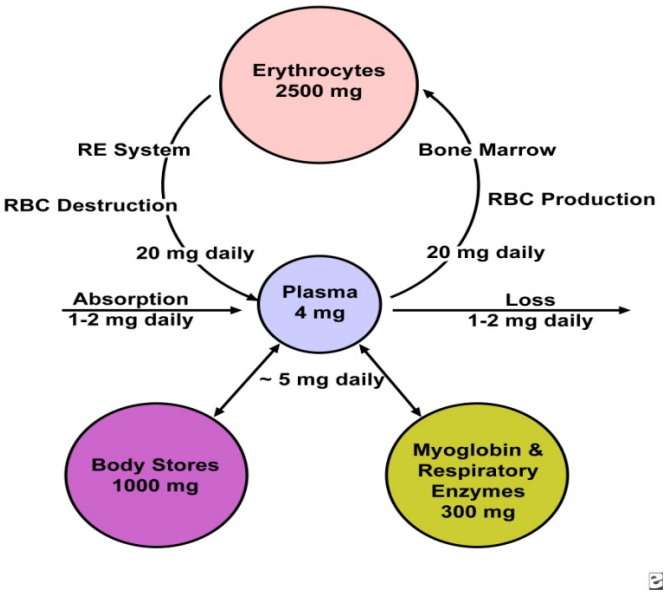

Figure (5): The balance of the iron in the body ***(Harper & Conrad, 2012)***.

Iron balance is achieved primarily by mechanisms affecting intestinal absorption and transport, rather than urinary or fecal excretion. In adults, 5% of daily iron needs comes from dietary sources and equals the iron loss that primarily occurs from the gastrointestinal tract. However, in infants and