HEMOGLOBIN AND IRON STORE STATUS IN LOW BIRTH WEIGHT BABIES AND EFFECT OF EARLY IRON SUPPLEMENTATION ON THEM-RANDOMIZED AND OPEN LABEL STUDY.

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Dissertation submitted to

BLDE (Deemed to be University) Vijayapur, Karnataka

In partial fulfillment of the requirements for the degree of

DOCTOR OF MEDICINE

IN

PEDIATRICS

Under the guidance of

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HOSPITAL & RESEARCH CENTRE, VIJAYAPUR

KARNATAKA

2020

"Haemoglobin and Iron store status in Low Birth Weight babies And Effect of early Iron supplementation on them- A Randomized and Open label study."

MD IN PEDIATRICS

LIST OF ABBREVIATIONS

IDA	-	Iron deficiency Anemia
NICU	-	Neonatal Intensive Care Unit
IUGR	-	Intra uterine growth retardation
EPO	-	Erythropoietin
LBW	-	Low Birth Weight Infants
ROP	-	Retinopathy of prematurity
NEC	-	Necrotizing Enterocolitis
FTT	-	Failure to Thrive
Hb	-	Hemoglobin
HbA	-	Hemoglobin A
CBC	-	Complete Blood Count
MCV	-	Mean Corpusclar Volume
MCH	-	Mean corpuscular Hemoglobin
MCHC	-	Mean Corpuscular Hemoglobin Concentration
TIBC	-	Total Iron Binding Capacity
LDH	-	Lactate dehydrogenase
ELBW	-	Extremely Low Birth Weight Infant
Tf	-	Transferrin

r-HuEPO - recombinant erythropoietin

VLBW - very low birth weight

EI - Early Iron Supplementation

LI - Late Iron Supplementation

RDA - Recommended dietary allowance

HM - Human Milk

IFF - Iron fortified formula

MDI - Mental Developmental index

PDI - Pscychomotor developmental index

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INTRODUCTION

Iron and heme-proteins are pivotal in a wide range of metabolic and synthetic functions that are essential for the normal functioning at cellular level. Iron deficiency during development has a deleterious impact on the growth and functionality of numerous organs, including the heart, erythrocytes, GIT, and skeletal muscles. The effect of iron deficiency on the developing neurological system is the most alarming one. Early iron deficiency has a deleterious effect on cognitive functioning in humans that is long-lasting, according to neuropsychological research.

Low birth weight neonates, especially preterm infants, are more likely to develop iron deficiency anemia (ID) because they have smaller iron stores at birth and higher requirements of iron in accordance with a rapid exapansion in the red cell population and size of the organs than term infants. Increased hemolysis, low erythropoietin levels, shortened red blood cell life span, frequent blood draws, and various medical and surgical procedures can all cause anemia in these low birth weight and premature infants. These conditions alter the existing at-birth iron levels in the preterm infant while in the NICU, where a significant number of events may further disrupt the normal iron balance. Significant maternal iron deficiency anemia, maternal hypertension (without IUGR), intrauterine growth restriction (IUGR), pre- or post-partum diabetes in the mother, and maternal smoking all lower fetal iron store status. (1) Maternal hypertension causes placental insufficiency resulting in IUGR, which is a significant contributing

factor in majority of preterm births and hence is important determinant of iron status in a newborn. Whereas conditions like Congenital hemochromatosis (2) and frequent blood transfusions can lead to iron overload in LBW infants, culminating in ferritin levels above 1000 ng/ml.

In a low birth weight infant, the factors that cause decrease in iron levels after birth; include frequent phlebotomy loss of blood, a late initiation or even low dosage of oral iron supplementation, use of erythropoietin (EPO) in NICU, and relatively faster growth rates than term babies. Blood loss by phlebotomy is one of the major contributing factors to anemia of prematurity.(3) 3.46 mg of elemental iron is lost for each one gram of phlebotomy loss of hemoglobin. Loss of 10–40 mg of iron per kg body weight per week owing to phlebotomy procedures, is rather common in the NICU. An international panel on nutrition has advised beginning oral iron supplementation in preterm infants between the ages of 2 weeks and 2 months.(4) When the initiation of iron supplementation is delayed until two months of post-natal age, the probability of developing iron deficiency anemia at 6 months of age increases. Accordingly, a dose of 2-3 mg/kg body weight in a day has been advised; and smaller doses than these appear to have a negative impact on the iron store status. The use of erythropoetin (EPO) has been proposed to reduce the requirement for blood transfusions. This EPO induced erythropoiesis, however, necessitates greater availability of iron. If the iron supplementation is not raised during EPO therapy to at least 6 mg/kg body weight/day, the iron stores get exhausted. Finally, as NICU now focus on enhancing growth rates in preterm neonates, the effect of these

improved post-natal growth rates on iron levels and iron stores, should not be underestimated for neurodevelopmental reasons.(5) Improved faster growth leads to a rapid increase in RBC count, and the increased hemoglobin synthesis necessitates further supplementation. On the other hand, a positive iron balance can be acheved by early initiation and correct dosage of iron supplementation, reducing frequent phlebotomy, and non-restrictive blood and iron transfusion guidelines. With positive iron balance there is a very fine line delineating it from iron overload, particularly when several blood transfusions or parenteral iron are used. Preterm neonates are the most susceptible to risk for iron deficiency and iron deficiency anemia due to smaller iron stores at birth (iron transfer occurs mainly in the third trimester, hence this iron accretion is affected when the baby is delievered before term) and their increased demand (owing to the higher post natal growth rates than that of term infants) (6).

Since iron plays an important role in neurodevelopment, iron deficiency has been proven to result in long-term poor neurocognitive outcomes (7, 8).

Around 25% to 85% of preterm infants develop Iron Deficiency Anemia (IDA) during the first year of life. Unlike Term neonates, who typically develop iron deficiency after 6 months of age, preterm neonates are at risk for iron deficiency anemia during their first six months soon after birth. In general, the lower the gestational age, the greater is the risk of iron deficiency at an ealier post natal age. Iron deficiency is more common in LBW infants who do no receive any iron supplementation and in preterm neonates of developing nations.

The negative effects of Iron deficiency are anticipated to be more severe in preterm neonates given the low maturity and higher growth rates. However, free iron plays a role as an oxidant stressor and preterm newborns have poor anti-oxidant capacity, so iron supplementation in LBW neonates should be practised with caution.

Recent studies have shown that preterm low birth weight babies are more likely to develop neurological and cognitive problems owing to iron deficiency, reinforcing the importance of iron supplementation in preterm LBW neonates.

Currently, the guidelines for supplementation of iron in infants who were born prematurely or with low birth weight are ill-defined. Many international associations have made varying recommendations pertaining to the intiation. According to the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition Committee on Nutrition, recommendation; preterm infants should receive iron fortified human milk or separate oral iron supplementation between the ages of 2 and 6 weeks (ELBW infants at 2-4 weeks). The Canadian Pediatric Society has suggested that infants less than 1000g get a total of 3–4 mg/kg body weight per day starting at post natal age of 6^{th}- 8^{th} week . According to the American Academy of Pediatrics (AAP), a low birth weight infant who is breastfed should receive elemental iron at a dose of 2 mg/ kg body weight per day, beginning at 4 weeks of age and continuing through the first year of life.

Extreme caution is to practised when starting iron in LBW infants due to the significant possibility of iron overload. High iron intake through dietary supplements or transfusions causes production of free Fe^{2+}. These ions cause oxidative stress leading to

the formation of ROS (reactive oxygen species) that damage developing organs. Therefore, careful cautious provision of iron supplementation is essential to enable optimal development without causing oxidative damage and overload.

As of right now, there is still a lack of global agreement regarding recommendations for iron supplementation in LBW babies. Different countries have different protocols for dosages, timing of starting, and duration of iron supplementation. Oral Iron, at 2 mg/kg body weight per day, is advised for preterm neonates in China started at first 2–4 weeks after birth through one year of corrected gestational age. This preventive approach, however, needs to be further studied and looked into.

AIM AND OBJECTIVES

AIM:

To study the effect of early iron supplementation in LBW babies.

OBJECTIVES:

- To study whether low birth weight (LBW) infants receiving early iron(EI) supplementation will have reduced incidence of iron deficiency anemia (IDA).

- To evaluate effect of early iron (EI) supplementation on iron stores(serum ferritin) levels.

- To study the incidence of neonatal morbidity, blood transfusion requirements in LBW infants receiving early enteral iron (EI) supplementation.

RESEARCH HYPOTHESIS

- Supplementing iron at 2 weeks of age or prior in LBW infants results in relatively less drop in serum ferritin and hematological parameters at short interval follow up (≥6 weeks of age) when compared to controls
- Early iron supplementation significantly decreased the incidence of iron deficiency anemia (IDA) at 6 weeks when compared to controls who did not receive any supplementation
- Iron supplements do not adversely affect infant growth, or cause increase in in infections, ROP, NEC or other morbidity.

REVIEW OF LITERATURE

Infancy is a period of rapid development second only to the fetal period. Nutritional supplementation is needed to promote adequate growth and organ development. The Barker hypothesis highlighted the effects of adverse nutrition in early life, on the developmental origins of diseases .(9) Undernutrition during the fetal period as a result of placental, maternal, or fetal conditions may result in growth restriction with retarded organogenesis and low birth weight. While fetal nutrition is not affected until extreme maternal malnutrition occurs, the postnatal growth is easily affected by undernutrition. Hence undernutrition in infancy results in growth failure or failure to thrive (FTT) as well as metabolic derrangements that can last into adulthood.

Due to the intrinsic difficulties associated with immaturity, preterm infants are particularly at risk of experiencing post-natal growth failure. It has been demonstrated that better preterm care, including recent advancements in neonatal and infant nutrition, have improved growth and development outcomes.

Micronutrients or trace elements are nutritional components that are needed in very small quantities often in micrograms. The most prevalent micronutrients are iron, zinc, copper, copper, chromium, manganese, and selenium. These trace elements necessitate age, size, and disease-specific adjustments in children as opposed to adult guidelines. While the absorption of micronutrients is closely regulated across the gastrointestinal mucosa, providing nutrients parenterally bypasses this regulation, increasing the risk of overload if excessive quantities are provided.

ROLE OF IRON

Iron is essential to human survival. It is the fundamental component of hemoglobin and myoglobin and is crucial for important processes such as DNA synthesis. It is a crucial element in the heme component of hemoglobin, the protein responsible for oxygen transport. Erythrocytes, or red blood cells, are produced by hematopoietic stem cells of the bone marrow. Through a hormone called erythropoietin, the kidneys stimalate RBC production in the bone marrow. In addition to the basal secretion of erythropoietin, this secretion and synthesis is augmented by hypoxia. The absence of iron therefore causes microcytic anemia and failure to thrive (FTT). Growing preterm infants need a larger dose of iron to meet their growth requirements and to support the increased erythropoiesis. Breastfed infants may need iron supplementation after 4-6 months of life, but iron-fortified-formula-fed infants do not.

HEMOGLOBIN

Hemoglobin being the oxygen-binding protein transports oxygen molecules to tissue level. Each hemoglobin molecule is made up of four globin chains. A heme moiety made up of a protoporphyin ring and a central ferrous ion (Fe^{2+}) comprise each globin subunit.

Each Fe^{2+}ion has the ability to bind and unbind oxygen, contributing to the oxygen transportation. In adults the predominant Hb is HbA, consistin of two α globin and two β globin chains. Each type of globin subunit is encoded by a different globin gene. (10)

Globin synthesis and heme synthesis are the two primary components of hemoglobin production. These chains are formed by transcription and translation in the RBC cytoplasm. Research has demonstrated that globin chain production is induced by presence of heme moiety. Genes for the α and β chain are located on chromosome 16 and 11 respectively. Both the RBC cytoplasm and mitochondria are the sites of heme production. Glycine and succinyl coenzyme serve as the building blocks. It culminates in the production of a protophorpyrin IX ring. The final heme molecular structure is created by the binding of protophorpyrin to a ferrous (Fe^{2+}) ion (11)

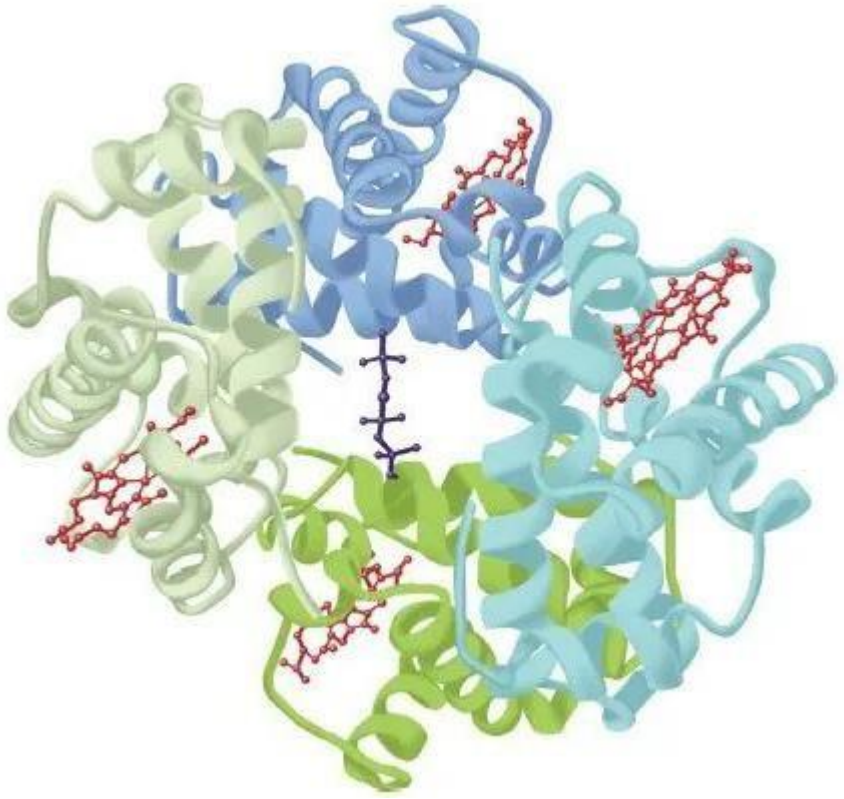

Figure 1. Hemoglobin: A molecule of 2,3-bisphosphoglycerate (dark blue) bound to deoxyhaemoglobin, shown in 3D. The two α subunits are colored in dark green and blue shades, the two β subunits in light green and light blue shades, and the heme prosthetic groups are colored red.

NEONATAL HEMATOLOGIC SYSTEM

Fetal haemoglobin (HbF), the predominant haemoglobin at birth, circulates in the blood until approximately three months of age, after which it is gradually replaced by adult haemoglobin (HbA).The oxygen-hemoglobin dissociation curve shifts to the left due to strong affinity of HbF for oxygen. Leading to low oxygen pressures in the

arterial blood in neonates compared to adults. When compared to adults where 50% of hemoglobin is saturated with oxygen at 27mmHg, whereas in neonates 19 mm Hg is the oxygen partial pressure at which 50% of haemoglobin is saturated with bound oxygen. This left shift is further influenced by the fact that 2.3-bisphosphoglyceric acid (2,3 BPG) has less affinity towards HbF. HbF also protects the RBCs from sickling. Normal neonatal hemoglobin levels range from 18 to 20 g/dL.

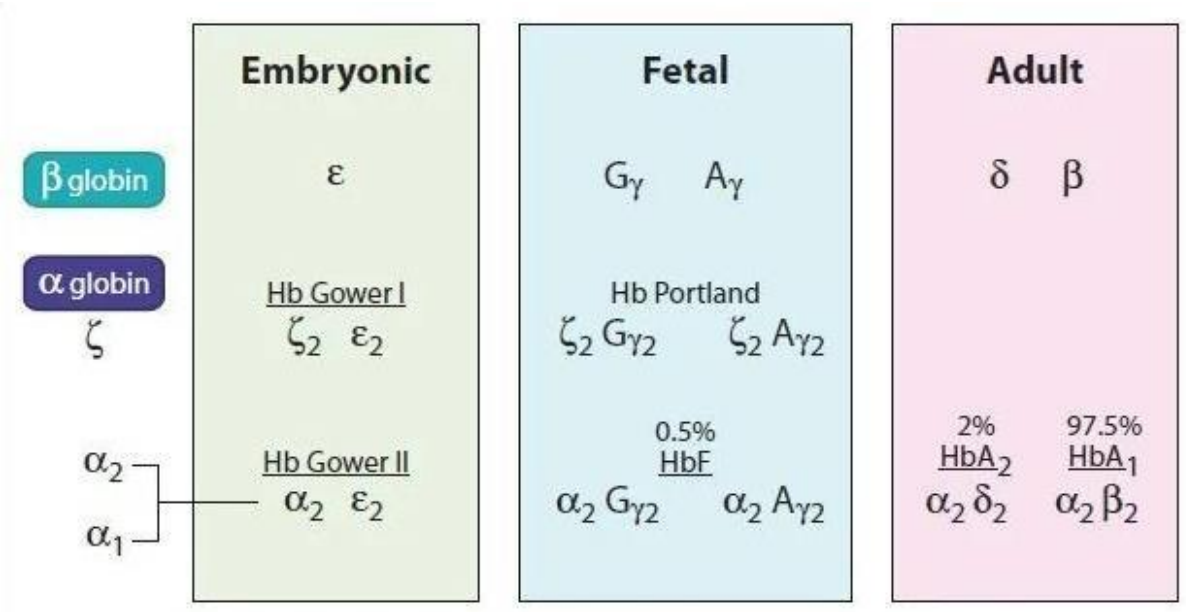

Figure 2 Major types of hemoglobins found during embryonic, fetal & adult life

PHYSIOLOGY OF HAEMATOPOIESIS

Hematopoiesis first starts in the yolk sac, as early as the third week of gestation.(12) The liver is the site of hematopoiesis from 11 to 12 weeks of gestation. (13) Around 30 weeks of intrauterine life, RBC production site shifts to the bone marrow. (14)

The oxygen saturation in fetal blood is low i.e. 45%, erythropoietin levels are high, leading to more RBC production. After delivery, the oxygen saturation increases to 95%, which in turn downregulates the production of red blood cells by fall in erythropoietin levels. By three days after birth, erythropoietin levels are not detectable resulting in reduced reticulocyte counts and, consequently a fall in Hb levels.

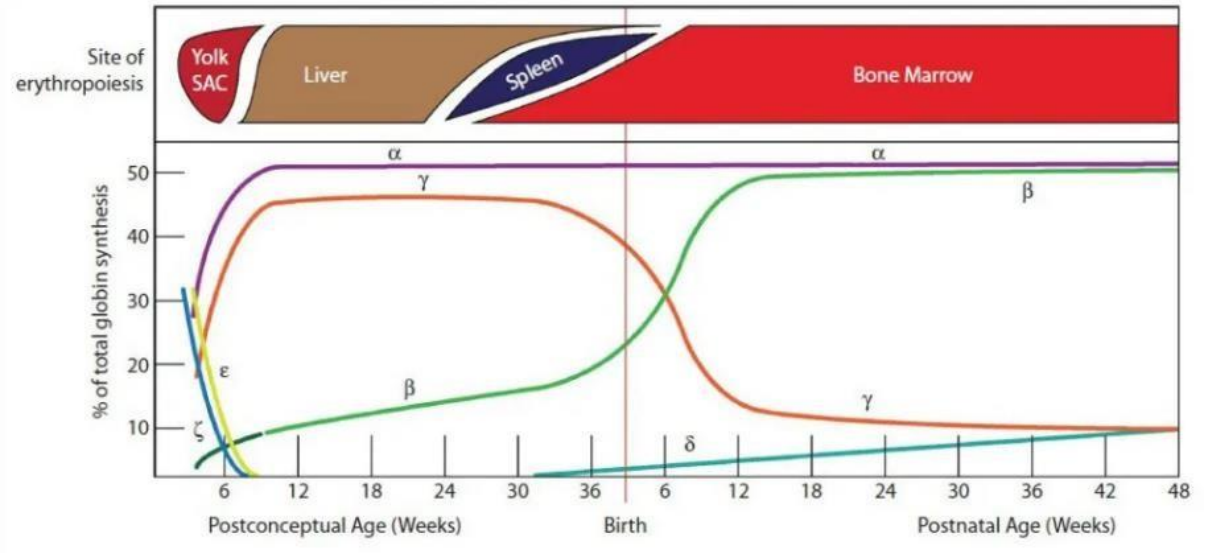

Figure 3: Shift in pattern and location of haemoglobin synthesis in fetal and post-natal period

Hb levels at birth range from 14.9 - 23.7 g/dl in term infants and from 19.1 g/dl- 22.1 g/dl in preterm infants. By 9–11 weeks after birth, it drops sharply to 9.5–11 g/dl in term babies and to 6.5–9 g/dl in preterm babies at 4-8 weeks of post natal age.(16) At this level, the bone marrow is stimulated to start producing RBCs. The liver stores the iron produced during the destruction of these RBCs, which is then used for erythropoiesis. Usually, term babies have enough iron reserves for this RBC production until they are five months old, at which point, iron needs to be supplemented through their diet. Preterm and LBW babies need iron supplementation starting at around 2-3 weeks postnatally and continuing through 12 months of age. As HbA replaces HbF during the first 6 months of life, there is an increase in 2,3- BPG in the red blood cells.(17,18) The oxygen supply to the tissues actually increases even with falling hemoglobin levels owing to the comparatively low affinity of HbA towards oxygen as opposed to HbF.

Preterm and LBW babies have additional issues such as overall poor health, short erythrocyte life span (35-40 days in preterm neonates vs. 60-70 days in term neonates)(19), frequent phlebotomy procedures in NICUs, comparatively faster growth rates, poor iron sores, and inadequate bone marrow erythropoiesis, which puts them at risk of negative iron balance and exposure to transfusions. Generally, by the time term neonates have doubled their birth weight, even these term infants with normal hemoglobin at birth would have used up all of their iron stores. (20)

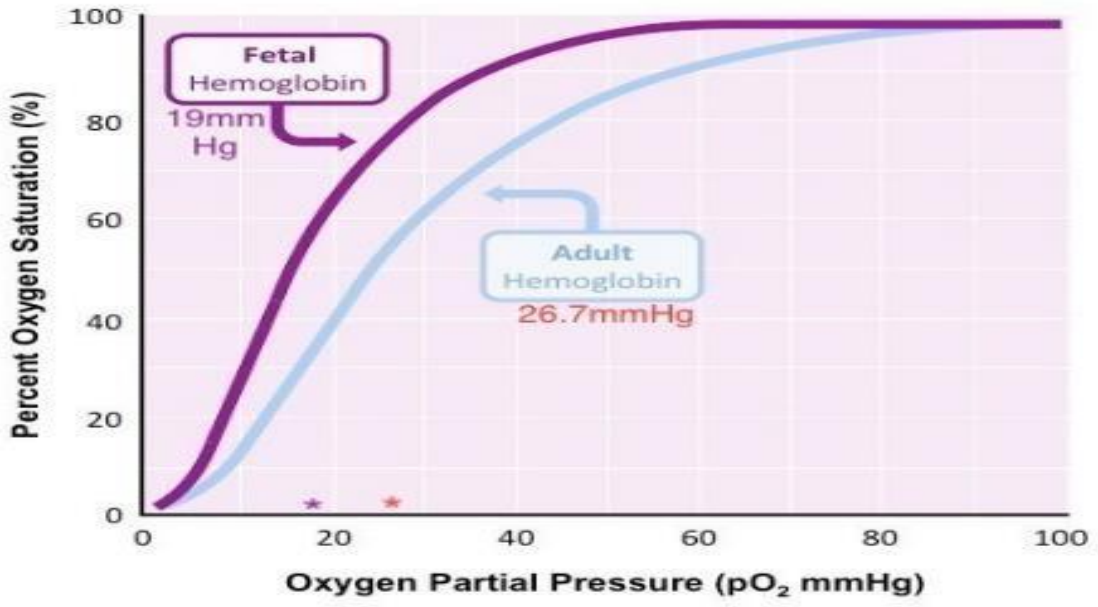

Figure 4 Oxygen Dissociation Curve in fetus and adults

MAGNITUDE OF IRON DEFICIENCY

Iron deficiency is a major public health challenge. Children, adolescents, and pregnant women, as well as those with impaired absorption, or who have undergone GI surgeries have greater physiological demand for iron, hence are at a greater risk of developing Iron deficiency.

According to estimates, the incidence of iron deficiency varies between 25% and 80% in preterm infants.(21) LBW neonates are especially vulnerable to developing IDA due smaller iron stores at birth and rapidly increase in red cell mass requiring more iron supply. Other factors causing iron deficiency anemia in LBW infants include preterm birth (as fetal accretion of iron occurs majorly during 3rd trimester), maternal diabetes

mellitus, maternal hypertension or smoking, etc. leading to uteroplacental insufficiency, reduced life span and subsequent increased destruction of RBCs, low circulating EPO levels, increased blood sampling and surgical procedures.

EVALUATION OF IRON STATUS

The best way to assess iron status is to measure serum ferritin and transferrin saturation. The body iron stores are quantified by serum ferritin. Ferritin level less < 30 ng/mL is considered diagnostic of iron deficiency; and a ferritin level of < 10-15 ng/mL holds 99% specificity for IDA, despite the fact that the normal ferritin levels vary with age. The drawback with ferritin is that it is an acute-phase reactant, and its levels may be impacted by any ongoing inflammation in the body. Another measure of iron status is transferrin saturation, which indirectly indicates the amount of iron that can be transported to tissues. Under 20% transferrin saturation generally indicates iron deficiency. A complete blood count (CBC) may also show signs of iron deficiency in the form of a microscopic, hypochromic red blood cell (RBC) picture;but early stages of ID may show a normal peripheral blood picture, so red blood cell characteristics should not be the only factor used to determine an individual's iron status.

Evaluation of iron status–

1. Hemogram / complete blood count (CBC):

a. Includes hemoglobin (Hb), Hematocrit, Red blood cell indices

2. Corrected reticulocyte count:

a. estimates the new RBC production in marrow

3. Iron profile:

a. serum iron, serum ferritin and total iron-binding capacity (TIBC).

4. Peripheral smear:

a. Helps study RBC morphology

5. Hemolysis profile:

a. Haptoglobin, LDH and unconjugated bilirubin levels.

6. Macrocytosis profile:

a. Vitamin B 12, folate levels, methylmalonic acid, and homocysteine levels.

7. Hb electrophoresis:

Evaluates the globin chains

8. Abdominal ultrasound:

To determine size of liver, spleen

9. Bone marrow Aspiration

Several studies have found that iron supplementation improves the hematological parameters of iron status and decreases the incidence of anemia or iron deficiency in

infants with low birth weight or preterm birth. Major concern associated with iron supplementation is the increase oxidative stress due to Fe2+ ions through the release of free radicals. Therefore, both iron deficiency and iron overload must be prevented. Currently, the appropriate time to start oral prophylactic iron supplements in LBW infants is unclear.

IRON STATUS IN NEWBORNS

Approximately 14% of newborns worldwide are born LBW i.e. < 2500g. The occurrence ranges from 5% in developed nations to 28% in developing nations such as India. The term “LBW infant” encompasses term SGA infants (small for gestation) and preterm newborns, and is a potential risk for behavioural and psychological dysfunction. The majority of LBW neonates weigh between 2 - 2.5 kg and seldom require ICU care. Guidelines on their iron supplementation are widely variable.(22) During the final trimester of pregnancy, the fetus accumulates vast amounts of iron (23) into the body by its active transfer from the maternal circulation via the placenta.(24) Throughout the third trimester, this transfer hepls to maintain the total iron content of around 75 milligrams per kilogram of fetal body weight.(23) Thus, a 1000g neonate (ELBW) delivered before the third trimester accretion of iron, has only 37.5 mg of total iron content in the body, but a term infant with a birth weight of >3.5 kg has 262.5 mg at birth. Iron in the body is divided amongst three sections: red blood cells, storage pools,

and non-red blood cell tissue. RBCs contain the major percentage of the body's iron content, determined by the Hb and Packed cell volume.

The Iron stores, mostly located in the liver's reticulo-endothelial system are best indicated by the serum ferritin levels. From 24 to 40 weeks of gestation, there is a marginal increase in ferritin levels. The mean value in a term infant at birth is 170 ng/mL, with a 5th centile cut off level of 59.8 ng/mL.(25) During the first few months of life, ferritin concentrations are much higher than infants >6m of age and toddlers (1, 26, 27), and full term newborns have more iron reserves per kg weight than older children (1, 26, 27). Even though tissue stores are the smallest, this pool is crucial because the iron in tissue pool is necessary for metabolism at cellular level. These Hemoproteins and enzymes are required for intracellullar oxygen transport, oxidative phosphorylation, and neurotransmitter production in the brain. Unfortunately, there are currently no markers that evaluate iron stores in tissue, whereas most of the symptoms of ID, including the neuro-cognitive effects, are caused by tissue iron store delpletion(28, 29)

FACTORS COMPROMISING FETAL IRON STATUS

In a neonate with LBW, several factors can alter the iron levels. Severe IDA in the mother, intrauterine growth retardation (IUGR), hypertension in the mother (without IUGR), pre-gestational or gestational diabetes in the mother, and smoking all diminish

iron stores of the fetus(1). In a significant proportion of full-term babies, IUGR related to maternal hypertension is a risk factor due to uteroplacental insufficiency.

LBW and preterm neonates have lesser body and liver iron levels, hemoglobin, and serum ferritin levels compared to term neonates. (22, 27, 30, 31) SGA babies and preterm neonates may have normal serum iron and serum transferrin -Tf levels in the umbilical cord blood(32), but reduced organ stores.(33) After birth, the hemoglobin level falls by 30–50% because RBC production ceases, the vascular volume expands, and due to shorter RBC lifespan of 45–60 days compared to term neonates.(27) The iron released during hemolysis (3.4 mg of iron per gram of hemoglobin) gets stored and is evident by a transient increase in stored iron levels i.e. ferritin.(34, 35) During physiological anemia in preterm infants, the Hb level drops to a lower and earlier nadir.(35-37) In preterm infants, erythropoiesis starts 1-3m prior compared to term neonates. With this beginning of RBC production, the ferritin concentrations drop.(35-38) Extremely low birth weight (ELBW) infants when not supplemented with may develop a negative-iron balance during these first 30 days after birth as a result of active erythropoiesis.(39) Without an external iron supplement, preterm infants can maintain sufficient erythropoiesis only until their birth weight has doubled due to low iron stores at birth(35). The catch-up growth spurt after birth in LBW neonates, with expansion in red cell mass and Hb requirement, necessitates an increase in postnatal iron acquisition. Low birth weight infants and preterm infants need to increase their iron levels by 3-6

times during first year of life, to reach the physical growth and iron sufficiency status same as that of term neonates.(40, 41)

Blood loss due to Phlebotomy procedures, late initiation of oral iron therapy, low doses of IV iron, administration of erythropoietin (Epo), and catch up growth spurt all contribute to drop iniron levels in a preterm or LBW infant. Phlebotomy losses are a major contributor to the condition known as "anemia of prematurity" (3) Each 1g of Hb lost results in the loss of 3.46 mg of elemental iron. In a neonate admitted at the NICU, approximately 10–40 mg/kg/week of iron is lost. Two weeks to two months is the recommended age to begin enteral iron administration in these neonates. (42) Delaying iron therapy until 8 weeks of age increases the prevalence of iron insufficiency at the end of first half of infancy. (43) The recommended dose of enteral iron is 2–3 mg per kg body weight per day (42), and lower doses do not seem to be effective enough in neonates.

Blood loss due to phlebotomy, exchange transfusion(44), and use of erythropoietin (r-HuEPO) lower the LBW infants' already small iron stores. EPO is administered in an effort to stimulate marrow production of RBCs.(45, 46) It reduces the requirement for RBC transfusions hence this practice deprives the preterm neonate of this external iron source. The acquisition of iron for erythropoiesis (47) reduces the body's iron stores, particularly in the absence of concurrent external iron supplementation. (48) Following administration of r-HuEPO, serum ferritin (45, 49) and iron levels (49) fall, and

erythrocytes become hypochromic (45), despite high-dose iron supplementation, and irrespective of the dose of r-HuEPO. (50, 51)

The majority of transfusions are administered within the first month of life, either to compensate blood loss due to phlebotomy or to maintain the packed cell volume. The iron given this way increases the body's iron stores. During the first month of life, serum ferritin increases in preterm infants who have received more than two transfusions, with levels quadrupling in those who have received seven transfusions.(57) Thus, ELBW children who have undergone numerous transfusions can maintain significant iron reserves without iron supplementation until the age of 6 months without iron supplementation.(52, 53) Infants born prematurely receiving > 100 ml of PCV transfusion have increased levels of ferritin, than those who get smaller transfusion volumes or none at all.(53, 54) On the other hand, a cautious transfusion strategy, as is being practiced now-a-days(51,55), could negatively impact iron storage if LBW infants are not given adequate iron supplementation. LBW children who have not received blood transfusion have lower stores iron at 6 m of age compared to term infants at the same age who have not received blood transfusion.

Lastly, catch up growth rates should not be overlooked or undervalued (5, 56), especially in light of the present emphasis on enhancing the catch up growth in premies for neurological development. Rapid expansion of the red cell mass is caused by rapid growth, and increased haemoglobin production necessitates extra iron. The tissue iron

stores of preterm newborns with the faster growth rate decrease more rapidly. This is especially true of immature preterm newborns, who display the quickest catch-up growth. Intriguingly, iron status at birth has no effect on postnatal growth rate(57), indicating that preterm newborns will grow regardless of their iron status at delivery. Increases in blood volume and Hb mass accompanying rapid postnatal catch-up growth, necessitates extra iron. Unless supplemented by external sources, the endogenous iron stores of preterm newborns at birth are insufficient to supply their iron requirements beyond the doubling of birth weight, or beyond roughly 8-12 weeks of age.

In contrast, sufficient iron levels can be achieved by starting early and adequate oral supplementation of iron, minimising blood loss due to phlebotomy, relaxed transfusion guidelines, I.V. iron. Improved iron levels are advantageous, but risk of overload increases, especially when frequent transfusions or IV iron are supplied.

The hematological parameters of iron store status at discharge determine the further requirement of iron. At discharge, haemoglobin and ferritin levels can guide the supplementation of iron.

To conclude, the above mentioned factors can either further increase the deficiency of iron in preterm neonates or exacerbate the possibility of iron overload.

CONSEQUENCES OF IRON DEFICIENCY

Low body Iron levels affect various organs across the systems. Iron deficiency is associated with poor growth, GI disturbances, thyroid abnormalities, decreased immune response, and temperature instability in infants < 1.5 kg birth weight (58). Anemia most often manifests late. The effects of early iron deficiency on the developing brain are a major cause for concern.

POTENTIAL EFFECTS OF IRON DEFICIENCY ON NEURODEVELOPMENT

Inspite of adequate supplementation of iron, iron deficiency during first six months of life has been linked to detrimental affects on neuro-development that are irreversible. It has been noted that term neonates with deficiency of irom exhibit long-term cognitive impairments.(5)

Few studies have found that iron deficiency in first 6m of age in preterm babies may affect neurological functioning and development. Greater proportion of LBW infants with Hb levels < 10 g/dL and ferritin levels below 76 ng/mL were reported to have abnormal neurologic reflexes at PMA of 37 weeks, than their counterparts with normal Hb and normal iron stores.(59) A recent study compared the “development of neurologic abnormalities in preterm infants who received iron supplementation at ages 2 months and preterm infants who received iron supplementation at 2 weeks after birth”.(60) The study revealed a higher occurrence of neurological abnormalities in children who began

iron supplementation at 2 months. Those who were supplemented late also exhibited diminished cognitive function.(60) Therefore, iron deficiency <6 months of age seems to have detrimental effect on neurodevelopment in infants born before term.(1,60)

DETERMINING IRON REQUIREMENTS OF PRETERM INFANTS

The goal here is to mirror intra-uterine acquisition of iron and achieve normal iron levels in blood.(61) This estimation of the iron requirement of infants < 2.5 kg birth weight as well as those with < 1.5 kg birth weight can be calculated by two methods. The first is a factorial method, which assumes that "the objective is to match the iron status of the breastfed term infant at a later time point (e.g., 1 year of age). Because the status of the two major compartments for iron, red blood cells and storage pools, is well-documented for the term infant during the first postnatal year, iron requirements for the preterm infant can be calculated using discharge weight, expected growth rates, and discharge haemoglobin and ferritin concentrations." The second method involves making guidelines based on previous studies on iron supplementation.

Assuming achieved weight of 7.5 kg at 6 months, for an infant with 2kg weight at birth, with approximate volume of blood - 80 mL/kg, and estimated tissue stores of iron of 7 mg/kg, the factorial methhod suggests that these stores of Iron would be used up by 3 months of age. Hence externally supplemented Iron required from 6 weeks to 24 weeks

would be 0.12 mg per kg each day. Considering the bioavailability of iron to be only 10%, the oral dose of iron required would be 0.12 x 10 i.e. 1.2 mg per kg per day.(62)

EARLY VS LATE IRON SUPPLEMENTATION

In a meta-analysis conducted by Hong-Xing Jin et al.(63), "preterm infants with birth weights between 1000 and 2000 g who receive iron supplementation at a dose of 2 mg/kg/day from 2 weeks of age tend to have less iron deficiency and require fewer red blood cell transfusions in the first 2 to 3 months of age when compared with unsupplemented preterm infants in the cohort". Initiating oral supplementation of Iron at 2 weeks of postnatal age at doses of 2 to 4 mg per kg body weight per day decreased the risk of ID and decreased the transfusion requirements by 30% in preterm babies weighing <1.3 kg at birth,in comparison to babies who received oral iron at 2 months onawards.(64) Iron supplementation seemed to have most positive affect in infants who have not been transfused > 2 weeks of postnatal age.(64) However, early iron administration neither prevents nor improves physiologic anaemia.(65)It is most beneficial when administered around time of onset of RBC production.(66) Even in non supplementaed infants, iron levels and Transferrin saturation reach a peak at 3weeks (67), and ferritin levels are high during the initial 6 weeks after birth.(68)

Lastly, in the neonatal period, absorption of iron across the GIT is weakly regulated hence leading to high iron levels in blood when higher concentration of supplements are administered.(69) A few studies support delaying enteral iron supplementation for four to eight weeks. However, this practise poses a significant risk of negative iron balance(69) in ELBW infants, the long-term consequences of which are unclear. Hence initiating supplementation of iron as early as 2 weeks seems to safe and effective in infants with birth weight <2.5 kg (LBW) who have not received blood transfusions.

PREVIOUS STUDIES

Few studies have examined role of iron supplementation in infants with marginally low birth weight. In **a 2010 study conducted by Berglund et al.**, "285 infants with birth weights between 2000 and 2500 g were randomly assigned to receive iron supplements [0 mg (placebo), 1 mg/kg/day, and 2 mg/kg/day] from 6 weeks to 6 months of age". At six months, a daily dose of 2 mg/kg significantly reduced the risk of iron deficiency anaemia (Iron deficiency Anemia) compared to placebo .(58) 36% of infants in the placebo group developed iron deficiency (ID), 10% of infants receiving 1 mg/kg/day developed ID and IDA, but only 4% of neonates in the group receiving 2 mg/kg body weight each day developed Iron Deficiency. Oral Iron at 1 or 2 mg/kg/day led to differences in iron status, but there was no significant difference in the proportion of infants who developed Iron deficiency or iron deficiency anemia, between the two groups. Supplemental iron had no negative effect on infant growth, infections, or other morbidity. The research found that a 0.25 mg/kg/day iron intake was sufficient to prevent IDA and that a 1 mg/kg/day iron intake prevented iron deficiency. (58)

In a **2013 follow-up study, Berglund et al.** followed up the infants enrolled in the 2010 study of derranged behavioural scores in infants who received placebo at 3.5 years of age, compared to those that received iron supplementation.(30) This study used a questionnaire (Achenbach Child Behaviour Checklist) and found "the prevalence of children with behavioural scores above the US subclinical cut-off was 12.7%, 2.9%, and

2.7% in the 0 mg/kg/day, 1 mg/kg/day, and 2 mg/kg/day groups, respectively, versus 3.2% in a reference group of children with normal birth weight. Adjusted for socioeconomic confounders, the risk of behavioural problems was 4.5 times higher (95% CI: 1.3–15.8) in the placebo group compared to the infants who received iron supplements." However, no statistically substantial variations in cognitive scores were noted.

In a RCT on "Early vs. Late enteral prophylactic iron supplementation in preterm very low birth weight infants"**by Rojo Joy, Sriram Krishnamurthy, Adhisivam Bethou, et al.**, 46 and 47 infants were assigned to the Early iron (EI) and Late iron (LI) groups, respectively, and their Serum Ferritin levels were measured at 2, 6, and 12 weeks postnatal age. At 12 weeks, the EI group had significantly higher serum ferritin levels. At 12 weeks, the concentrations of haemoglobin and MCH were also significantly higher in the EI group. On Comparing parameters at 2 weeks to 6 weeks, there was a "significant decrease in ferritin in the LI group and an increase in ferritin in the EI group at 6 weeks. Incidences of neonatal morbidities (necrotizing enterocolitis, periventricular leukomalacia, and retinopathy of prematurity), anthropometric parameters, and blood transfusion requirements did not differ significantly between the two groups".(70)

According to **the ESPGHAN recommendations**, iron supplementation at a dose of 1-2 mg/kg/day for the first six months of life is advised for infants with birth weights

between 2000 and 2500 grammes. (31) Additionally, after discharge, children with a birth weight between 1500 and 2000 g are advised to consume 2 mg per kg body weight of iron. Oral Iron or formula supplemented with iron should be continued for at least through 1st year of life, depending on the infant's diet. At following visits, haemoglobin and serum ferritin levels should be monitored and contrasted with age-specific reference levels. (26, 31, 71)

The results of a **2012 Cochrane review by Mills RJ and Davies MW,** which looked at 26 RCTs and quasi-controlled trials involving 2726 infants, showed that " oral iron supplementation improved Hb concentrations at 6 - 9 months of age and that dosages higher than the usual 2-3 mg/kg/day were not beneficial". (72)

In a cohort of Brazilian VLBW infants at 12 months of age adjusted for preterm, **Ferri et al' s observational study** found that "the risk of iron deficit (48%) and iron deficiency anaemia (26.5%) was significant." (72) Two risk factors included early cow milk drinking, which is known to have reduced iron content (relative risk = 1.7), and being small for gestational age at delivery (relative risk = 1.6). (73)

The iron levels of very low birth weight newborns (mean weight of birth = 1400 grams,fed iron-fortified formula vs infants fed solely human milk was compared in **2014 by Van de Lagemaat et al.** (66) "Up until three months of corrected age, the group received 2.7 mg/kg/day of total iron via formula and supplements, and 1.2

mg/kg/day up to six months of corrected age. When compared to LBW infants fed iron-fortified formula, a higher percentage of HM-fed babies had low ferritin levels (12 g/l) or high RDW (>14.5%) at the three and six-month follow-up visits". (66)

Griffin et al. (1999) examined the haemoglobin and ferritin levels in preterm neonates with mean birth weights under <1.4kg who were given formula feeds containing "1.17, 0.86, or 0.81 mg/kg/day of iron at 6 months (3 months of corrected gestational age)." (74) The three study groups showed "no statistically significant differences, and all three groups had haemoglobin concentrations >11 g/dL by 3 months corrected age. This developmental milestone was attained two months earlier in the group fed formula with a higher iron content than the other groups. Additionally, between 4 and 8 months of age, the percentage of ferritin that fell to levels below 10 ng/ml peaked at 14.8%, showing a negative iron balance in preterm children with mean birth weights below 1400 g". (74)

In 2001, Friel et al. looked at the effects of feeding 58 newborns with an average birth weight of <1.5kg neonates formulae containing 3-6 or 2-3 mg of iron/kg per day for nine months. (32) At 12 months, "there was no difference between the two groups in the incidence of anaemia or neurodevelopment. The group that consumed more iron, however, had higher levels of oxidative stress indicators like glutathione peroxidase, lower plasma concentrations of zinc and copper, and more frequent respiratory tract

infections, suggesting that the higher iron intake seemed to have negative effects."

According to **a 2003 study by Marriott et al.,** "VLBW infants with a mean birth weight of 1454 g who began weaning on a meal with greater iron had better haemoglobin levels at 6 months of age than the control group". (33)

Given that enteral iron absorption is only about 30%, a premature child would require a daily iron dose of 1.6-2.0 mg/kg intravenously(75) or 4-6 mg/kg orally. (76). However, this type of supplementation soon after birth is not physiologically necessary nor practicable. Nearly all newborns who are VLBW or LBW cannot acquire enteral nourishment. Growth and erythropoiesis stop immediately after birth, in contrast to intrauterine life. Under these circumstances, fewer people need iron. Similarly, factorial method estimations of daily needs (77) are probably not accurate. (78) Well-conducted, randomised, controlled trials are likely to be the most effective way for assessing the iron requirements of LBW newborns. These RCTs have shown that iron supplementation lowers the risk of iron insufficiency by the fortification of human milk, iron-fortified formula, and pharmaceutical iron. (78,79) The majority of research, however, have only looked at the short-term haematological advantages. It is unclear how long-term iron supplementation affects haematological and non-hematological characteristics like growth and neurodevelopment.

Early infancy iron supplementation was examined for both health advantages and

dangers in **2006 by Lora L. Iannotti et al**. The advantages and dangers of preventive oral iron supplementation in young children (aged 0-59 months) living in underdeveloped countries were examined by the authors of 26 randomised controlled trials. Investigations focused on anaemia, development, growth, morbidity, and mortality. Iron status and baseline haemoglobin levels were thought to be effect modifiers, although few research performed subgroup analyses. “Thirteen of the studies that were reviewed discovered that iron supplementation in early children significantly raised haemoglobin levels and decreased the prevalence of anaemia. Hematological parameters of Iron,such as serum iron, serum ferritin, transferrin saturation, and free erythrocyte protoporphyri,, improved in eleven investigations. Iron status markers improved in four of the five studies that revealed no discernible impact on haemoglobin levels. Children with iron deficiency and anaemia showed less cognitive and motor development abnormalities, especially with longer-duration, lower-dose regimens. Children with excess iron gained weight negatively as a result of iron supplementation, but their height was unaffected. Though few research had sufficient sample numbers or study designs to draw conclusions, the bulk of them did not find any effect on morbidity. The effect of oral iron supplementation on the outcome of infectious illness was assessed in 16 RCTs. An increase in dysentery episodes was noted in 49% of cases in Bangladesh that received oral iron supplements. According to a Tanzanian study, children with severe anaemia who got iron had a noticeably greater prevalence of pneumonia and an increase in infectious-cause mortality. While there were no effects on child mortality in Nepal, iron

supplementation was linked to an increase in major adverse outcomes in Zanzibar's malaria-endemic areas. There has to be more study done in areas where HIV and TB are prevalent. It may be required to identify iron-deficient kids in order to target iron supplementation in preventative programmes". (80)

"A double-masked, randomised control trial of iron supplementation in early infancy in term breastfed infants" who were healthy was carried out in **2003 by James K et al.** (81) The study's goal was to ascertain whether iron supplementation had an impact on the haematological, biochemical, and developmental status of fully developed breastfed infants. 77 term breastfed babies aged 1 to 6 months were randomly randomised to receive either a placebo or 7.5 mg/day of elemental iron in the form of ferrous sulphate. The group assignment was unknown to the families and the investigators. "The levels of ferritin, red cell superoxide dismutase, catalase, plasma ferric reducing antioxidant power, zinc, and copper in complete blood counts were assessed at ages 1, 3.5, 6, and 12 months. The Bayley mental and psychomotor developmental indices (MDI and PDI) and visual acuity (as measured by Teller acuity cards) were assessed in infants between the ages of 12 and 18 months. For the variance and t-test analyses, the intention to treat was examined."

The study concluded that "Iron supplementation improved haemoglobin and mean corpuscular volume at 6 months of age, and at 13 months of age, it significantly improved visual acuity and PDI (100 12 versus 93 9 [SD])." The inference made was

that there was no difference between the treatment and placebo groups in terms of anthropometric indices, compliance, biochemical state, or demographics. According to the study's findings, iron supplementation of breastfed newborns can be regarded as safe and may benefit some infants' haematological and developmental outcomes.

In **2011, Mishael K. Georgieff** and associates looked into "the long-term effects of early iron insufficiency on the brain and behaviour."(82) A significant amount of comparable human and animal research firmly supports the idea that early iron insufficiency leads to long-term neurobehavioral problems despite relatively quick detection and treatment. The problems happened in vital areas of the nervous system such myelination, energy metabolism, and dopamine metabolism, all of which are essential for the health of the adult brain. "Adult neurobehavioral dysfunction not only puts the individual at danger in terms of employment and educational success, but also for the coming generation."

In **2006, Betsy Lozoff et al.** investigated "the link between iron shortage and impaired brain growth. (83) Evidence from animal models suggests that early iron shortage affects neurotransmission and metabolism in important brain regions, including the hippocampus and basal ganglia, and disrupts the myelination process. Modified gene and protein profiles have also been discovered by recent research. When iron shortage is induced during pregnancy and/or breastfeeding, there are changes for each of these

systems before and after iron replenishment (brain growth spurt).” On the other hand, iron replacement during the third trimester of human pregnancy corrects regional and cerebral iron deficiencies and results in little functional abnormalities.

There is some evidence that iron deficiency anaemia in newborns between the ages of 6 and 24 months increases the risk of short-term cognitive, motor, social-emotional, and neuropsychological impairment in humans. Additionally, iron deficiency anaemia is frequently associated with worse long-term outcomes in this age range. Infant iron therapy does not always aid in development. The benefits of iron, notably on motor development and social-emotional behaviour, have been shown in recent large randomised trials of iron supplementation in impoverished countries. These findings indicate that iron supplementation early in development, or before iron deficiency anaemia becomes chronic or severe, may prevent and/or reverse negative effects. Prenatal iron insufficiency is a topic that is related to the timing of interventions. Recent research on fetal/neonatal iron shortage in infants of non-human primates and humans has shown worse outcomes, notably in the areas of neurocognition and social-emotional development.

The findings highlight the significance of protecting the developing brain against iron deficiency at a young age. Evidence that is consistent suggests that post-natal iron supplementation can reduce the prevalence of iron deficiency/iron deficiency anaemia and enhance developmental outcomes. Given the high global prevalence rates of iron

deficiency in pregnancy, intrauterine growth restriction, rising rates of gestational diabetes, and the global obesity epidemic, new findings from this study indicate the need for a greater focus on the fetal/neonatal iron deficiency's formative effects. According to new non-human primate models, maternal iron supplementation may help further protect the growth of the brain and behavioural systems by preventing fetal and neonatal iron insufficiency.

5. MATERIALS AND METHODS

5.1 STUDY DESIGN:

A randomized open label interventional study

5.2 STUDY PERIOD:

The study was conducted from December 2019 to July 2022

5.3 SOURCE OF DATA:

5.3.1 The study includes 96 Low Birth Weight infants delivered at Shri B M Patil MedicalCollege Hospital and Research Centre; 48 of whom received oral iron supplementation at 1- 2 weeks or as soon as the neonate tolerates full feeds. The other 48 babies enrolled as controls, did not receive any oral iron supplementation.

5.3.2 The infant's parents/ guardians were informed about the study in all respects and written informed consent was taken.

6. METHODOLOGY

6.1 Method of collection of Data (including sampling procedures if any):

This is a randomized open label interventional study in which 106 Low Birth Weight infants were enrolled, 53 of whom received Early Iron supplementation while the other 53 were enrolled as controls and did not receive any oral iron supplementation. The intervention used was administration of oral iron (Ferrous Fumarate), at 2mg/kg/dose of elemental iron PO given twice daily.

With a loss of 10 cases to follow up, 96 neonates (48 in each group) were assessed for hemoglobin level, RBC count and iron status indicators (PCV, Serum Ferritin) at or after 6 weeks of postnatal age.

6.2 SAMPLE COLLECTION

6.2.1 Oral and written consent was taken from the subjects prior to thecollection of samples.

6.2.2 A venous blood sample was collected at or before 2 weeks of postnatal age, when the neonate was able to tolerate full feeds i.e. before starting oral iron supplementation.

6.2.3 The second venous blood sample was drawn at or after 6 weeks of postnatal age.

INCLUSION CRITERIA:

- Low Birth Weight infants: 1.5 -2.5 kg ;
- Tolerating full feeds at 1-2 week of postnatal age;
- Born to mothers (irrespective of mother's iron store status) via any mode of delivery and
- Do not have severe anemia at birth or history of transfusions

EXCLUSION CRITERIA:

- Infants with Hb level <12 mg/dl at birth
- Infants with gross anomalies and Rh hemolytic disease

SAMPLE SIZE:

On the basis of the study: "Early iron supplementation in very low birth weight infants – a randomized controlled trial by Mari Jeeva Sankar, Renu Saxena, Kalaivani Mani, RameshAgarwal, Ashok K Deorari, Vinod K Paul"(7);

The anticipated Mean±SD of Early Iron and control group is 50.8±11.5 and 45.3±11.9 resp.

The minimum sample size is 48 per group (minimum total Sample size= 96) with 95% level of significance and 80% power. The formula used is:

$$N = 2\left[\frac{(Z_a + z_\beta) * S}{d}\right]^2$$

Z_a Level of significance=95%

Z_β--power of the study=90%

d=clinically significant difference between two

parametersSD= Common standard deviation

Sample size + drop out/loss of follow up of 10%= 53 per group

Though calculated sample size was 53 in each group, there was loss of follow up of 10 cases (5 in each group). Hence total sample collected was 96 (48 in each group).

Statistical analysis:

Numerical variables were presented as Mean ±SD, and categorical variables were presented as frequency(%) and diagrams

Comparison of numerical variables between groups was found using unpaired t test/Mann whitney U test ,and categorical variables by Chi square or Fisher's Exact test.

To compare results within group Paired t test/ Wilcoxon signed rank test were used and for categorical values Chi square or Fischer's Exact test were used.

OBSERVATIONS AND RESULTS

Table 1) Distribution of sample size between cases and controls

Group Description	No. of cases	% of cases
Cases	48	50.0
Controls	48	50.0
TOTAL	**96**	**100.0**

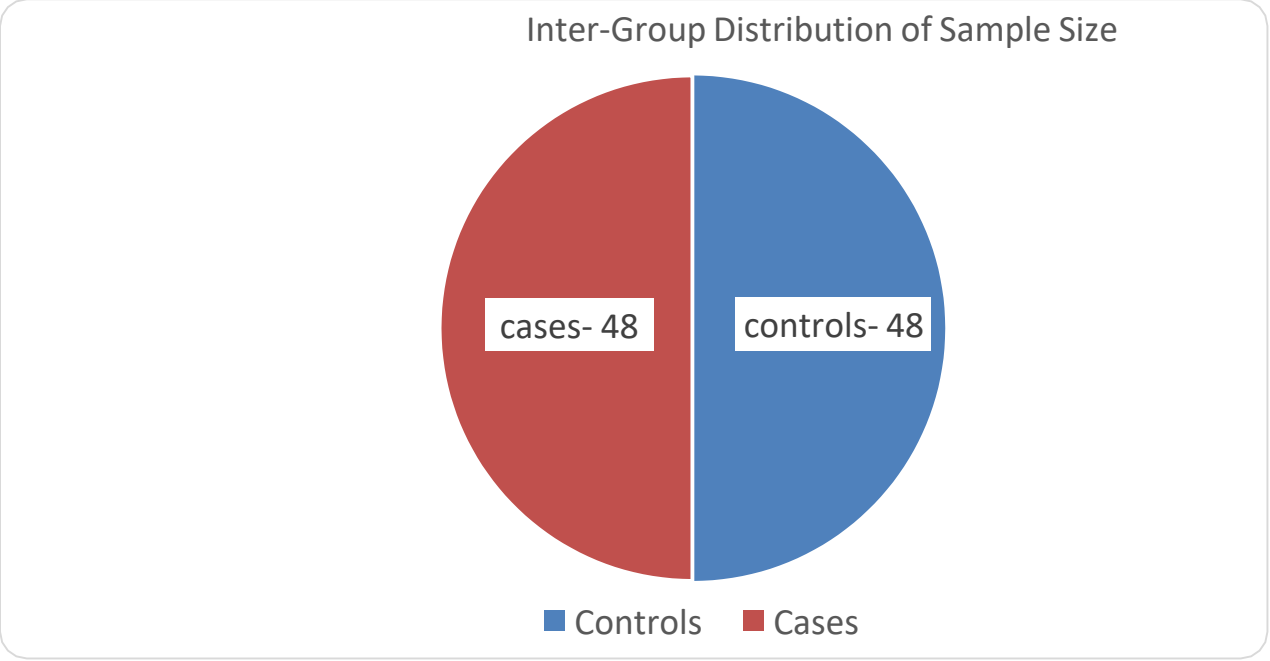

Figure 1)) Distribution of sample size between cases and controls

Table 2) Comparison of mean gestational age across study groups

Gestational Age (Weeks)	**[Cases] (n=48)**		**[Controls] (n=48)**		**P-value**
	Mean	**SD**	**Mean**	**SD**	
Gestational Age (Weeks)	36.05	1.68	36.90	1.45	0.009**
«Values are mean and SD, P-value by independent sample t test. P-value<0.05 is considered to be statistically significant. **P-value<0.01.»					

Comparison of mean gestational age across cases and controls

The mean ± SD of gestational age among cases and controls was 36.05 ± 1.68 weeks and 36.90 ± 1.45 weeks respectively. The minimum – maximum gestational age range was 30.86 – 38.71 weeks among Cases and 34.00 – 39.00 weeks among the Controls.

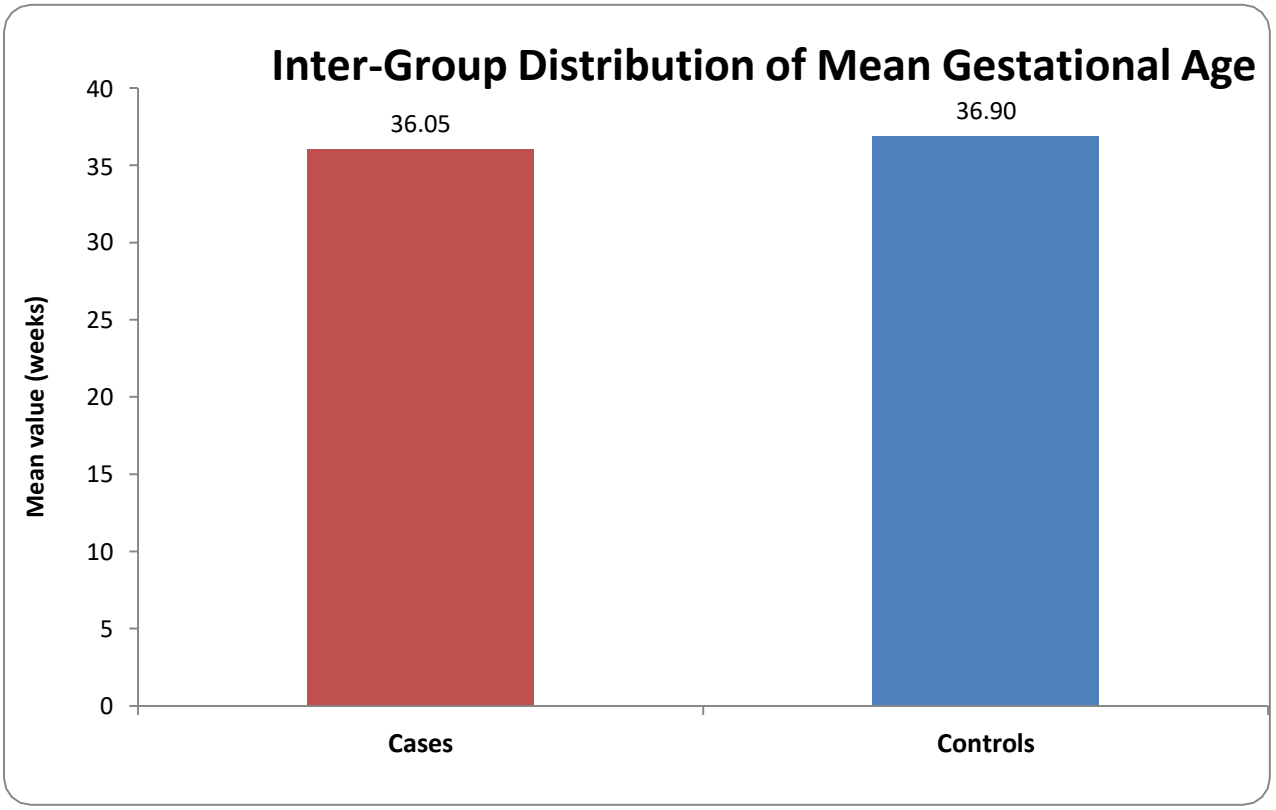

Figure 2) Comparison of mean gestational age across study groups.

Table 3) Distribution of sex of the infants across study groups

Sex	[Cases] (n=48)		[Controls] (n=48)		P-value
	N	%	n	%	
Male	26	54.2	24	50.0	0.683[NS]
Female	22	45.8	24	50.0	
Total	**48**	**100.00**	**48**	**100.0**	
«Values are n (% of cases), P-value by Chi-Square test. P-value<0.05 is considered to be statistically significant. NS – Statistically non-significant.»					

distribution of sex of the infants

Of 48 cases who received iron supplementation, 26 (54.2%) were male and 22 (45.8%) were female. Of the 48 controls, 24 (50.0%) were male and 24 (50.0%) were female.

Sex distribution of the enrolled infants did not vary significantly between cases and controls (P-value>0.05).

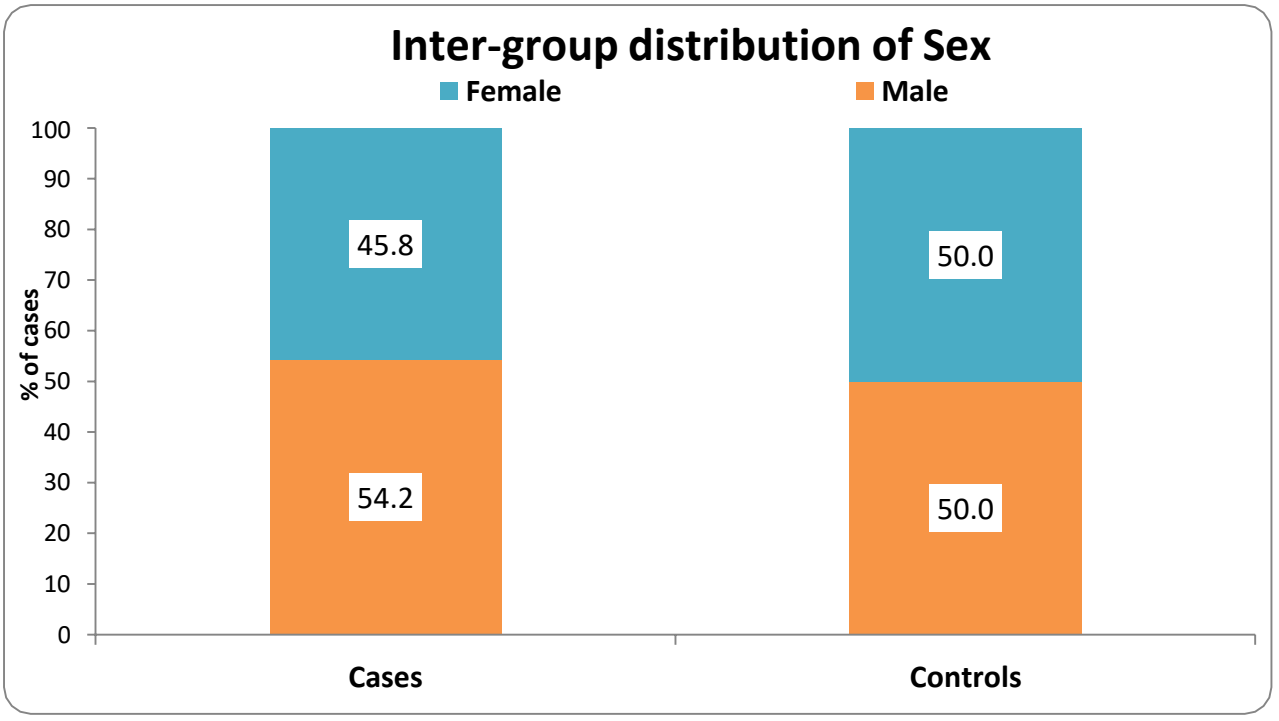

Figure 3)) Distribution of sex of the infants across study groups

Table 4) Inter-group comparison of mean birthweight.

Birthweight (kg)	**Cases (n=48)**		**Controls (n=48)**		**P-value**
	Mean	**SD**	**Mean**	**SD**	
Birthweight (kg)	2.16	0.26	2.19	0.24	0.630^{NS}
«Values are mean and SD, P-value by independent sample t test. P-value<0.05 is considered to be statistically significant. NS – Statistically non-significant.»					

Inter-group comparison of mean birthweight

The mean ± SD of birthweight in cases and controls was 2.16 ± 0.26 kg and 2.19 ± 0.24 kg respectively. The minimum – maximum birthweight range in cases and controls was 1.50 – 2.50 kg and 1.50 – 2.80 kg respectively.

The mean birthweight did not differ significantly between two study groups (P-value>0.05).

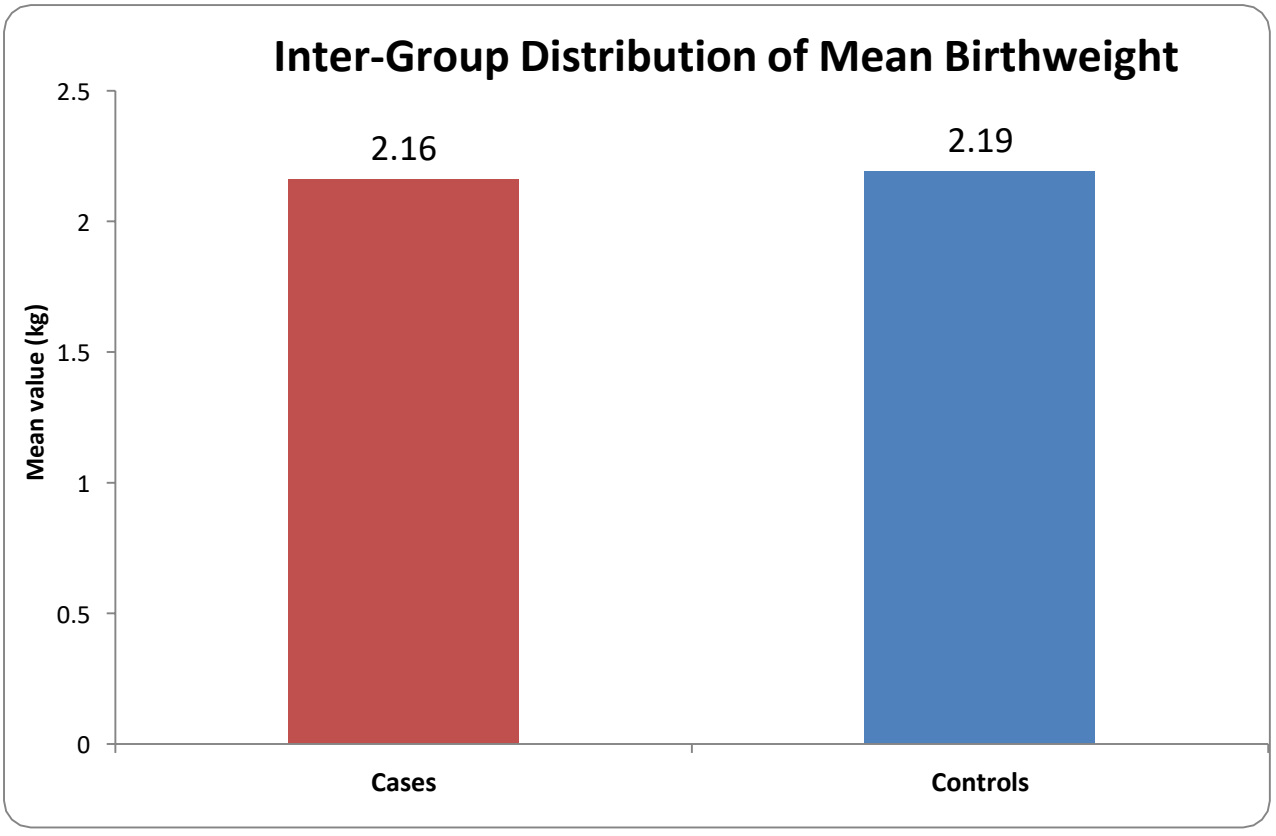

Figure 4) Inter-group comparison of mean birthweight.

Table 5) Inter-group distribution of mode of delivery.

Mode of delivery	Cases (n=48)		Controls (n=48)		P-value
	N	%	n	%	
Normal vaginal	10	20.8	16	33.3	0.168[NS]
LSCS	38	79.2	32	66.7	
Total	**48**	**100.00**	**48**	**100.0**	
«Values are n (% of cases), P-value by Chi-Square test. P-value<0.05 is considered to be statistically significant. NS – Statistically non-significant.»					

5. Inter-group distribution of mode of delivery

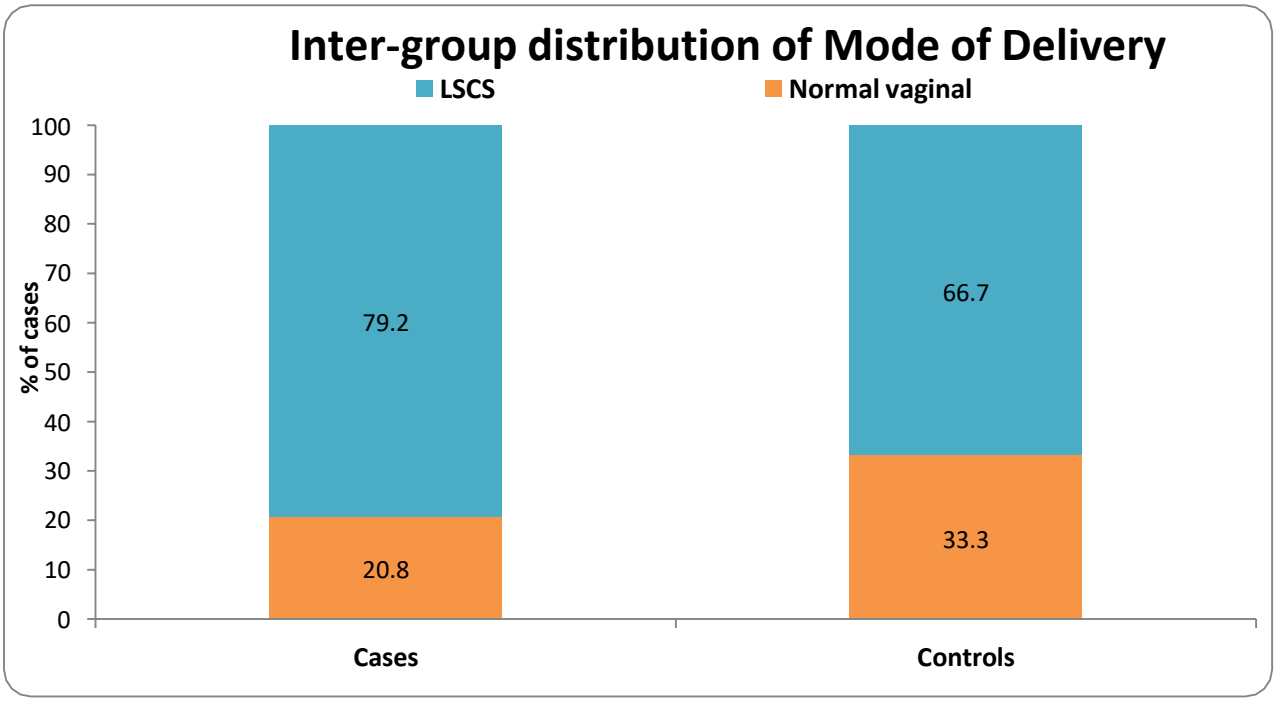

Figure 5) Inter-group distribution of mode of delivery.

Of the 48 cases who received Iron supplementation, 10 (20.8%) had normal vaginal mode of delivery and 38 (79.2%) had LSCS mode of delivery. Of 48 controls, 16 (33.3%) had normal vaginal mode of delivery and 32 (66.7%) had LSCS mode of delivery.

Distribution of mode of delivery did not differ significantly between two study groups (P-value>0.05).

Table 6) Inter-group and intra-group comparison of mean Hb levels.

	Cases (n=48)		**Controls (n=48)**		**P-value [Inter-group]**
Hb (gm%)	**Mean**	**SD**	**Mean**	**SD**	
Pre (0 – 2 weeks)	17.36	3.14	16.86	2.46	0.391^{NS}
Post (6 weeks)	12.68	1.51	11.38	1.03	0.001^{***}
% Change at 6-weeks	25.19%	--	31.45%	--	0.008^{**}
P-value [Intra-group]					
Pre vs Post	0.001^{***}		0.001^{***}		

«Values are mean and SD, P-value [Inter-group] by independent sample t test and P-value [Intra-group] by paired t test. P-value<0.05 is considered to be statistically significant. **P-value<0.01, ***P-value<0.001, NS – Statistically non-significant.»

6.1. Intra-group comparison of mean Hb levels

The mean ± SD of Pre-intervention Hb levels and Post-intervention Hb levels in Cases was 17.36 ± 3.14 gm% and 12.68 ± 1.51 gm% respectively. Distribution of mean Post-intervention Hb levels is significantly lower compared to mean Pre-intervention Hb levels in Cases (P-value<0.05).

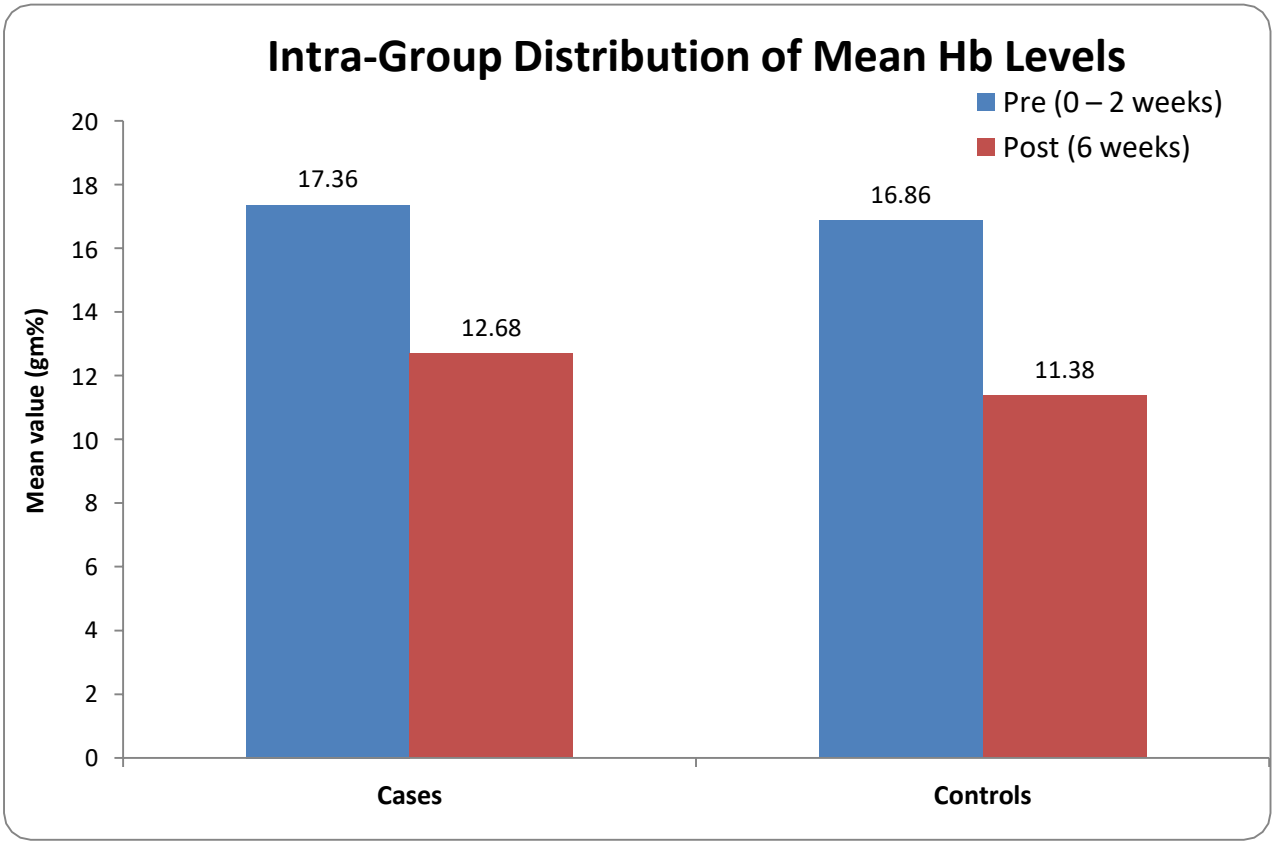

Figure 6.1) Intra-group comparison of mean Hb Levels

The mean ± SD of Pre-intervention Hb levels and Post-intervention Hb levels in Controls was 16.86 ± 2.46 gm% and 11.38 ± 1.03 gm% respectively. Distribution of mean Post-intervention Hb levels is significantly lower compared to mean Pre-intervention Hb levels in Controls (P-value<0.05).

Thus it can be said a significant fall in Hb levels is noted at follow up in both Cases and controls (p <0.005).

6.2. Inter-group comparison of mean Hb levels

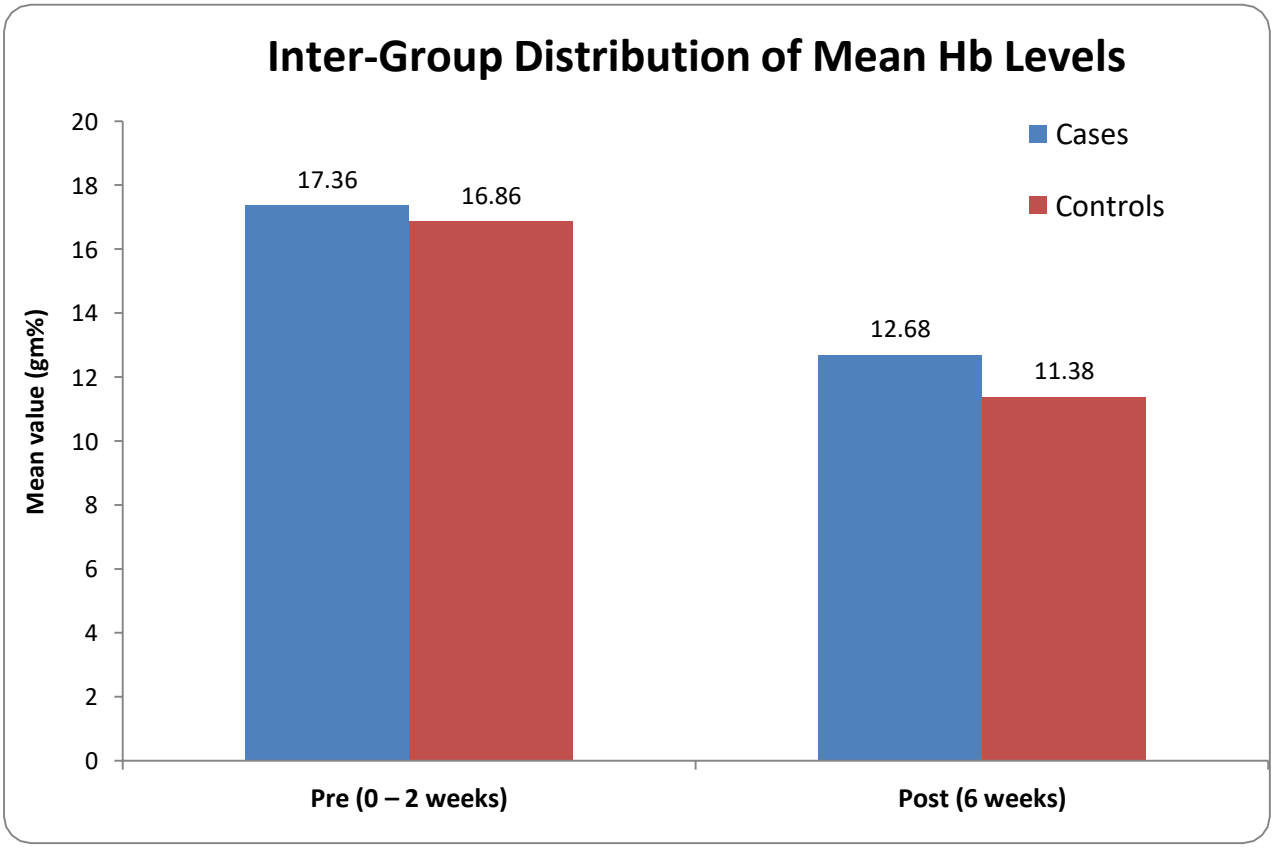

Figure 7.1) Inter-group comparison of mean Hb Levels

The mean ± SD of Pre-intervention Hb levels in Cases and Controls was 17.36 ± 3.14 gm% and 16.86 ± 2.46 gm% respectively. Distribution of mean Pre-intervention Hb levels did not differ significantly between two study groups (P-value>0.05).

The mean ± SD of Post-intervention Hb levels in Cases and Controls was 12.68 ± 1.51 gm% and 11.38 ± 1.03 gm% respectively i.e. higher Hb levels were seen in iron supplemented neonates at the ≥6 week follow up. This difference in mean Hb levels at 6 weeks follow up, between cases and controls was statistically significant. (P-value<0.05).

The mean % change in Hb levels in Cases and Controls at the Post-intervention follow-up was 25.19% and 31.45% respectively

This indicates a significantly larger drop in Hb levels among the neonates not receiving Iron supplememtation (P-value<0.05)

Table 7) Inter-group and intra-group comparison of median Serum Ferritin levels.

	Cases (n=48)		**Controls (n=48)**		**P-value [Inter-group]**
Serum Ferritin (ng/ml)	**Mean**	**SD**	**Mean**	**SD**	
Pre (0 – 2 weeks)	277.91	136.4 – 1116.6	263.06	53.4 – 707.4	0.835^{NS}
Post (6 weeks)	238.45	54.8 – 630.5	175.68	39.8 – 443.6	0.045^{*}
% Change at 6-weeks	15.13%	--	32.99	--	0.001^{***}
P-value [Intra-group]					
Pre vs Post	0.001^{***}		0.001^{***}		

«Values are median and min - max, P-value [Inter-group] by Mann-Whitney U test, P-value [Intra-group] by Wilcoxon's signed rank test. P-value<0.05 is considered to be statistically significant. *P-value<0.05, ***P-value<0.001, NS – Statistically non-significant.»

7.1. Intra-group comparison of median Serum Ferritin levels

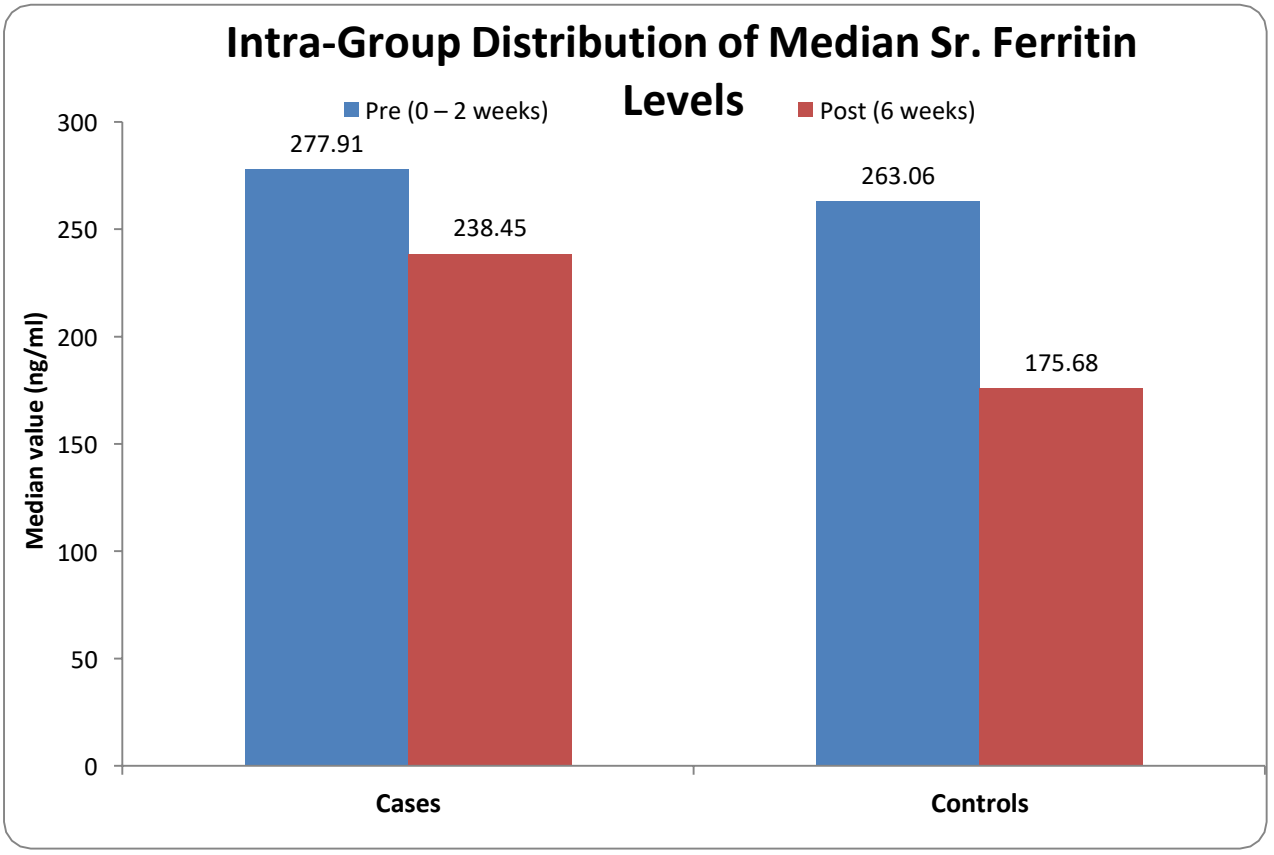

Figure 7.1) Intra-group comparison of mean Serum Ferritin Levels.

The median Pre-intervention Sr Ferritin levels and Post-intervention Sr Ferritin levels in Cases was 277.91 ng/ml and 238.45 ng/ml respectively. Distribution of median Post-intervention Sr Ferritin levels is significantly lower compared to mean Pre-intervention Sr Ferritin levels in Cases (P-value<0.05).

The median Pre-intervention Sr Ferritin levels and Post-intervention Sr Ferritin levels in Controls was 263.06 ng/ml and 175.68 ng/ml respectively. The median Post-intervention Sr Ferritin levels is significantly lower compared to mean Pre-intervention Sr Ferritin levels in Controls (P-value<0.05).

This shows that a significant fall in ferritin level is noted in both iron supplemented and non-supplemented babies.

7.2. Inter-group comparison of median Serum Ferritin levels

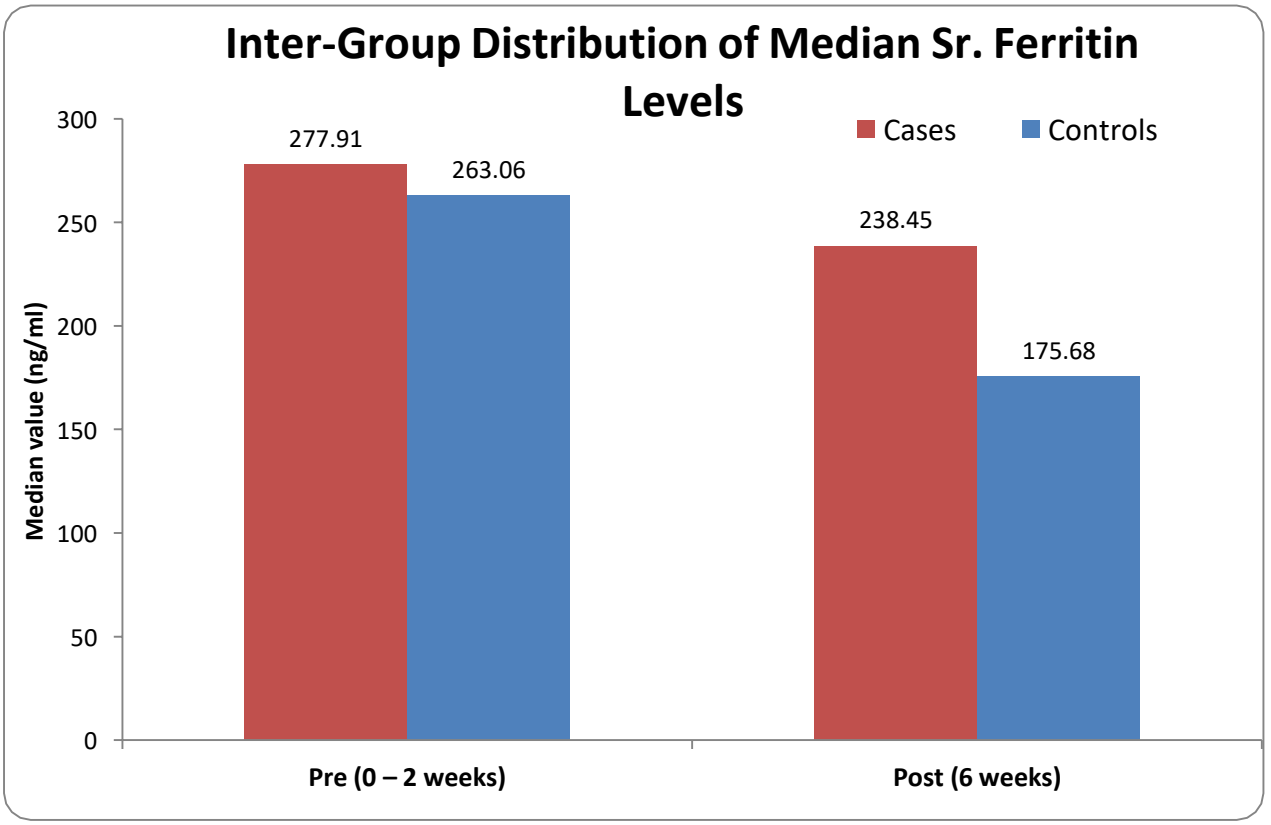

Figure 7.2) Inter-group comparison of median Serum Ferritin Levels.

The median Pre-intervention Sr Ferritin levels in Cases and Controls was 277.91 ng/ml and 263.06 ng/ml respectively. Distribution of median Pre-intervention Hb levels did not differ significantly between two study groups (P-value>0.05).

The median Post-intervention Sr Ferritin levels in Cases and Controls was 238.45 ng/ml and 175.68 ng/ml respectively. The median Post-intervention Sr Ferritin level is significantly higher in Cases compared to Controls (P-value<0.05).

The median % change in Sr Ferritin levels in Cases and Controls at the Post-intervention follow-up was 15.13% and 32.99% respectively. The median Post-intervention % change in Sr Ferritin levels is significantly lower in Cases compared to Controls (P-value<0.05).

Table 8) Inter-group and intra-group comparison of mean RBC count.

	Cases (n=48)		**Controls (n=48)**		**P-value [Inter-group]**
RBC count	**Mean**	**SD**	**Mean**	**SD**	
Pre (0 – 2 weeks)	4.91	0.85	4.79	0.67	0.473^{NS}
Post (6 weeks)	3.94	0.86	3.16	0.51	0.001^{***}
% Change at 6-weeks	19.21%	--	33.82%	--	0.001^{***}
P-value [Intra-group]					
Pre vs Post	0.001^{***}		0.001^{***}		
«Values are mean and SD, P-value [Inter-group] by independent sample t test and P-value [Intra-group] by paired t test. P-value<0.05 is considered to be statistically significant. **P-value<0.01, ***P-value<0.001, NS – Statistically non-significant.»					

8.1. Intra-group comparison of mean RBC count

The mean ± SD of Pre-intervention RBC count and Post-intervention RBC count in Cases was 4.91 ± 0.85 and 3.94 ± 0.86 respectively. The mean Post-intervention RBC count is significantly lower compared to mean Pre-intervention RBC count in Cases (P-value<0.05).

The mean ± SD of Pre-intervention RBC count and Post-intervention RBC count in Controls was 4.79 ± 0.67 and 3.16 ± 0.51 respectively. Distribution of mean Post-intervention RBC count is significantly lower compared to mean Pre-intervention RBC count in Controls (P-value<0.05).

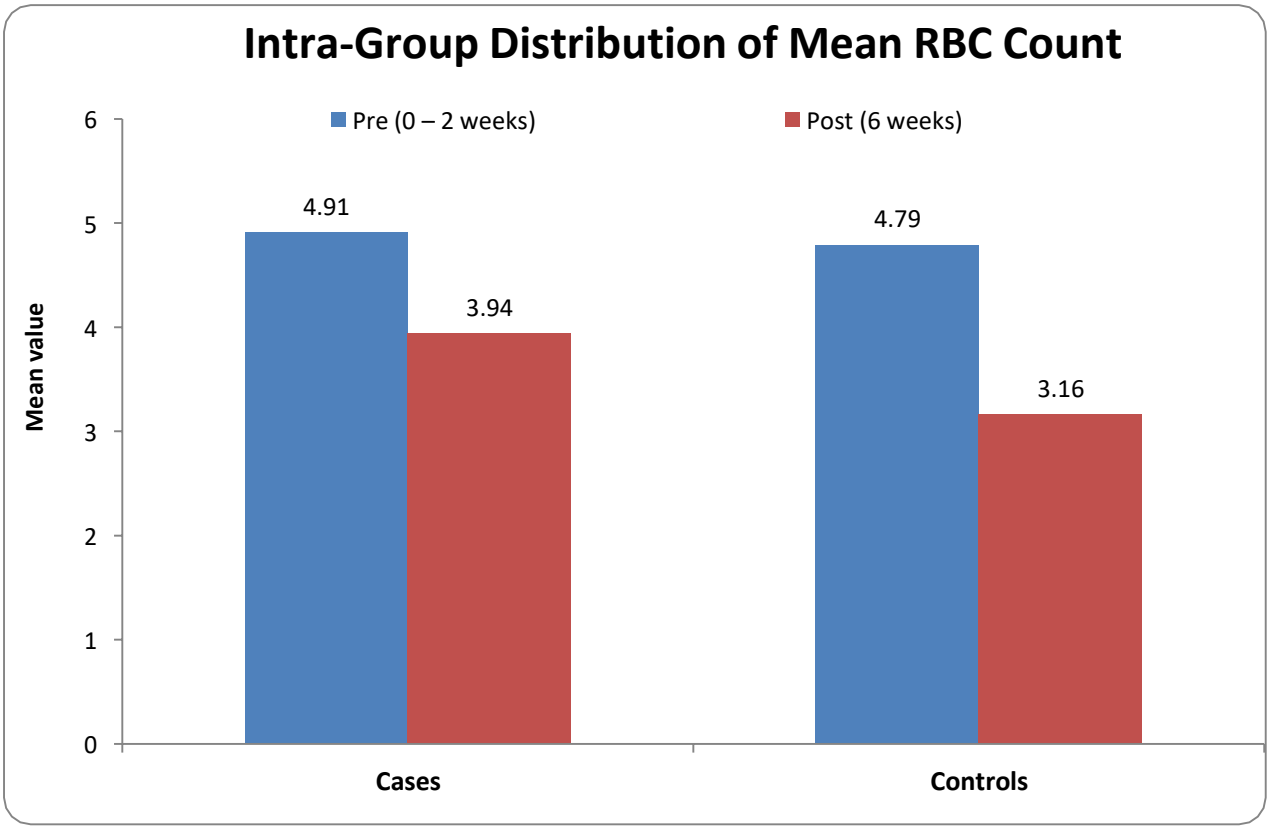

Figure 8.1) Intra-group comparison of mean RBC count.

There is a significant drop in the RBC count in both the groups at 6 week follow up.

8.2. Inter-group comparison of mean RBC count

The mean ± SD of Pre-intervention RBC count levels Cases and Controls was 4.91 ± 0.85 and 4.79 ± 0.67 respectively. Distribution of mean Pre-intervention RBC count did not differ significantly between two study groups (P-value>0.05).

The mean ± SD of Post-intervention RBC count in Cases and Controls was 3.94 ± 0.86 and 3.16 ± 0.51 respectively. Distribution of mean Post-intervention RBC count is significantly higher in Cases compared to Controls. (P-value<0.05).

The mean % change in RBC count in Cases and Controls at the Post-intervention follow-up was 19.21% and 33.82% respectively. The mean Post-intervention % change in RBC count is significantly lower in Cases compared to Controls (P-value<0.05).

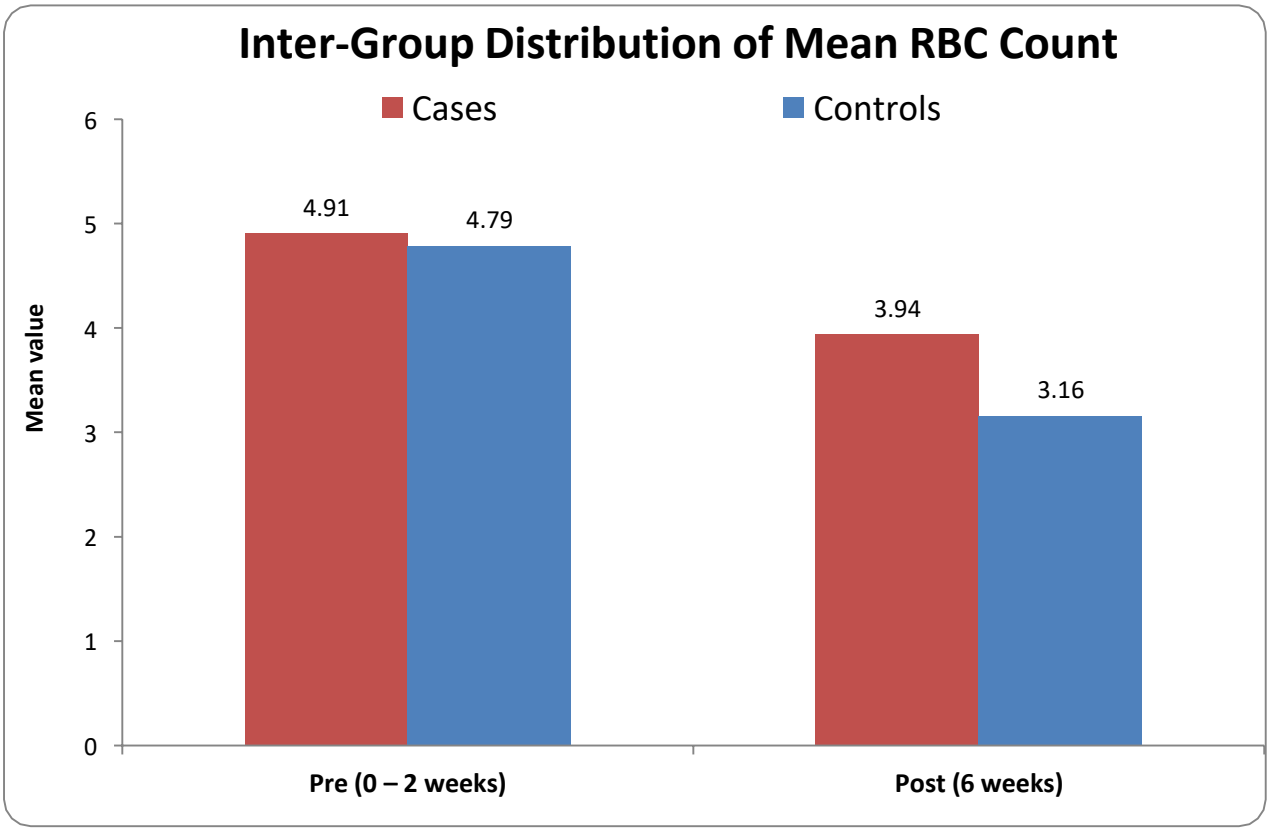

Figure 8.1) Inter-group comparison of mean RBC count.

The fall in the RBC count at the follow up among cases is significantly lower than the fall in RBC count among the controls at follow up.

Table 9) Inter-group and intra-group comparison of mean Hematocrit levels.

	Cases (n=48)		**Controls (n=48)**		**P-value [Inter-group]**
Hematocrit levels (%)	**Mean**	**SD**	**Mean**	**SD**	
Pre (0 – 2 weeks)	52.03	9.60	51.11	7.99	0.609^{NS}
Post (6 weeks)	33.16	3.75	34.04	3.45	0.234^{NS}
% Change at 6-weeks	34.32%	--	32.43%	--	0.402^{NS}
P-value [Intra-group]					
Pre vs Post	0.001^{***}		0.001^{***}		

«Values are mean and SD, P-value [Inter-group] by independent sample t test and P-value [Intra-group] by paired t test. P-value<0.05 is considered to be statistically significant. ***P-value<0.001, NS – Statistically non-significant.»

9.1. Intra-group comparison of mean Hematocrit levels

The mean ± SD of Pre-intervention Hematocrit levels and Post-intervention Hematocrit levels in Cases was 52.03 ± 9.60 % and 33.16 ± 3.75 % respectively. Distribution of mean Post-intervention Hematocrit levels is significantly lower compared to mean Pre-intervention Hematocrit levels among Cases (P-value<0.05).

The mean ± SD of Pre-intervention Hematocrit levels and Post-intervention Hematocrit levels in Controls was 51.11 ± 7.99 % and 34.04 ± 3.45 % respectively. The mean Post-intervention Hematocrit levels is significantly lower compared to mean Pre-intervention Hematocrit levels among Controls (P-value<0.05).

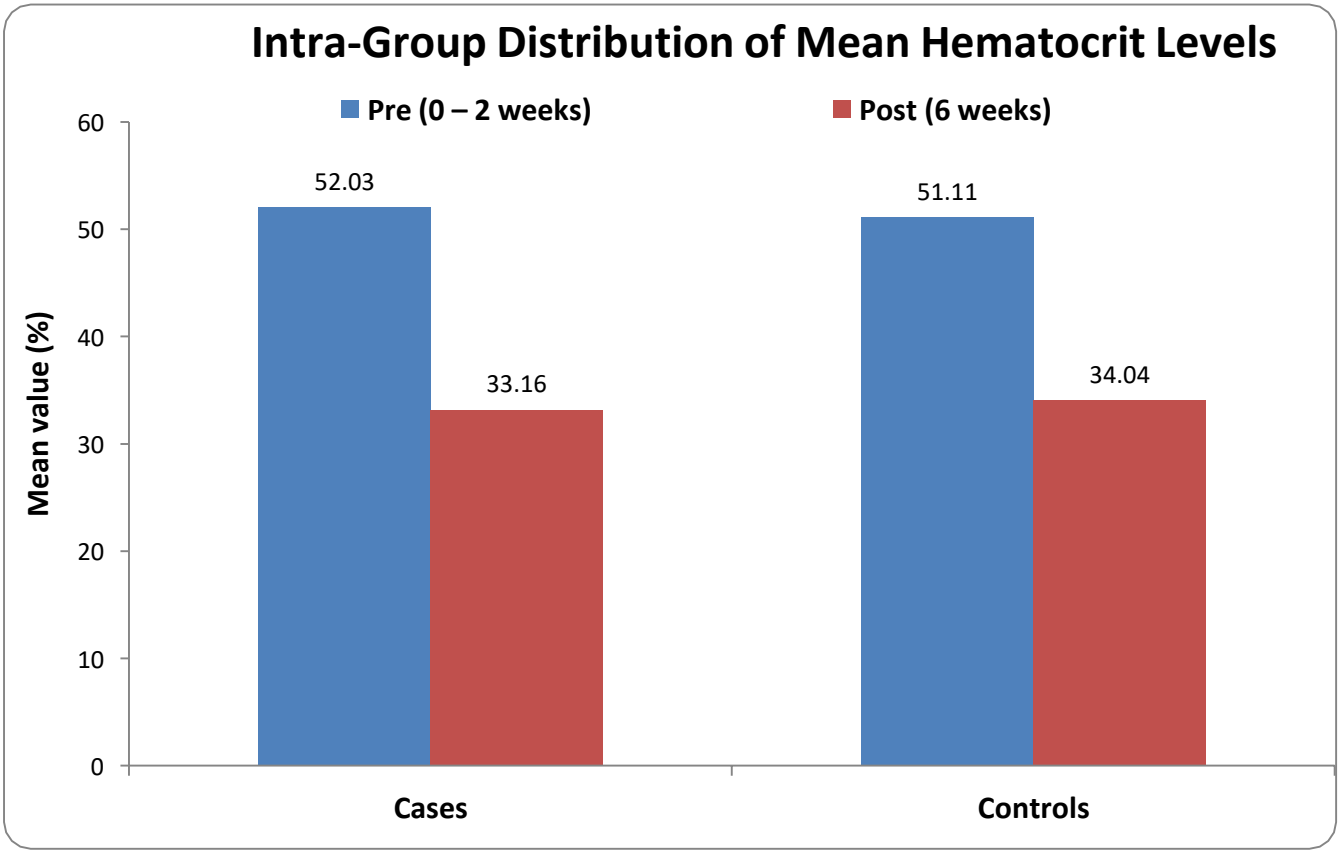

Figure 9.1) Intra-group comparison of mean Hematocrit levels.

9.2 Inter-group comparison of mean Hematocrit levels

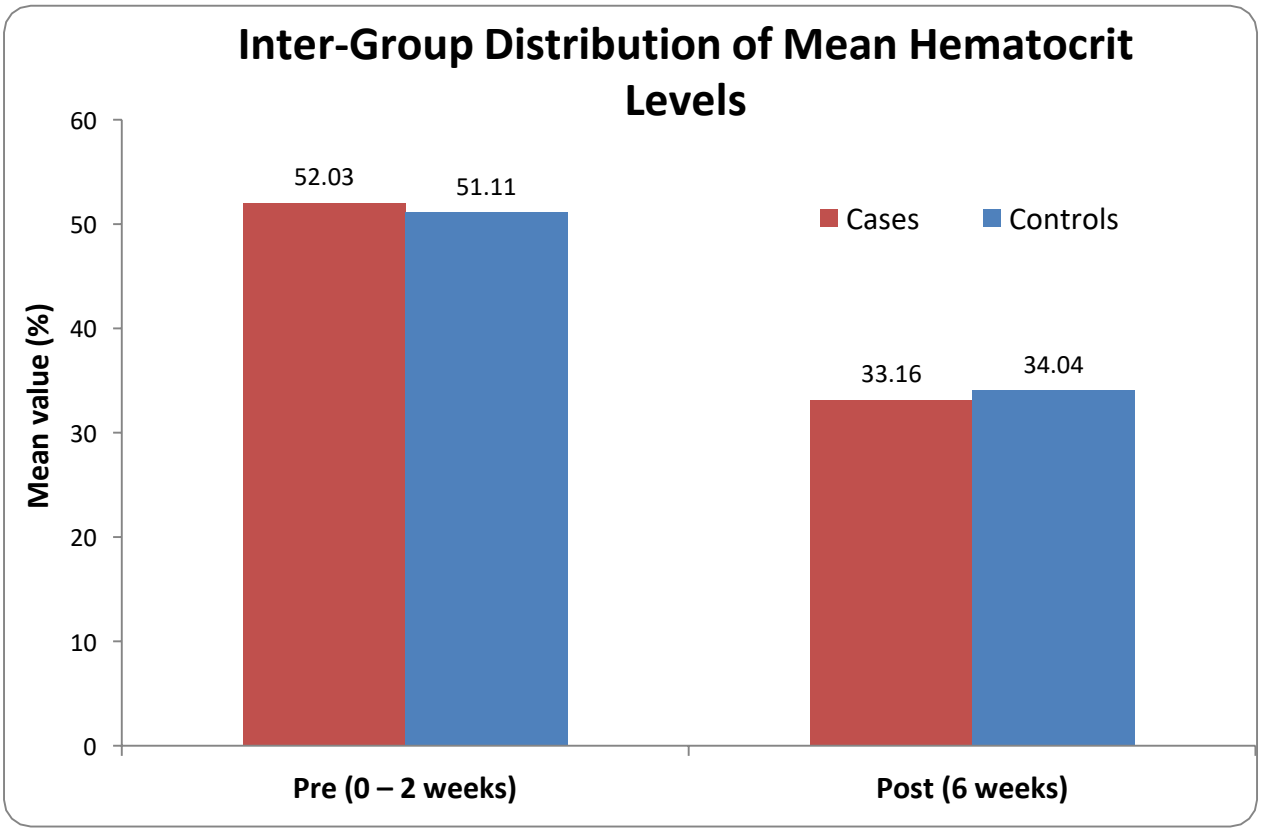

Figure 9.2) Inter-group comparison of mean Hematocrit levels.

The mean ± SD of Pre-intervention Hematocrit levels in Cases and Controls was 52.03 ± 9.60 and 51.11 ± 7.99 respectively. The mean Pre-intervention Hematocrit levels did not differ significantly between two study groups (P-value>0.05).

The mean ± SD of Post-intervention Hematocrit levels in Cases and Controls was 33.16 ± 3.75 % and 34.04 ± 3.45 % respectively. Distribution of mean Post-intervention Hematocrit levels did not differ significantly between two study groups (P-value>0.05).

The mean % change in Hematocrit levels in Cases and Controls at the Post-intervention follow-up was 34.32% and 32.43% respectively. Distribution of mean Post-intervention % change in Hematocrit levels did not differ significantly between two study groups (P-value>0.05).

10. Inter-group distribution of incidence of morbidity

Of 48 Iron supplemented cases , none had any incidence of any morbidity. Of 48 controls, none had any incidence of morbidity.

Distribution of incidence of morbidity did not differ significantly between two study groups (P-value>0.05).

11. Inter-group distribution of incidence of blood transfusion.

Of 48 babies enrolled as cases, none required blood transfusion. Of 48 babies among the controls, none required blood transfusion.

Incidence of requirement of blood transfusion did not differ significantly between two study groups (P-value>0.05).

DISCUSSION

Iron is a vital micronutrient in numerous cellular processes and functions, including developmental and growth processes. In addition to other micronutrients, it is believed that infants must have a sufficient amount of iron.(84) Low birth weight infants, including preterm infants, are particularly prone to developing iron deficiency anaemia (IDA) due to their relatively lower iron stores at birth and a higher iron requirement due to rapid increase in vascular cell mass than term infants.(85) Increased destruction of RBCs, a relatively short red blood cell life span, decreased EPO levels in circulation, frequent blood sample collection, and loss of blood linked with surgical and medical procedures may very well contribute to anaemia in infants < 2.5kg and those born prematurely.

Due to the high risk of iron deficiency anemia, supplementing iron in these babies is of utmost importance.(86) Early iron supplementation would possibly increase iron reserves and inhibit their depletion. However, previous research (87) by Franz *et al* notes that when iron supplements were given to neonates as soon as >100ml feed/kg each day were tolerated, the serum ferritin levels at 61 days in these infants were not statistically different from those who weren't given iron supplementation. The study also concluded that group of infants in whom the supplementation was delayed by 61 days (LI -late iron supplementation) had more incidence of Iron deficiency (26/65 vs 10/68) and also transfusion requirements at >14 days of life was higher in this LI group. Similar

contradicting results were noted in a few other studies. Therefore, we designed a randomised open label study to determine whether iron supplementation starting 2 weeks of life or earlier when full feeds are tolerated, in low birth weight (LBW) infants would increase the hematologic indicators of iron status and iron stores (serum ferritin) at 6 weeks of postnatal age.

In the present study, LBW infants were administered 2mg/kg/dose BD of oral iron. The pre-intervention values of mean haemoglobin, mean serum ferritin, mean RBC count, and mean hematocrit did not differ significantly between the two groups.

The post-intervention values of mean haemoglobin (*CASES* Hb: 12.68 ± 1.51, $\text{CONTROLS} - 11.38 \pm 1.03$), mean serum ferritin (*CASES*: 238.45, CONTROLS-175.68), mean RBC count (*CASES*: $3.94 \pm 0.86, CONTROLS\ 3.16 \pm 0.51$), and mean hematocrit (*CASES*: $33.16 \pm 3.75, CONTROLS\ 34.04 \pm 3.45$), were considerably lower than the pre-intervention values

(mean Hb: *CASES* $17.36 \pm 3.14, CONTROLS\ 16.86 \pm 2.46$;

mean sr. ferritin $CASES: 277.91, CONTROLS\ 263.06$; mean RBC count: $CASES\ 4.91 \pm 0.85, CONTROLS\ 4.79 \pm 0.67$, mean hematocrit $CASES\ 52.03 \pm 9.60$,

$CONTROLS\ 51.11 \pm 7.99$) in both cases and control groups, indicating a drop in their levels regardless of supplementation.

The infants who received **early iron supplementation had significantly higher haemoglobin, serum ferritin, and RBC levels at the 6-week follow-up** than those who did not receive any supplementation.

[% change at 6 weeks follow up- mean Hb: *CASES* 25.19%, *CONTROLS* 31.45% (*p value:*<0.01); mean ferritin: *CASES* 15.13%, *CONTROLS* 32.99%(*p value:*<0.001); mean RBC count: *CASES* 19.21%, *CONTROLS* 33.82% (*p value:*<0.001)] Indicating that, despite the fact that iron store status and haematological parameters decrease in both groups, the levels are comparatively higher in the cases compared to the controls, and iron store status is better in iron-supplemented infants. The percentage % decline of each of these values was significantly greater among the controls. Babies who received early iron supplementation had improved haematological parameters and iron stores at 6 weeks. There was no statistically significant difference between the pre-intervention and post-intervention values for the mean hematocrit levels(% change in mean heamtocrit: CASES 34.32%, CONTROLS 32.43 (*p value:>0.05 not significant*). Recent research did not find any indication of morbidity, such as NEC/ROP, in infants receiving early iron supplementation. None of the infants in either group were transfused with blood.

Joy R et al		**EI group At 2 weeks**	**EI group at 6 weeks**	**EI group at 12 weeks**	**LI group at 12 weeks**
	Hemoglobin (g/dl)	12.9 ± 0.8	Not mentioned	10.1± 0.4	9.2 ± 0.4
	Serum ferritin (*n*g/mL)	112 ± 5	130 ±4	82 ± 5	63 ± 3
Our study					**CONTROLS At 6 weeks**
	Hemoglobin (g/dl)	17.36 ± 3.14	12.68 ± 1.51	NA	11.38± 1.03
	Serum ferritin (*n*g/mL)	277.91 (136.4 - 1116.6)	238.45 (54.8 – 630.5)	NA	175.68 (39.8 – 443.6)

Our results were similar to a study conducted by Joy R et al in 2013, (88) which evaluated "the effect of early iron supplementation vs late iron supplementation in low birth weight infants". Though our study did not include comparison with a late iron supplementation group, the results of early iron supplementation followed up at ≥6 weeks were similar to the results noted in the EI (early iron) group of this study.

In the study by Joy R et al, "the serum ferritin levels initially improved at 6 weeks (130 ±4) in the EI group compared to the 2 weeks values (112 ± 5). The ferritin levels dropped at the 12 weeks follow up (82 ± 5)" most likely denoting the increased utilization of iron stores for post natal catch up growth. We noted a similar drop in serum ferritin values at the 6 week follow up [238.45 (54.8 -630.5)] in our study compared to the 2 weeks values [277.91 (136.4 - 1116.6)]

Joy R et al noted that inspite of the drop in ferritin levels at 12 weeks(82 ± 5) and drop in hemoglobin at 12 weeks (10.1± 0.4), these levels were still better when compared to LI (Late Iron supplementation) group values of serum ferritin(63 ± 3) and hemoglobin (9.2 ± 0.4) at 12 weeks , indicating a statistically improved iron store status in the early iron supplementation group.

Similar findings of improved iron store status at 6 weeks [238.45 (54.8 – 630.5)] and improved hemoglobin levels (12.68 ± 1.51) were noted among the cases in our study when compared to controls at 6 weeks [ferritin 175.68(39.8-443.6); hemoglobin- (11.38 ± 1.03)].

The incidence of neonatal morbidities (NEC, ROP, or periventricular leukomalacia) and requirement of blood transfusions did not vary significantly between the two groups. This finding is also similar to our outcome of similar incidence of morbidity and transfusion requirements between the cases and controls.

Hence, oral iron supplementation at 2mg/kg twice daily started at 2 weeks of age or earlier when baby tolerates full feeds, leads to improved hematological parameters and iron store status in LBW infants and reduces the risk of Iron deficiency/iron deficiency anemia in low birth weight /preterm infants.

CONCLUSION

• In our recent study, early iron supplementation at 2mg/kg twice daily in LBW neonates improved iron store status, accompanied by a decrease in iron deficiency and anaemia among preterm and LBW infants.

• No adverse effects of supplementation were observed.

• Thus reinforcing iron supplementation as early as 2 weeks of postnatal age in LBW babies (started at 2 weeks of age or when baby is tolerating full feeds) helps to improve the iron store status and reduces the incidence of iron deficiency among them within them.

SHORTCOMINGS OF THE STUDY:

• The sample size of the study was small; larger studies are necessary to strengthen the position.

• The study compares the outcomes of iron supplemented babies to those of unsupplemented babies; further studies comparing the outcomes of early and late iron supplementation would provide a clearer understanding of the effects of iron supplementation.

• In this study, the effect of iron supplementation on neurodevelopment,

cognitive function, or growth was not followed up, which might have given better support for early iron supplementation of LBW babies.

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ANNEXURE I

INFORMED CONSENT FORM

TITLE OF RESEARCH :

Haemoglobin and Iron store status in Low Birth Weight babies and effect of early Iron supplementation on them. A Randomized and Open label study

GUIDE : DR R.H. GOBBUR

PG STUDENT :DR SAFOORA UMAIMA A. G.

PURPOSE OF RESEARCH:

I have been informed that the purpose of this study is to assess the effect of early iron supplementation on the haemoglobin and iron store status in Low Birth Weight infants.

PROCEDURE:

I understand that after having obtained a detailed clinical history, thorough clinical examination and relevant investigations will be done.

RISKS AND DISCOMFORTS:

I understand that there is no risk involved in this study and my baby may experience mild pain during the above mentioned procedures. This is mainly the result of my baby's condition and the procedures are not expected to exaggerate these feelings which are associated with the usual course of treatment.

BENEFITS:

I understand that my baby's participation in this study will help to study the effect of Early Iron supplementation on Haemoglobin and Iron store status in Low Birth Weight infants.

CONFIDENTIALITY:

I understand that the medical information produced by the study will become a part of hospital record and will be subjected to confidentiality and privacy regulations of hospital. Information of sensitive personal nature will not be part of medical record but will be stored in investigations research file. If the data is used for publication the identity will not be revealed; other identifiers such as photographs will be used only with special permission. I understand that I may see the photograph before giving my permission.

REQUEST FOR MORE INFORMATION:

I understand that I may ask for more information about the study at any time and Dr. Safoora Umaima at the Department of Paediatrics will be available to answer my questions or concerns. I understand thatI will be informed of any significant new findings discovered during the course of the study, which might influence my baby's continued participation. A copy of this consent form will be given to me to keep for careful reading.

REFUSAL OR WITHDRAWAL OF PARTICIPATION:

I understand that my baby's participation is voluntary, and I may refuse to participate or withdraw consent and discontinue participation in the study at any time without prejudice. I also understand that Dr. Safoora Umaima may terminate my participation in the study after she has explained the reasons for doing so.

INJURY STATEMENT:

I understand that in the unlikely event of injury to my baby resulting directly from my participation in this study, if such injury were reported promptly, the appropriate treatment would be available to the baby. But, no further compensation would be provided by the hospital. I understand that by my agreements to participate in this study and not waiving any of my legal rights.

I have explained to_____the purpose of the research, the procedures required and the possible risks to the best of my ability.

Dr. Safoora Umaima Parent/Gaurdian

(Date) (Date)

PARENTS / GUARDIAN CONSENT STATEMENT:

We confirm that Dr. Safoora Umaima, is conducting a study on “Haemoglobin and Iron store status in Low Birth Weight babies And effect of early Iron supplementation on them. A Randomised and Open label study.”

Dr.Safoora Umaima, has explained to us the purpose of research and the study procedure. We are willing to give as much as information required for the study and consent for investigations and the possible discomforts as well as benefits. We have been explained all the above in detail in our own language and we understand the same.

Therefore we agree to give consent for our baby’s participation as a subject in this research project.

_____ _____

(Parents / Guardian) Date

_____ _____

(Witness to signature) Date

ANNEXURE II

PROFORMA

Name:

Day Of Life:

Sex :

IP/OP no.

Address:

Phone No.

DOB:

Mother's Obstetric history:

Ante-natal history-

Antenatal check ups done/ not

Natal & Neonatal history:

Mode of
Delivery:

Gestational age

Birth weight:

Vitals: HR RR Temp CFT

General Examination:

Pallor/ Jaundice/ Cyanosis, Edema/ Clubbing

Systemic examination:

CVS:

RS:

PA:

CNS:

Date of starting Iron supplementation:

Dosage and frequency of administration:

Route of administration:

INVESTIGATIONS:

	At 2 weeks/ when full feeds tolerated	At 6 weeks
RBC Count-		
Haemoglobin level		
Hematocrit		
Serum Ferritin		

Any other investigation to be done for treatment of the baby:

Any incidence of NEC / evidence of ROP:

Any Blood Transfusion done:

CONCLUSION;

SIGNATURE

DATE:

ANNEXURE III

ETHICAL CLEARANCE CERTIFICATE

IEC/No-131/2019
22-11-2019

B.L.D.E. (DEEMED TO BE UNIVERSITY)
(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act, 1956)
The Constituent College
SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The ethical committee of this college met on 13-11-2019 at 3-15 pm to scrutinize the synopsis of Postgraduate students of this college from Ethical Clearance point of view. After scrutiny the following original/corrected and revised version synopsis of the Thesis has been accorded Ethical Clearance

Title: Haemoglobin and Iron store status in low birth weight babies and effect of early iron supplementation on them – A randomized and open label study.

Name of PG student: Dr Safoora Umaima Abdul Gaffur, Department of Paediatrics

Name of Guide/Co-investigator: Dr R H Gobbur, Professor Department of Paediatrics

DR RAGHVENDRA KULKARNI
CHAIRMAN
Institutional Ethical Committee
B.L.D.E.U's, Shri. B.M. Patil
Medical College, B'LR-586103

Following documents were placed before Ethical Committee for Scrutinization:

1. Copy of Synopsis / Research project
2. Copy of informed consent form
3. Any other relevant documents.

MASTERCHART (CASES)

sl no.	case no.		name	IP No.	sex	gestational age	birth weight	MOD	Dose and frequency of iron supplement	Hb(0-2wk)	Hb (6wk)	ferritin (0-2 wk)	ferritin (6wk)	RBC (0-2wk)	RBC (6 wk)	Hct(0-2wk)	Hct(6 wk)	ROP/ NEC/ morbidity	transfusion
1	1	case	b/o Ashwini H	227790	Female	36+5	2.2	LSCS	0.2ml PO BD	17.4	14.8	231.6	189.4	4.91	4.86	54.2	34.3	no	No
2	2	case	b/o bhagyashree	226803	Female	37+5	2.3	LSCS	0.2ml PO BD	18.4	13.2	470.4	413.5	5.24	5.22	58.4	33.7	no	No
3	3	case	b/o Ashwini J Goni	229396	Male	38+5	2.3	LSCS	0.2ml PO BD	18.9	12.5	488.5	428.3	5.77	5.36	60.8	35.6	no	No
4	4	case	b/O Meghana	226842	Male	35+5	2.5	LSCS	0.2ml PO BD	14.1	13.4	239.9	175.6	4.42	4.29	40.7	36.2	no	No
5	5	case	b/o husainbee T2	227803	Male	38+1	2.2	LSCS	0.2ml PO BD	18.2	14.3	136.4	97.8	5.3	4.98	57.2	33.2	no	No
6	6	case	b/o Gurubai B	265101	Female	34+6	2.3	LSCS	0.2ml PO BD	17.9	13.1	300.6	257.3	5.4	5.18	56.4	38.9	no	No
7	7	case	b/o Sushmita T1	274017	Female	37+1	1.9	LSCS	0.15ml PO BD	18.1	14.8	171.2	124.2	5.03	4.86	58.3	37.4	no	No
8	8	case	b/o Sushmita T2	274018	Male	37+1	2.2	LSCS	0.2ml PO BD	19.2	14.3	366.7	310.5	5.55	4.69	60.1	34.5	no	No
9	9	case	b/o Savitri B	278646	Male	37+4	2.48	LSCS	0.2ml PO BD	16.4	14.2	415.6	369.4	4.52	4.56	49.8	37.6	no	No
10	11	case	b/o Rajeshri A K	270111	Male	33	2.3	LSCS	0.2ml PO BD	14.2	13.7	363.1	308.3	4.09	3.97	45.9	32.5	no	No
11	14	case	b/o Jyothi A R	272325	Male	35	1.9	LSCS	0.15ml PO BD	19.3	12.8	227.5	185.7	5.89	5.29	57.8	36.6	no	No
12	15	case	b/o Devamma Kamble	295841	Male	36+1	1.85	LSCS	0.15ml PO BD	15.2	14.9	254.5	200.6	4.5	4.26	48.6	30.9	no	No
13	16	case	b/o Indirabai K	275786	Female	36+4	2.3	LSCS	0.2ml PO BD	14.8	13.4	617.0	540.6	4.31	3.98	45	38.5	no	No
14	19	case	b/o Hameeda S	275788	Female	36	1.54	NVD	0.1ml PO BD	19.4	12.5	138.8	102.8	5.22	5.17	60.8	28.9	no	No
15	20	case	b/o Devamma T1	271634	female	33	2.1	LSCS	0.2ml PO BD	17.7	14.6	254.6	198.3	5.04	4.56	55.9	35.1	no	No
16	21	case	b/o Aishwarya Irayya M	277117	Female	30+6	2.45	LSCS	0.2ml PO BD	15.4	13.7	361.7	307.5	4.17	4.11	46.7	32.7	no	No
17	22	case	b/o Akshata G	290838	Male	36+1	1.8	LSCS	0.15ml PO BD	24.5	12.8	505.7	470.6	6.85	4.59	68.9	35.6	no	No
18	23	case	b/o Gangavva	290832	Female	37+5	2.2	LSCS	0.2ml PO BD	23.4	12.4	288.8	239.4	5.41	4.34	69.1	37.8	no	No
19	24	case	b/o Laxmi Sidappa J	281769	Male	36	2.45	LSCS	0.2ml PO BD	17	13.6	344.5	302.6	4.31	4.19	51.1	30.4	no	No
20	25	case	b/o Kenchamma	280260	Male	37	2.4	LSCS	0.2ml PO BD	12.9	11.7	593.6	345.7	4.1	3.67	40.8	37.8	no	No
21	28	case	b/o Rashmi Rathod	282271	Female	35+5	2.1	LSCS	0.2ml PO BD	17.3	13.9	276.5	237.5	4.97	4.35	54.9	36.5	no	No
22	29	case	b/o rekha parshuram	254093	Male	36	2.33	LSCS	0.2ml PO BD	13.6	12.2	665.0	630.5	3.62	2.98	40.7	29.5	no	No
23	30	case	b/o Shobha Ganachari	104722	Male	32	1.7	LSCS	0.1ml PO BD	16.3	13.2	159.0	127.6	5.56	4.26	58	31.4	no	No
24	31	case	b/o Boramma	110126	Female	36+1	2.2	LSCS	0.2ml PO BD	16.1	12.6	516.0	485.4	4.77	3.51	51.3	29.8	no	No
25	32	case	b/o Samreen	127577	Female	36	1.8	LSCS	0.2ml PO BD	16.6	14.5	268.4	245.9	4.98	3.42	55.5	30.5	no	No
26	33	case	b/o mohina	126471	Male	34+4	1.89	LSCS	0.2ml PO BD	16.5	13.8	218.0	124.9	5.29	4.95	54.3	32	no	No
27	34	case	b/o pavitra	127585	Female	36	1.9	LSCS	0.2ml PO BD	19	14.2	220.9	187.4	5.34	3.19	62.2	39.5	no	No
28	35	case	b/o sayara	126601	Male	37	2.14	LSCS	0.2ml PO BD	21.7	14.4	217.4	159.3	6.26	4.31	63.4	37.4	no	No
29	36	case	b/o Jayashree	126621	Female	37	2.48	LSCS	0.2ml PO BD	13.5	9.5	205.9	178.6	4.03	2.91	41.7	29.3	no	No
30	37	case	b/o kavita jadhav	133904	Male	36	2.3	LSCS	0.2ml PO BD	16.3	12.4	279.3	252.3	4.56	2.23	50.2	28.4	no	No
31	38	case	b/o anasuya	136079	Female	34-36	2.4	LSCS	0.2ml PO BD	20.9	11.9	258.9	171.8	5.75	3.45	60.9	25.8	no	No
32	39	case	b/o danamma	281013	Male	38+5	2.38	NVD	0.2ml PO BD	17.4	14.2	390.2	365.8	4.98	3.56	49.5	34.7	no	No
33	40	case	b/o soniya	159623	Male	32-34	2.3	NVD	0.2ml PO BD	13.9	10.1	137.8	115.4	4.41	2.78	40.7	31.6	no	No
34	41	case	b/o kallubai	136101	Female	34-36	1.9	LSCS	0.2ml PO BD	17.7	12.6	137.8	123.6	5.18	3.86	53.6	29.9	no	No
35	42	case	b/o girija	133746	Female	34-36	2.3	LSCS	0.2ml PO BD	16.2	11.5	173.7	123.1	4.78	3.24	51.1	38.6	no	No
36	43	case	b/o roopa T2	155099	Female	32-34	1.5	LSCS	0.1ml PO BD	16.8	10.8	359.4	342.6	4.36	3.15	47.6	33.7	no	No
37	44	case	b/o roopa T1	155100	Male	32-34	1.7	LSCS	0.1ml PO BD	12.3	8.9	1116.6	106.7	3.41	2.64	32.8	34.9	no	No
38	45	case	b/o seema rathod	160724	Female	34-36	2.2	NVD	0.2ml PO BD	22.4	11.3	294.5	272.8	6.5	4.15	66.9	31.4	no	No
39	46	case	b/o malashree	131567	Male	36	2.1	NVD	0.2ml PO BD	20.2	10.5	161.6	54.8	5.99	3.93	63.8	30.2	no	No
40	47	case	b/o shruthi	150477	Male	32-34	2	NVD	0.15ml PO BD	12.5	10.2	396.2	342.5	3.63	2.31	34.8	27.9	no	No
41	48	case	b/o Girija	277097	Male	37+1	2.4	NVD	0.2ml PO BD	11.7	10.3	394.3	343.2	3.44	2.56	34.8	28.5	no	No
42	49	case	b/o Rekha Naikodi	223957	Male	37+1	2.38	NVD	0.2ml PO BD	14.4	11.2	395.4	351.2	4.27	3.52	48.3	29.1	no	No
43	50	case	b/o Reshma	246639	Male	37+4	2.42	NVD	0.2ml PO BD	21.8	12.1	236.2	226.3	6.31	5.25	65.3	30.2	no	No
44	51	case	b/o Kalpana Watar	264901	Male	38+1	2.48	NVD	0.2ml PO BD	12.3	11.1	370.39	351.2	3.31	2.69	37.1	24.8	no	No
45	52	case	b/o Arathi Pawar	229274	Female	36	2.21	LSCS	0.1ml PO BD	20.2	11.9	175.8	122	3.92	2.85	38.1	31.5	no	No
46	53	case	b/o Tejaswini S B	248377	Female	36	2.2	LSCS	0.1ml PO BD	21.3	12.2	176.7	123.7	4.2	3.6	37.7	31.2	no	No
47	54	case	b/o Akshatha T1	246661	Male	36-38	2.1	LSCS	0.2ml PO BD	21.8	13.7	399.7	267.8	5.92	3.85	58.5	38	no	no
48	55	case	b/o Akshatha T2	246662	female	36-38	2.46	LSCS	0.2ml PO BD	20.2	12.7	201.8	135.12	5.68	3.69	57.3	37.2	no	no

MASTERCHART(CONTROLS)

SrNo	case no.		name	IP No.	sex	gestational age	birth weight	MOD	Dose and frequency	Hb (0-2wk)	Hb >6wk	Ferritin (0-2wk)	ferritin >6wk	RBC count (0-2)	RBC >6wk	Hct (0-2wk)	Hct >6wk	ROP/ NEC/ morbidity	transfusion
1	18	control	b/o Ashwini Amar Kurle	282389	female	38	2.13	LSCS	NA	19.3	12.2	230.74	154.16	5.46	3.55	60.9	39.1	NA	no
2	10	control	b/o Kallawwa Pujari	280213	female	36-38	2.1	LSCS	NA	19.4	12.1	214.22	143.15	5.22	3.39	60.1	36.2	NA	no
3	12	control	b/o Laxmi Anand Kane	280253	female	38	2.1	LSCS	NA	18.1	11.4	254.46	170.5	5.31	3.31	58.8	37.2	NA	no
4	13	control	b/o Nazmeen	275764	Male	34-36	1.95	LSCS	NA	11.8	10.4	477.47	182.19	3.24	2.11	36.2	33.5	NA	no
5	17	control	b/o Bhagyashree R P	281815	female	36-38	2.3	NVD	NA	16.7	10.5	166.58	111.46	4.54	2.95	51.9	33.7	NA	no
6	26	control	b/o Savita Annigeri	297701	female	36-38	2.3	LSCS	NA	17.5	11.0	707.37	173.9	4.9	3.19	50.2	32.6	NA	no
7	27	control	b/o Ranjitha P H	294775	Male	36-38	2.18	NVD	NA	18.5	11.7	356.04	238.5	5.12	3.33	54.2	35.2	NA	no
8	56	control	b/o Geetha	248519	female	36-38	2.01	LSCS	NA	14.4	10.0	557.7	373.27	4.27	2.78	48.3	31.4	NA	no
9	57	control	b/o Roopa	248193	Male	36-38	2.3	LSCS	NA	15.8	10.0	460.1	308.3	4.65	3.02	45.1	29.3	NA	no
10	58	control	b/o Laxmi Donur	248163	Male	36-38	2.4	LSCS	NA	18.4	11.6	386	258.62	5.01	3.26	51.9	33.7	NA	no
11	59	control	b/o Danamma Math	213486	female	36-38	2.4	NVD	NA	16.4	12.5	262.7	191	4.51	4.13	49.1	37.6	NA	no
12	60	control	b/o Tejaswini Chetan B	256820	Male	34-36	2	NVD	NA	19.5	12.4	158.4	133.2	5.31	4.6	59.7	32.3	NA	no
13	61	control	b/o Shantabai T1	250772	Male	34-36	2.15	LSCS	NA	18.3	11.5	91.54	61.23	5.14	3.34	51.4	33.4	NA	no
14	62	control	b/o Shantabai T2	250774	Male	34-36	2.1	LSCS	NA	15	12.1	373	249.91	4.5	2.93	45	29.3	NA	no
15	63	control	b/o Yallawwa T1	250949	Male	34-36	2.1	LSCS	NA	18.6	11.7	235.77	158.11	5.71	3.71	57	37.1	NA	no
16	64	control	b/o Yallawwa T2	250948	female	34-36	2.06	LSCS	NA	20.4	12.9	299.71	200.8	5.08	3.97	59.2	37.5	NA	no
17	65	control	b/o malashree	255597	Male	36-38	2.2	NVD	NA	16.8	10.6	53.8	43.99	5.22	3.39	51.4	33.4	NA	no
18	66	control	b/o shruthi	255636	Feemale	36-38	2.17	LSCS	NA	15.8	13.5	242	162.51	4.65	3.02	45.1	30.3	NA	no
19	67	control	b/o nilamma	249484	Female	36-38	2.48	LSCS	NA	16.8	12.5	225.15	150.92	4.63	3.01	46.8	30.4	NA	no
20	68	control	b/o laxmi	255588	Male	34-36	2.3	NVD	NA	15.2	12.6	239.8	160.87	4.33	2.81	43.6	29.3	NA	no
21	69	control	b/o Deepa Gondali	246030	female	38	2.1	LSCS	NA	18	11.3	569	381.52	5.27	3.43	55.9	36.3	NA	no
22	70	control	b/o Sudharani	249732	Male	37	2.48	LSCS	NA	17.5	11.0	243.98	163.57	4.74	3.08	52.2	33.9	NA	no
23	71	control	b/o Papi Vikas Rathod	251588	female	39	2.4	LSCS	NA	16.3	10.3	260.77	174.87	4.48	2.91	49.1	31.9	NA	no
24	72	control	b/o Sana Kathik	245444	female	39	2.3	LSCS	NA	17	10.7	237.28	159.85	5.02	3.26	52.1	32.9	NA	no
25	73	control	b/o Shivavva T1	244612	female	32-34	1.9	NVD	NA	16	10.1	367.07	245.9	4.43	2.88	49	35.9	NA	no
26	74	control	b/o Shivavva T2	244611	Male	32-34	2	NVD	NA	12.3	11.1	322.1	215.8	3.32	2.16	36.3	28.6	NA	no
27	75	control	b/o Mandakini	300638	female	38	2.1	LSCS	NA	18.2	11.5	662.16	443.6	5.3	3.45	57.2	37.2	NA	no
28	76	control	b/o Mahananda T1	299321	female	34-36	2.4	LSCS	NA	15.9	10.0	105.99	91.25	5.03	3.27	55.5	36.1	NA	no
29	77	control	b/o Mahananda T2	299322	female	34-36	2	LSCS	NA	21.3	13.4	249.44	167.88	5.74	3.73	63.9	41.5	NA	no
30	78	control	b/o Prema Rathod	293151	Male	34-36	2.08	NVD	NA	13.4	11.5	53.4	39.81	3.9	2.54	40.6	31.4	NA	no
31	79	control	b/o Ranjita harijan	294775	Male	34-36	2.81	NVD	NA	15.6	11.8	202.5	135.17	4.44	2.89	46.1	30.0	NA	no
32	80	control	b/o Sampa Pujari	296171	Male	36-38	2.3	NVD	NA	16	10.1	645.45	432.5	4.75	3.09	46.3	30.1	NA	no
33	81	control	b/o Swaliya	286249	male	34-36	2.3	LSCS	NA	17.5	11.0	237.66	159.21	4.88	3.17	50.5	32.8	NA	no
34	82	control	b/o Pratibha Badiger	222621	female	32-34	1.54	LSCS	NA	19.4	12.2	557.4	373.5	5.54	3.6	61.1	39.7	NA	no
35	83	control	b/o Ashwini Kallu B	249680	Male	36-38	2.3	NVD	NA	18.2	11.5	547.88	367.11	4.98	3.24	57.1	37.1	NA	no
36	84	control	b/o Nasreen Banu	236376	female	38	2.49	LSCS	NA	17.4	11.0	238.21	159.6	4.37	2.84	53.8	35.0	NA	no
37	85	control	b/o Sunanda naik	277111	female	34-46	2.2	NVD	NA	16.7	10.5	198.72	133.13	5	3.25	52.7	34.3	NA	no
38	86	control	b/o Shilpa B Patil	296164	Male	32-34	1.8	LSCS	NA	16.2	10.2	263.43	176.5	4.69	3.05	48.3	31.4	NA	no
39	87	control	b/o Chinakka Iranna S	297802	female	36-38	2.3	NVD	NA	20.3	12.8	346.1	231.9	5.59	3.63	59.4	38.6	NA	no
40	88	control	b/o Heena Mustak P	297864	female	34-36	1.8	LSCS	NA	19	12.0	501.12	335.8	5.2	3.38	50.8	33.0	NA	no
41	89	control	b/o Vidyashree R	300773	Male	36-38	2.48	LSCS	NA	10	10.0	531.47	356.1	2.79	2.01	28.6	30.0	NA	no
42	90	control	b/oRashmi S Gachi	304169	Male	36-38	2.4	LSCS	NA	16.1	10.1	79.29	75.1	4.73	2.07	50.5	32.8	NA	no
43	91	control	b/o Nilabayi Jambagi	292128	Male	37	2.48	NVD	NA	22	13.9	251.2	168.3	6.42	4.17	67.5	43.9	NA	no
44	92	control	b/o Anita S Barakhade	299256	Male	32-34	1.8	NVD	NA	14.2	10.1	288.7	193.64	4.4	2.86	43.3	30.9	NA	no
45	93	control	b/o Jagadevi	244555	male	36-38	2.47	LSCS	NA	15.1	10.5	269.52	180.86	4.24	2.76	45.6	31.2	NA	no
46	94	control	b/o Ashwini Navi	246318	Male	37+2	2.41	LSCS	NA	13.4	11.6	344.96	231.11	4.59	2.98	45.2	34.1	NA	no
47	95	control	b/o Rekha Kalburaki	233355	female	32-34	1.76	LSCS	NA	13.9	10.7	403	299	3.72	3.01	40.5	33.0	NA	no
48	96	control	b/o Pallavi Walikar	227463	female	36-38	2	LSCS	NA	19.9	12.1	563.1	370.7	5.67	3.43	68.1	37.9	NA	no