INTRODUCTION

Iron deficiency (ID) is the most important cause of nutritional anemia and is the most common micronutrient deficiency worldwide, especially in developing countries. Pregnant women are particularly vulnerable to ID because of the increased metabolic demands imposed by pregnancy involving a growing placenta, fetus and maternal tissues, coupled with associated dietary risks ***(Betelihem et al., 2015).***

Iron serves many functions in the body both systemically, as well as on a tissue specific level. In the central nervous system (CNS), iron is an essential cofactor for many enzymes including those involved in energy metabolism, neurotransmitter synthesis and the process of myelination ***(Rao et al., 2013).***

Therefore, the impact of ID during vulnerable periods of neurodevelopment can be significant and early gestational ID has been associated with abnormalities in neuronal development in children ***(Jougleux et al., 2014).***

This is increasingly recognized as a special concern for hearing development, as mild gestational iron deficiency anemia (IDA) is associated with detrimental effects on cochlear hair cells ***(Yu et al., 2014),*** amplitudes and auditory nerve velocity ***(Jougleux et al., 2014).***

This is of great concern as infants born with suboptimal iron stores may be at a greater risk of impaired neural

maturation. Therefore, there is a paramount need for diagnostic neurofunctional screening that could identify low CNS iron stores, despite normal systemic clinical endpoints ***(Greminger and Mayer-Pröschel, 2015).***

The auditory brainstem response (ABR) provides a noninvasive means to delineate the rapid development and maturation of the central nervous system. It represents the progressive activation of different levels of the auditory pathway from the cochlear nerve (wave I and II) to the cochlear nuclear complex (wave III) and the lateral lemniscus (wave V) ***(Lou et al., 2015).***

During late fetal and early postnatal life, there is a rapid maturation of the ABR that parallels an important period of brainstem myelination, neuronal development and axonal growth. ABR maturation is characterized by shorter absolute and interpeak latencies. The decrease in interpeak latencies reflects increased nerve conduction velocity. Both absolute and interpeak latencies are influenced by degree of myelination, axonal growth and synaptic function ***(Lou et al., 2015).***

Neonatal observational studies suggest that latent or brain iron deficiency during in utero life as evaluated by cord ferritin concentration is associated with long-term adverse effects on the developing brain in near-term and term infants ***(Amin et al., 2010).*** However, the effect of maternal iron deficiency anemia on neonatal auditory neural myelination remains to be elucidated.

Aim of the Work

This study aimed to evaluate the effect of maternal iron deficiency anemia and the in utero iron status on auditory neural myelination in full term neonates.

MATERNAL IRON DEFICIENCY ANEMIA

Anemia with Pregnancy

Anemia is defined as decreased hemoglobin level, or circulating red blood cells and it is the most common hematological disorder during pregnancy. Inadequate intake or absorption of iron in conjunction with blood loss during pregnancy may contribute to anemia. Iron deficiency and consequent anemia during pregnancy could be associated with severe complications like increased risks of maternal mortality and morbidity, premature delivery and low birth weight. Thus, routine screening tests for anemia are recommended in pregnant women ***(Gautam et al., 2008; Malee, 2008).***

The ***World Health Organization (WHO)*** defines anemia as hemoglobin below 13 g/dL in men over 15 years, below 12 g/dL in non-pregnant women over 15 years and below 11 g/dL in pregnant women. The normal range for hemoglobin also varies between different populations. Therefore, it is reasonable to use the lower limit of the normal range for the laboratory performing the test to define anemia ***(WHO, 2008).***

The most common causes of anemia in pregnancy include iron deficiency, folate deficiency, vitamin B12 deficiency, hemolytic diseases, bone marrow suppression, chronic blood loss and underlying malignancies ***(Reveiz et al., 2007).*** 30-50% of women become anemic during pregnancy,

with iron deficiency being the most common form of anemia in more than 90% of the cases ***(Johnson, 2010).***

Because anemia is the most common indicator used to screen for iron deficiency, the terms anemia, iron deficiency and iron deficiency anemia are sometimes used interchangeably. There are, however, mild to moderate forms of iron deficiency in which, although anemia is absent, tissues are still functionally impaired ***(WHO, 2001).***

About 1000 mg of iron is required during pregnancy. 500-600 mg for red blood cells (RBC) expansion.300 mg for fetus and placenta and the rest for the growing uterus. As a result of amenorrhea there is a saving of about 150 mg of iron and therefore, about 850 mg of extra iron is required during pregnancy. Diet alone can not provide the extra iron and stores which have around 500 mg of iron get depleted. But if iron stores are already deficient, iron deficiency anemia manifests ***(Sharma et al., 2010).***

In Egypt, not many studies have been done on this problem in children and little progress has been made in combating anemia and IDA, particularly in aboriginal and rural communities. A national survey, recently conducted on adolescents, detected overall prevalence of anemia of 46.6% among the age group 10-19 years ***(Barduagni et al., 2004; Al Ghwass et al., 2015).*** In Another clinic based Egyptian study showed that 43% of the study population from 6 to 24 months had IDA ***(Elalfy et al., 2012; Al Ghwass et al., 2015).***

I. Erythrocyte Mass

The increase in RBC mass does not begin until approximately 20 weeks, but then increases more rapidly than the plasma volume (PV) until 28 weeks. From 28 weeks to term, the RBC mass rises only slightly, but the slope of erythrocyte increase begins to exceed that of the PV (a situation opposite that found earlier in pregnancy).

The RBC mass is approximately 30% higher than its maximum in the non pregnant state. In the early postpartum period, the RBC mass remains approximately 10% above non pregnant levels for 1–2 weeks but returns to normal by 6 weeks. The decrease is principally related to blood loss at delivery and a decline in erythrocyte production. There is no evidence of increased RBC destruction during the puerperium. Bone marrow erythropoiesis assumes a normal level of RBC production by the end of the postpartum period ***(Morrison and Parrish, 2011).***

The increase of RBC mass during pregnancy is accomplished by a complex interaction of several hormonal and physiologic factors, but in general it follows the erythropoietin production. In normal pregnancy, the erythropoietin level begins to rise slowly at 15 weeks, but the effects of this stimulation on RBC mass are first documented at 18–20 weeks. The maximal activity for erythropoietin occurs between 20 and 29 weeks, corresponding with the maximal increase in uterine blood flow and basal oxygen consumption. The level of

erythropoietin begins to decrease slowly after birth in spite of blood loss at delivery ***(Morrison and Parrish, 2011).***

Erythropoietin production and the subsequent size of the erythrocyte mass are directly related to increased basal oxygen consumption, an event associated with pregnancy. Other factors, such as elevation of cardiac output, decrease in peripheral resistance, reduction in viscosity and increased erythrocyte content of 2, 3- diphosphoglycerate, are also related to the increased need of maternal and fetal tissues for oxygenation ***(Morrison and Parrish, 2011).***

This oxygen requirement stimulates the kidneys as well as other organs to elaborate renal erythropoietic factor. This precursor transforms a dormant circulating pre hormone into erythropoietin, a glycoprotein with a molecular weight of 60,000–70,000 dA that is found in the plasma and urine. It stimulates the genetically predetermined precursor stem cells in the bone marrow to differentiate by way of the erythroid cell line into erythrocytes (figure 1). Prolactin also appears to enhance the effect of the erythropoietin already produced. Human Placental Lactogen (HPL), by its general anabolic action, may support endogenous erythropoietin. The site of HPL action appears to be at the level of the stem cell, whereas prolactin seems to act on the late erythroid precursors ***(Morrison and Parrish, 2011).***

Therefore, during normal pregnancy, the PV rises early and the RBC mass rises to its maximal level during the third

trimester and in iron-sufficient women the difference in packed cell volume (PCV) and the concentration at term is minimal. The total blood volume (TBV) is composed of and follows the increase in PV and erythrocytes ***(Little et al., 2005).***

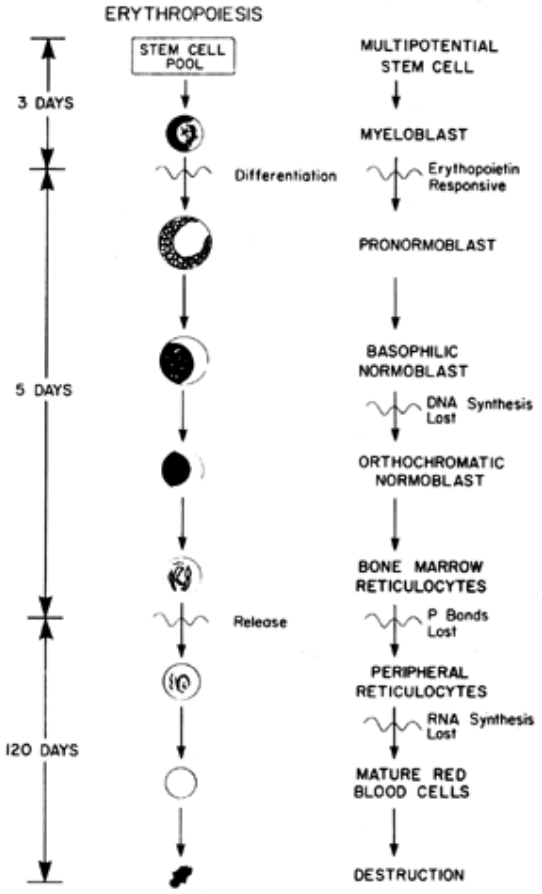

Figure (1): Time sequence of the maturation process of erythrocytic cell lines (erythropoiesis) ***(Morrison and Parrish, 2011).***

II. Hemoglobin Structure

Hemoglobin is a large protein found within red blood cells. Synthesis is initiated in the bone marrow by erythroblasts and is completed once the cells become reticulocytes. It is composed of four subunits with each subunit consisting of a polypeptide with a heme moiety. There are two pairs of polypeptide chains. In normal adult hemoglobin (HbA) these are named alpha and beta chains. The four chains comprise the globin part of the molecule. The heme moiety consists of a porphyrin ring with a central iron atom (figure 2). Oxygen binds to each iron atom to form oxy-hemoglobin, enabling each hemoglobin molecule to carry four molecules of oxygen ***(Karen, 2011).***

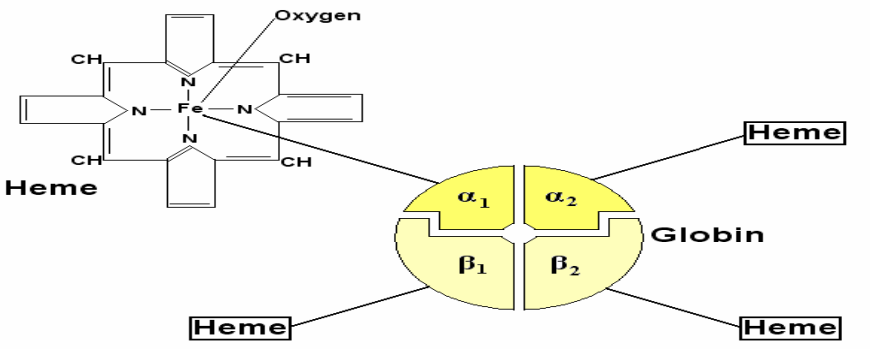

Figure (2): Structure of a hemoglobin molecule ***(Yekoye et al., 2006).***

A. Heme

The heme structure consists of a ring of carbon, hydrogen and nitrogen atoms called protoporphyrin IX with an atom of divalent/ferrous iron (Fe2+) attached (ferroprotoporphyrin). Each hemegroup is positioned in a pocket of the polypeptide chain near

the surface of the Hb molecule of oxygen. The heme component also renders blood red ***(Rodak et al., 2007).***

B. Globin

The globin in the Hb molecule consists of two pairs of polypeptide chains. These chains comprise 141 to 146 amino acids each. Variations in the amino acid sequences give rise to different types of polypeptide chains. Each polypeptide chain is designated by a Greek letter (table 1) ***(Schumacher et al., 2003).***

Each of the polypeptide chains is divided into eight helices and seven nonhelical segments. The helices, designated A to H, contain subgroup numberings for the sequence of amino acids in each helix and are relatively rigid and linear ***(Rodak et al., 2007).***

The nonhelical segments are more flexible and lie between the helical segments, as reflected by their designations: NA for the residues that lie between the N terminus and the A helix. AB for the residues between the A and B helices and so forth with BC, CD, DE, EF, FG, GH, HC and the carboxy terminus ***(Rodak et al., 2007).***

Table (1): Nomenclature of normal hemoglobins

Hemoglobin classification	Globin defect	Chain Present	Common name	Official designation
Adult hemoglobin*		2α 2β	HbA	$\alpha_2^A\beta_2^A$
Adult hemoglobin*		2α 2δ	HbA_2	$\alpha_2^A\delta_2^A$
Fetal hemoglobin*		2α 2γ	HbF	$\alpha_2^A\gamma_2^f$
Embryonic fetal hemoglobin*		2α 2ε	Hb Gower II	$\alpha_2^A\varepsilon_2^G$

(Morrison and Parrish, 2011)

Hemoglobin is the oxygen-carrying protein that is found within all RBCs. It picks up oxygen where it is abundant (the lungs) and drops off oxygen where it is needed around the body. Hemoglobin is also the pigment that gives RBCs their red color.

Iron is an essential component for hemoglobin production. Most of the iron for this process comes from the recycling of other iron stores with only 1-2 mg per day being absorbed from the diet ***(Karen, 2011).***

III. Iron homeostasis:

For a 70-kg male individual, total body iron is about 3.5 g (50 mg/kg). Most of the iron in the body is distribute within RBC hemoglobin (65%; 2300). Approximately 10% is present in muscle fibers (in myoglobin) and other tissues (in enzymes and cytochromes) (350 mg). The remaining body iron is stored in the liver (200 mg), macrophages of the reticuloendothelial system (RES; 500 mg) and bone marrow (150 mg). In premenopausal

women, total body iron (especially the stored fraction, 250-300 mg) is lower than in men. The normal diet contains 15-20 mg of iron and the body absorbs 1-2 mg/d of dietary iron. This is balanced with losses via sloughed intestinal mucosal cells, menstruation and other blood losses. Therefore, internal turnover of iron is essential to meet the bone marrow requirements for erythropoiesis (20-30 mg/d) ***(Muñoz et al., 2009).***

A. Iron absorption

Absorption of iron occurs in the duodenum and is tightly controlled by duodenal enterocytes. Iron is absorbed in three forms: Ferrous ion (Fe 2+), Ferric iron (Fe 3+) and heme. Within the enterocytes Fe 2+ forms ferritin where it is stored as Fe 3+ by binding to apoferritin. It is then transported across the basolateral membrane by ferroportin. Ferroportin is coupled to feroxidase, which converts the ferrous ion to its ferric form.

Only Fe 3+ can pass freely from the enterocyte into the bloodstream. Once in the bloodstream Fe 3+ is carried by transferrin to its target cell. The iron is released by endocytosis of the transferrin complex and after removal of the iron the transferrin molecule returns to the circulation. The levels of transferrin are directly affected by the iron stores, with adequate stores resulting in lower levels of transferrin. This in turn results in decreased transfer of iron across the duodenal mucosa ***(Karen, 2011).***

B. Iron distribution

Iron released into the circulation binds to transferrin and is transported to sites of use and storage. Transferrin has two binding sites, binding one iron atom each (thus three forms can be found in plasma: apo-transferrin which contains no iron, mono ferric-transferrin and diferric-transferrin). About 30%-40% of these sites are occupied under normal physiological conditions. Thus, transferrin-bound iron is about 4 mg, but this is the most import dynamic iron pool ***(Crichton et al., 2008).***

Transferrin- bound iron enters target cells mainly erythroid cells, but also immune and hepatic cells through a process of receptor-mediated endocytosis. As diferric-transferrin has a much higher affinity for transferrin receptor (TfR) than does monoferric-transferrin, it binds to the TfR at the plasma membrane and patches of cell surface membrane that carry receptor ligand complexes invaginate to form clathrin coated endosomes (siderosomes) ***(Crichton et al., 2008).***

After clathrin is removed, the siderosomes become acidified through an ATP-dependent proton influx, which leads to conformational changes in transferrin and TFR1 and promotes iron release of Fe 3+ from transferrin. Fe 3+ is then reduced to Fe 2+ by a ferri reductase and transported to the cytoplasm through the divalent metal transporter 1(DMT-1), whereas the TfR is recycled to the cell membrane and transferrin shed back to the circulation ***(Andrews, 2008).***

In the erythroid precursors, the expression of TfR1, DMT-1 and ferritin are regulated reciprocally through cytosolic iron regulatory proteins (IRP1) and (IRP2), which act on the iron regulatory elements (IREs) present in their RNA. Thus, when increased iron uptake is needed, the expression of TfR1 and DMT-1 is increased, whereas the synthesis of ferritin is halted ***(Muñoz et al., 2005).***

A truncated form of the TfR can be detected in human serum. The serum concentration of this soluble form of TfR (sTfR; normal median concentration: 1.2-3.0 mg/L, depending on the assessment kit used) is proportional to the total amount of surface TfR. Increased sTfR concentrations indicate ID even during the anemia of chronic disease (ACD), as well as increased erythropoietic activity without ID, whereas lower sTfR concentrations may reflect decreased numbers of erythroid progenitors ***(Muñoz et al., 2005).***

C. Iron storage

Hemoglobin iron has substantial turnover, as senescent erythrocytes undergo phagocytosis by RES macrophages. Within the phagocytic vesicles, heme is metabolized by heme oxygenase and the released iron is exported to the cytoplasm through the action of natural resistance associated macrophage protein-1, a transport protein similar to DMT-1. Macrophages can also obtain iron from bacteria and apoptotic cells, from plasma through the action of DMT-1 and TfR1 and from other sources. Within the cell, iron can be stored in two forms: in the

cytosol as ferritin and, after breakdown of ferritin within the lysosomes, as hemosiderin. Hemosiderin represents a very small fraction of normal body iron stores, mostly in macrophages, but increases dramatically in iron overload ***(Crichton et al., 2008).***

Importantly, iron storage at the macrophages is safe, as it does not lead to oxidative damage. EPO reduces iron retention in macrophages by decreasing DMT-1 and increasing ferroportin 1 expression ***(Kong et al., 2008)***

The liver is the other main storage organ for iron. In iron overload, free radical formation and generation of lipid peroxidation products may result in progressive tissue injury and eventually cirrhosis or hepatocellular carcinoma ***(Muñoz et al., 2009).*** Iron is sequestrated in hepatocytes predominantly in the form of ferritin or hemosiderin ***(Siah et al., 2006)***.

D. Sources of Iron

Lean red meat, fish, poultry and iron-fortified grains, such as WIC-approved cereals, are good sources of iron. Legumes and dark green vegetables, such as kale, collard greens and spinach, are part of a healthy diet. They also have iron. It is easier for the body to use the iron in meat than the iron in plant foods. To help the body use iron from plant foods, vitamin-C-rich foods, such as citrus fruits/juices, or a small amount of meat should be eaten at the same meal. Using cast-iron skillets for cooking can add iron to the food cooked. Coffee, tea, soda and excessive milk intake can reduce iron absorption and should be avoided, especially at mealtimes ***(CDC, 2012).***