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The Effects of Oral Iron Supplementation on Maternal and Infant Iron Status and on Breast Milk Iron Concentration in Benguet, Philippines

Pelin Bugtong-Belino*

Abstract : A prospective investigation was conducted to determine the effects of oral iron supplementation on maternal iron status and on breast milk iron concentration of lactating women and the iron status of their exclusively breastfed infants. Thirty two lactating women and their exclusively breastfed infants who were selected purposively during their prenatal consultations participated in the study. Lactating women were supplemented with oral iron supplements for three months. Women who were still anemic at three months continued taking iron supplements until six months. Infants found to be anemic were supplemented with 0.3 ml oral iron drops containing 15 mg. elemental iron daily for three months. Blood samples were drawn from both mother and child at three time points for the determination of iron status indices. Breast milk samples were also collected for the determination of breast milk iron. Results were analyzed for statistical significance using GLM procedure, correlation coefficient (Pearson *r*) and *t*-test. At baseline, 31% of lactating mothers were anemic and 44% had depleted iron stores (serum ferritin < 15 ng/ml). After 3 months of iron supplementation, hemoglobin concentration increased among lactating mothers with a significant increase among the anemic mothers. At six months, all the lactating women had normal hemoglobin levels. Eighty percent had normal iron stores but 20% still had depleted iron stores as indicated by their serum ferritin levels (<15 ng/ml). Results showed that generally the anemic women who have very low iron status responded more efficiently to the oral iron supplementation. However, some anemic women fall short of achieving normal hemoglobin levels after three months. Overall, 80% of the participants had normal iron stores as indicated by their serum ferritin but 20% still had depleted iron stores after 3 to 6 months of iron supplementation implying that a longer period of supplementation is necessary for them to build on their iron stores. Among iron-supplemented anemic infants, hemoglobin increased significantly with a mean incremental change of 27g/L higher than the incremental change of 3g/L among the non-supplemented infants. In conclusion, oral iron supplementation significantly improved iron status of lactating women and their exclusively breastfed infants. Oral supplementation among anemic infants at an early age warrants improvements in iron deficiency anemia among infants.

Keywords: Benguet iron supplementation, Benguet breast milk iron, Philippine serum ferritin.

1. INTRODUCTION

Iron-deficiency anemia is one of the most widespread public health problems and has an important health and welfare, social, and economic consequences. These include impaired cognitive development, reduced physical work capacity, and in severe cases increased risk of mortality, particularly during the perinatal period. There is also evidence that anemia may result in reduced growth and increased morbidity.

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In the Philippines, iron deficiency anemia (IDA) is still a public health concern among Filipino infants, children, pregnant and lactating women. Among infants below 6 months, as in other countries, little is known about the prevalence of this problem. It has been generally assumed that breastfed infants born at term and with an adequate birth weight have adequate iron stores for the first 6 months of life. Several studies however in other countries show that the rate of iron-deficiency anemia at 3-4 months of age was rather high. Evidence has accumulated that infants with adequate birth weight but born to anemic mothers have low iron stores and are likely to develop anemia, which challenges the current thinking that there are adequate iron stores to protect against iron deficiency up to six months of age (Faqih, A.M. and Qazag, H.S., 1999; De Pee et al., 2002)

The high prevalence of anemia among the > 6- to< 1 year old children raises the concern that iron stores in some infants are inadequate to sustain growth and development through the first 6 months of life, and postnatal factors such as maternal iron deficiency and anemia may be contributory to early depletion of iron stores and development of anemia. Hence, this study was designed to validate results of previous studies in other countries.

Given the magnitude of the problem in the Philippines and in the province of Benguet in particular, it is important that we have information on the iron situation among young infants (below 6 months). Iron deficiency should be prevented and/ or treated at a young age as possible to prevent consequences. Greater efforts are likewise needed to develop and implement programs both to prevent and control anemia. In program development, it is essential to understand the effectiveness of each program and intervention. Thus, this study was conducted to assess the changes in the maternal iron status and breast milk iron concentration of iron-supplemented lactating women in Benguet Province and in the iron status of their exclusively breastfed infants.

2. LITERATURE REVIEW

Iron Metabolism

Distribution and Function of Iron

Iron is the most abundant trace element in the body. The body contains about 75 mg/kg. fat-free body weight of iron which is about 3 to 5 grams. The amount varies with age, sex, nutrition, general health and size of iron stores. Of the total amount, 60-75% is present as part of the hemoglobin and 5% as myoglobin. About 25% is found in the liver, spleen and bone marrow (as ferritin or as hemosiderin); and small amounts in transport form (as transferrin) in the blood serum; and about 5% in every cell as constituent of certain enzymes and chromatin (Claudio, 2004).

Iron is essential as a cofactor of oxygen transport, respiration, amino acid, lipid, alcohol, vitamin A, and sulfite metabolism, and various other redox reactions (Kohlmeier, 2003).

Iron Absorption and Utilization

Factors affecting iron absorption. Iron absorption refers to the amount of dietary iron that the body obtains and uses from food. Healthy adults absorb about 10% to 15% of dietary iron, individual absorption is influenced by several factors.

Majority of the iron present in the food is ferric iron. There are several factors that affect the absorption of iron. These include the form of iron, type of iron, body needs, bulk in the diet, size of dose, presence of phytic and oxalic acids, presence of citrates, sugars and some amino acids, presence of tannins, intake of coffee, presence of ascorbic acid, infections and malabsorption syndromes (Claudio, 2004).

Uptake of iron from the intestinal lumen uses three distinct pathways, one for heme-bound iron through a largely uncharacterized pathway, another one for ferrous iron (Fe^{2+}) via the divalent metal iron transporter 1 (DMTI), and one for ferric iron (Fe^{3+}) via the beta3-integrin-mobilferrin pathway (Conrad et al., 2000 cited by Kohlmeier, 2003). Absorption is most effective in the duodenum, slightly less in the remainder of the small intestine, and least in the colon.

Iron absorption from human milk. Iron in human breast milk is well absorbed by infants. It is estimated that infants can use greater than 50% of the iron in breast milk as compared to less than 12% of the iron in infant formula.

Domellof and his co-workers (2002) investigated the effects of age, iron status, and iron intake on iron absorption in healthy, term, breast-fed infants. Iron absorption was measured in 25 infants at 6 months of age and was repeated in 18 of them at 9 months of age. At 6 months, the mean fractional absorption of iron from human milk was 16.4 ± 11.4%, with no significant difference between iron-supplemented infants (11.9 ± 7.4%) and unsupplemented infants (17.8 ± 12.2%). At 9 months the mean fractional iron absorption was found to be significantly higher in unsupplemented infants (36.7 ± 18.9%) than in iron-supplemented infants (16.9 ± 9.3%). This was expected by the researchers because the unsupplemented, breast-fed infants had significantly smaller iron stores as assessed by plasma ferritin concentration at 9 months than did the iron-supplemented infants. Low iron stores are thought to increase intestinal iron absorption.

Hicks et al. (2006) studied 2 groups of human milk-fed infants using iron given as ferrous sulfate without any milk and iron given at the time of breast milk feeding to determine whether healthy infants at risk for iron deficiency would regulate their iron absorption based on their iron status. The researchers studied 20 Peruvian infants at 5-6 months of age and 18 infants at 9-10 months of age.

In this study, infants with lower iron status, as measured by serum ferritin, exhibited an upregulation of iron absorption at both 5-6 and 9-10 months of age. Infants with serum ferritin <12 mg/L had significantly higher absorption than those with serum ferritin >12 mg/L. Results from the study suggest that infants with low iron status are able to compensate for their impaired status by increasing iron absorption. The researchers concluded that iron absorption in infants is related to iron status as assessed by serum ferritin but not hemoglobin concentration. Infants with low iron status upregulate iron absorption from breast milk at both 5-6 and 9-10 months of age.

Utilization of iron. Iron absorbed and carried by the blood is bound to with a plasma protein, transferrin and enters several pathways. Some will be released for the synthesis of respiratory enzymes and other vital cellular constituents that require iron. Much of the iron in the blood plasma comes from dietary iron or it comes from the breakdown of body iron cells and from the storage depots and will be removed by the bone marrow to form hemoglobin necessary for the formation of red blood cells. About 40% of the iron from phagocytosed red blood cells is used for hemoglobin synthesis again within 12 days; the remainder goes into storage (Kohlmeier, 2003).

About 20 mg. of iron is needed for the formation of hemoglobin. Excess iron is stored in the liver as ferritin and as hemosiderin in the bone marrow, and the rest in spleen muscles. If the iron supply from the diet and from breakdown of red blood cells is not enough for body needs, iron will be mobilized from the storage areas, and will be bound as transferrin in the blood. It is then recirculated to the different parts of the body. The iron stores are sufficient to last for 3 to 6 months (Claudio, 2004).

Pathogenesis of Iron Deficiency Anemia

Depletion of iron stores is the first phase in the development of iron deficiency that leads to anemia (Figure 1). In the first phases of iron deficiency the iron stores in the body are progressively depleted. Iron is essential to synthesize hemoglobin, which is a protein in the blood that transports oxygen. Once the body stores of iron are used up, an individual begins to produce less hemoglobin. This is the second phase of iron deficiency. As iron deficiency develops further, it progressively leads to anemia. In the third phase, anemia is diagnosed when an individual's hemoglobin concentration falls below a specific cutoff value. Iron deficiency anemia is a reduction in the amount of red blood cells, which is caused by a lack of iron and which decreases the amount of oxygen transported to the cells of the body. In an individual with iron deficiency anemia, red blood cells are generally smaller than normal (microcytic) and paler than normal (hypochromic).

The two main factors that contribute to iron deficiency are : (*a*) loss of red blood cells as a result of blood loss, and (*b*) low iron intake. Certain parasitic infections cause blood loss that result in iron deficiency. The dietary intake of iron is especially critical during phases of the life cycle when the need for iron is high, such as infancy and early childhood and the adolescent growth spurt, and during pregnancy and lactation.

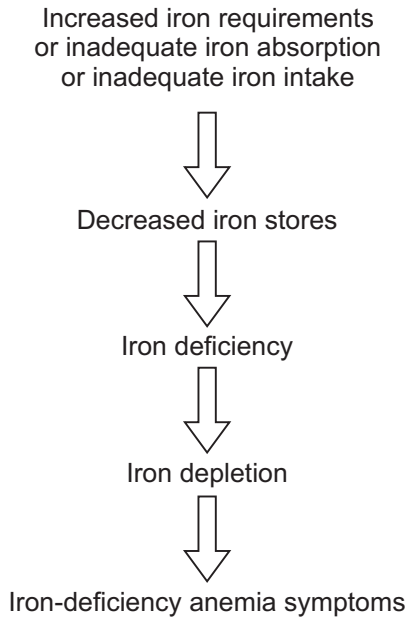

Figure 1: Progression of iron deficiency (Brown, 2005)

3. METHOD

Research Design

The study was a prospective investigation on the effect of oral iron supplementation during early lactation and infancy on the iron status of lactating mothers and young infants (Figure 2).Thirty two pregnant women who satisfied the inclusion criteria participated in the study. The study was approved by the Cordillera Health Research and Development Ethics Review Committee.

Participants of the Study

The participants were lactating women and their exclusively breastfed infants who were previously selected purposively during their prenatal consultations at the Municipal Health Unit, in the barangay health stations and at the Benguet General Hospital. Participants were informed about the study protocol, including the need for drawing blood samples from them and their infants at three time points *i.e*. after delivery, after 3 months and at 6 months.

Selection Criteria

Inclusion criteria were as follows:

For the mother :

Permanent residence of Benguet, aging between 20 and 40 years old

Gestation of 37-42 weeks (9 months)

Singleton pregnancy

Mothers who were decided to exclusively breastfed their infants for at least six months

Informed consent

For the infant:

Full term infants

Normal birth weight (>2.5 kg.)

Exclusion Criteria : Lactating women who have medical or obstetric problems, including women with anemia due to hemoglobinopathies, chronic bleeding, parasitosis, diseases of the liver, cardiovascular system and kidney; women who had any form of parenteral iron therapy for anemia of pregnancy; women with antepartum hemorrhage, and women with malaria and history of malaria were excluded from participating in the study..

Infants who were of low birth weight and who had medical problems were excluded in the study.

Procedures

Upon delivery, blood samples were drawn from both the lactating women and their infants for the determination of their baseline iron status. Breast milk samples were also collected through house to house collection from the mothers 2-3 weeks after delivery.

All the participating mothers were given oral iron supplements. The lactating women were advised to take an oral dose of 1 tablet (coated) containing 60 mg. elemental iron and 400 ug. folic acid daily for 3 months or 90 days as prescribed in the Guidelines on Micronutrient Supplementation of the Department of Health.

After 3 months of oral iron supplementation, lactating women with low iron status and have iron deficiency anemia i.e. hemoglobin level of <122 g/L (adjusted for high altitude) continued to take oral iron supplements up to 6 months of lactation.

On the other hand, at 3 months of age, infants with iron deficiency anemia i.e. hemoglobin level of < 105 g/L were supplemented with 0.3 ml of iron drops containing 15 mg. elemental iron/0.6 ml once a day following the guidelines for low birth weight infants prescribed by the Department of Health until 6 months.

Blood samples were drawn from the lactating women and their infants 3 times during the period of the study *i.e*. after delivery, after 3 months (90 days) and at 6 months for analysis of hemoglobin and serum ferritin. Changes in the hemoglobin levels were assessed in both mother and infant while serum ferritin was assessed among the mothers only due to the difficulty of extracting enough blood serum from the infants.

Breast milk samples were also taken from the lactating mothers 3 times at the same interval *i.e*. 2-3 weeks after delivery, after 90 days and at 6 months for analysis of breast milk iron concentration. Breast milk iron concentration was assessed during lactation and was correlated with the maternal iron status since breast milk is known to be constantly changing its composition, dependent on the interaction with the baby.

Collection of Blood Samples

Three venous blood samples (5 ml.) were obtained from each mother. The first was obtained at baseline after delivery, the second after three months (after 90 days of oral iron supplementation) and the third at six months of lactation. For the infants, three blood samples (about 1 ml.) was drawn by heel prick, first after birth, second at three months old and third at six months. The blood samples from mothers were analyzed for hemoglobin and serum ferritin levels. Hemoglobin levels were immediately analyzed at the Benguet General Hospital using an automated cell analyzer (CELL-DYN 1700 Multiparameter Hematology Analyzer). After centrifugation, serum were placed in tubes and were stored frozen at -20 ºC and were delivered to the Biomedical Nutrition Division of the Food and Nutrition Research Institute for the analysis of serum ferritin by radioimmunoassay (Ferritin IRMA-COAT-A-COUNT). Baseline,

midline and endline samples from the participants were assayed on the same day to minimize interassay variation. No sample was brought abroad nor stored for future research.

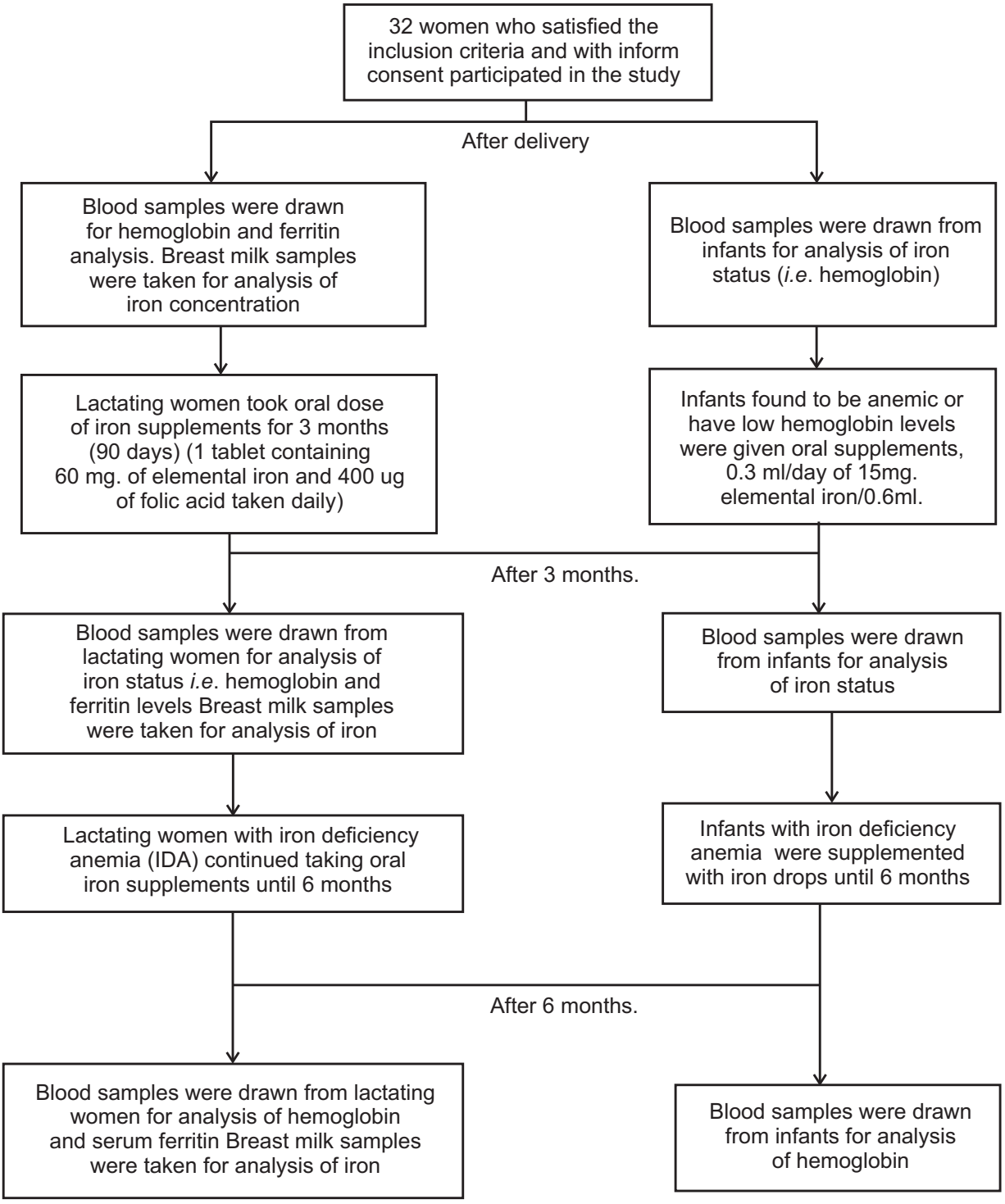

Figure 2: Flowchart of study

Collection of Breast Milk Samples

Breast milk samples were collected three times, first at 2-3 weeks postpartum, second at three months postpartum and third at six months by the researcher. Breast milk samples (100 ml.) were expressed by hand or manual pumps into plastic cups, transferred into plastic containers, frozen rapidly and stored in a freezer. These samples were brought to the Food Analytical Service Laboratory of the Food and Nutrition Research Institute, Department of Science and Technology for the analysis of the iron content. This

laboratory is an accredited testing laboratory (FNS ISO/IEC 17025:2005). Iron content was determined using atomic absorption spectrophotometry (AAS) following the AOAC 985.35 (modified) Method. Atomic absorption spectrophotometry is the recommended method of analysis by the Codex Alimentarius Commission in 2006. It is a sensitive technique with a detection limit of 10 to 20 ppb and a linear range between 1 and 20 ppm of iron. It is highly reliable, accurate and precise (Nalubola and Nestel, 2000).

Data Processing and Analysis

Determination of Iron Status

Iron statuses of the participants were determined whether they have normal iron status, iron deficient, and iron deficiency with anemia. According to the WHO (2001), iron deficiency is defined as a condition in which there are no mobilizable iron stores and in which signs of a compromised supply of iron to tissues are noted. Less ferritin is made in cells when cellular iron content is low. Thus, serum ferritin is used as an index of body iron stores:1 ng ferritin/ml serum equals 10 mg body iron stores. Normal serum ferritin concentrations (for adults) exceed ~12 ng/ml (Gropper et al., 2005),

The iron status of lactating women were determined using the results of the hemoglobin and serum ferritin levels obtained at baseline using the standards set by the World Health Organization. For serum ferritin, the cutoff value for depleted iron stores is 15 ng/ml. The cutoff value for anemia at high altitude was used for hemoglobin concentration because the normal hemoglobin concentration increases with altitude to compensate for the lower concentration in the air. Benguet Province has an altitude of about 1500 meters, so for the participants in this study, adjustment was done such that for the lactating women aging 20-40 years, the cutoff or -2SD for iron deficiency anemia was 122 g/L (117 + 5 g/L for 1500 meter altitude) (WHO, 2001).

The iron status of infants was likewise determined by comparing these with standards. For hemoglobin, since there is no established cut-off value for infants < 6 months old, the suggested cut-off value of 100g/L by Lonnerdal et al. (2001) cited and used by De Pee et al. (2002) was used. This was adjusted accordingly for the high altitude of Benguet. The cutoff value for infants below 6 months that was then used was 105 g/L (100 g/L + 5 g/L). For the cutoff for the hemoglobin level of infants taken at 6 months, the -2SD set by the WHO adjusted for the altitude was used *i.e*. 110 g/L (105 g/L + 5 g/L).

The results of the succeeding blood analysis were also compared with standards for possible improvements and changes.

Data Analysis

Results of the analyses of both maternal and infant iron status and breast milk iron concentrations were subjected for statistical significance. Statistical analyses were performed using Statistical Analysis System (SAS). Mean ± standard deviation (SD) was reported unless otherwise stated. The GLM procedure was used for the analysis of covariance. Correlation between iron status variables and breast milk iron was done using correlation coefficient (Pearson r). t-test was used to determine statistical significance of the observed differences between the anemic and non-anemic participants as well as differences within the same group between time points.

4. RESULTS AND DISCUSSION

Iron Status of Participants Hemoglobin Level of Lactating Mothers and Their Exclusively Breast fed Infants

The baseline hemoglobin concentration of lactating mothers taken within 24 hours after delivery at the hospital ranged from 80g/L to 184g/L with a mean of 130 ± 21g/L (Figure 3).Using hemoglobin levels as indicative of anemia, 31% (10) of the lactating mothers were found to be suffering from anemia (Figure (4) following the World Health Organization standard for hemoglobin level which is below 2 standard deviation (-2SD).

Among the infants, baseline hemoglobin concentration ranged from 130g/L to as high as 224g/L with a mean of 179 ± 26 g/L. At baseline, none of the infants was found to be anemic.

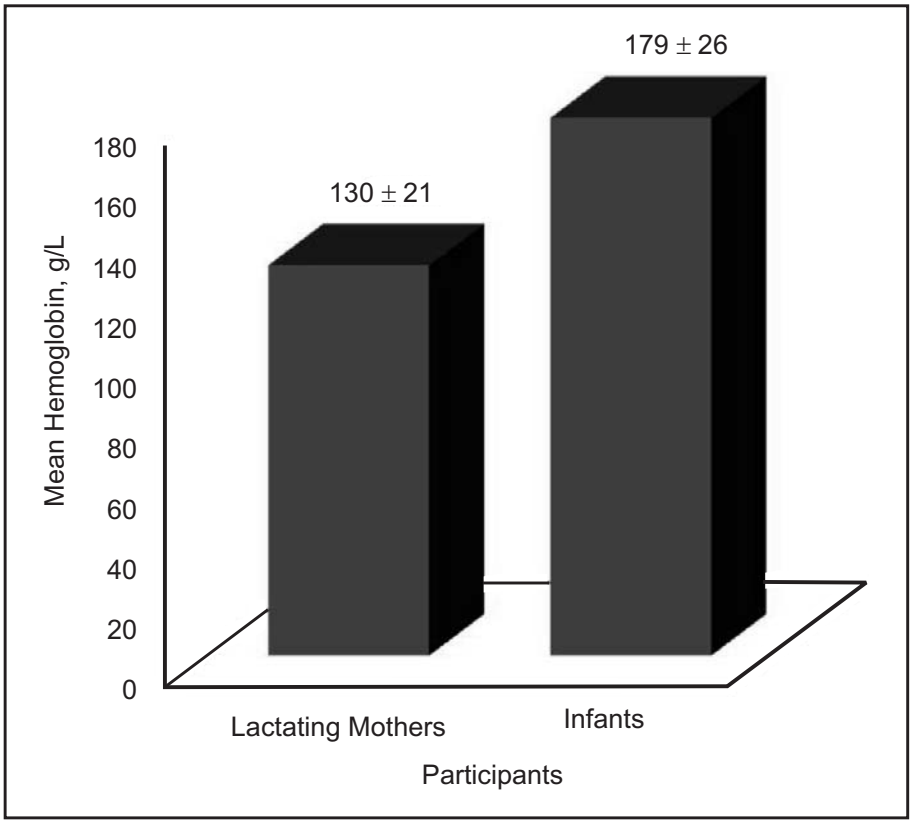

Figure 3: Hemoglobin level of lactating mothers and their infants at baseline

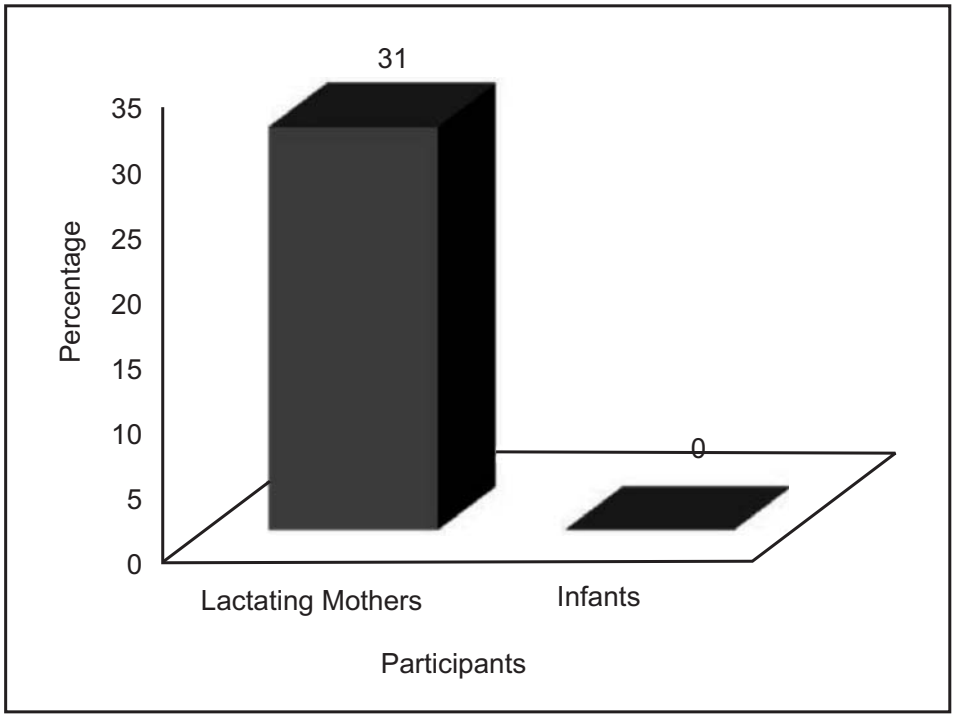

Figure 4: Percentage anemia among lactating mothers and their infants after delivery

Results showed that in deed, full-term infants are born with ample amounts of body iron in the form of hemoglobin iron. All of the infants had normal hemoglobin concentration at birth. At birth the infant of normal weight is expected to have iron stores in the form of serum ferritin that supply most of the

requirements for iron during the first four to six months of life.

Effect of Oral Iron Supplementation on Maternal and Infant Iron Status and on Breast Milk Iron Concentration

Iron Status Indices of Anemic and Non-anemic Lactating Mothers

Hemoglobin concentration. The iron indices, hemoglobin and serum ferritin levels, used in the study to determine iron status were compared in the anemic and non-anemic lactating mothers. At baseline, there was a significant difference between the mean hemoglobin of anemic and non-anemic mothers ($P < 0.01$). The mean hemoglobin concentration of anemic lactating mothers of 107 ± 15.29g/L was very much lower compared to the mean hemoglobin concentration of 140 ± 14.18g/L among non-anemic lactating mothers (Figure 5).

After 3 months of iron supplementation, the mean hemoglobin concentration among all lactating mothers increased by 10 g/L. The lactating mothers had hemoglobin concentration ranging from 110g/L to 167g/L with a mean of 140 ± 14.66 g/L. Among the anemic mothers, the hemoglobin concentration increased significantly to 130 ± 14.38 g/L ($P < 0.05$). Among the non-anemic lactating mothers, the mean hemoglobin increased to148 ± 9.75 g/L but not significant. The average increased in the hemoglobin concentration among the anemic lactating women was 27g/L, higher as compared to 8g/L among the non-anemic lactating women.

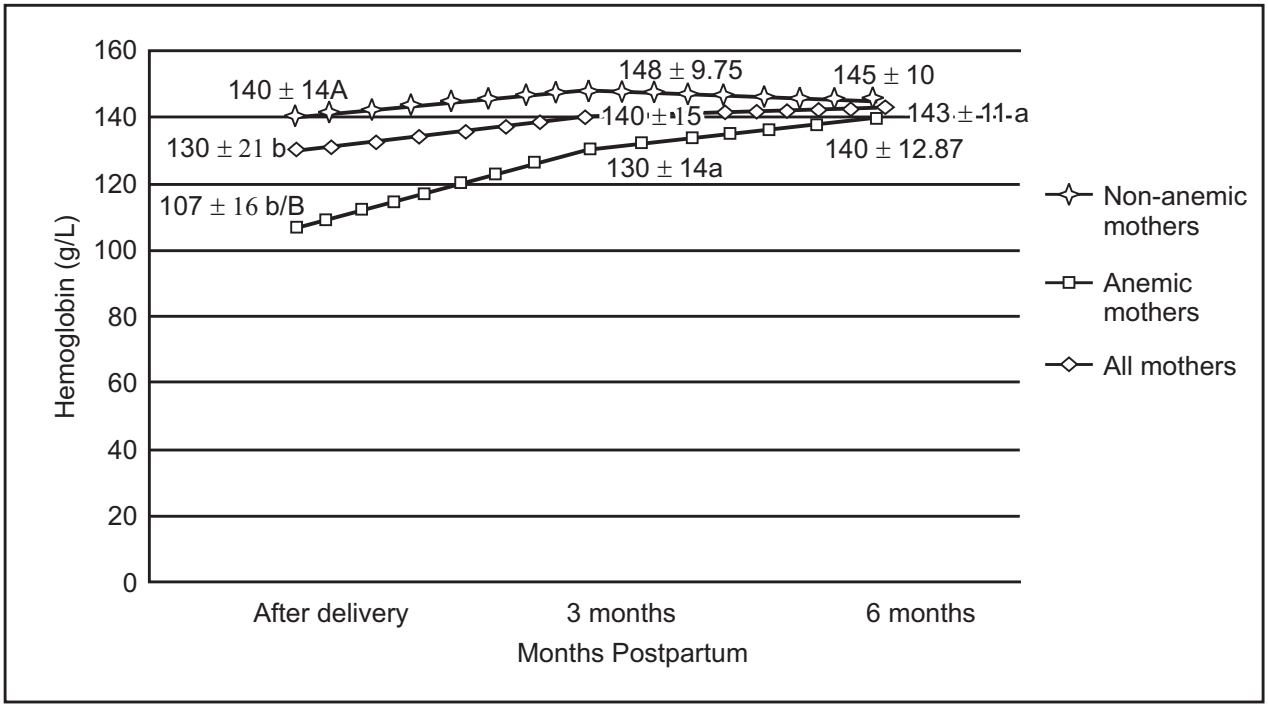

Figure 5: Comparison of the mean hemoglobin level of all mothers,anemic and non-anemic lactating mothers (Note: *a, b*: $p = 0.05$; A,B: $p = 0.01$)

Seventy five per cent (8) of the lactating mothers found to have iron deficiency anemia have improved hemoglobin concentration and were not considered anemic while 25% (2) had increased hemoglobin concentration but still below the cut-off value. However, another 11% (3) mothers developed iron deficiency anemia at 3 months. Overall, there were 18% (5) who were anemic at 3 months who continued taking iron supplements until 6 months.

At 6 months lactation, the mean hemoglobin concentration of all the lactating mothers increased by 3g/L, ranging from 127g/L to 170g/L with a mean of 143 ± 11g/L. Among all the participants, the increase in the hemoglobin level from baseline (130 ± 21.02 g/L) to 6 months (143 ± 11.39 g/L) was found to be

statistically significant at 5% level of significance. At this time, all the participants had normal hemoglobin levels. Among the anemic mothers, the hemoglobin concentration continued to increase (140 ± 12.87 g/L) as five anemic women continued to take iron supplements until 6 months. The mean hemoglobin concentration among the non-anemic lactating women who stopped taking the oral iron supplements slightly decreased to 145 ± 10.21g/L. The difference however was not statistically significant.

These higher increases in the hemoglobin concentration of anemic mothers compared to the non-anemic mothers could be attributed to the increase in the absorption of iron. The iron state of the individual is an important factor that affects the absorption of this element in the human body. Iron-deficient adults absorb 10 to 20% of dietary iron compared to 5 to 10 % absorption rate among healthy adults (Chatterjea and Shinde, 2002). Persons who are iron deficient tend to absorb iron more efficiently and in greater quantities than do normal individuals (Latham, 1997).With replete iron status more iron gets from blood into enterocytes and the intracellular iron concentration is maintained at a higher level than in iron deficiency. Accordingly, people with full iron stores absorb a much lower percentage of ingested iron than iron-depleted people (Kohlmeier, 2003).

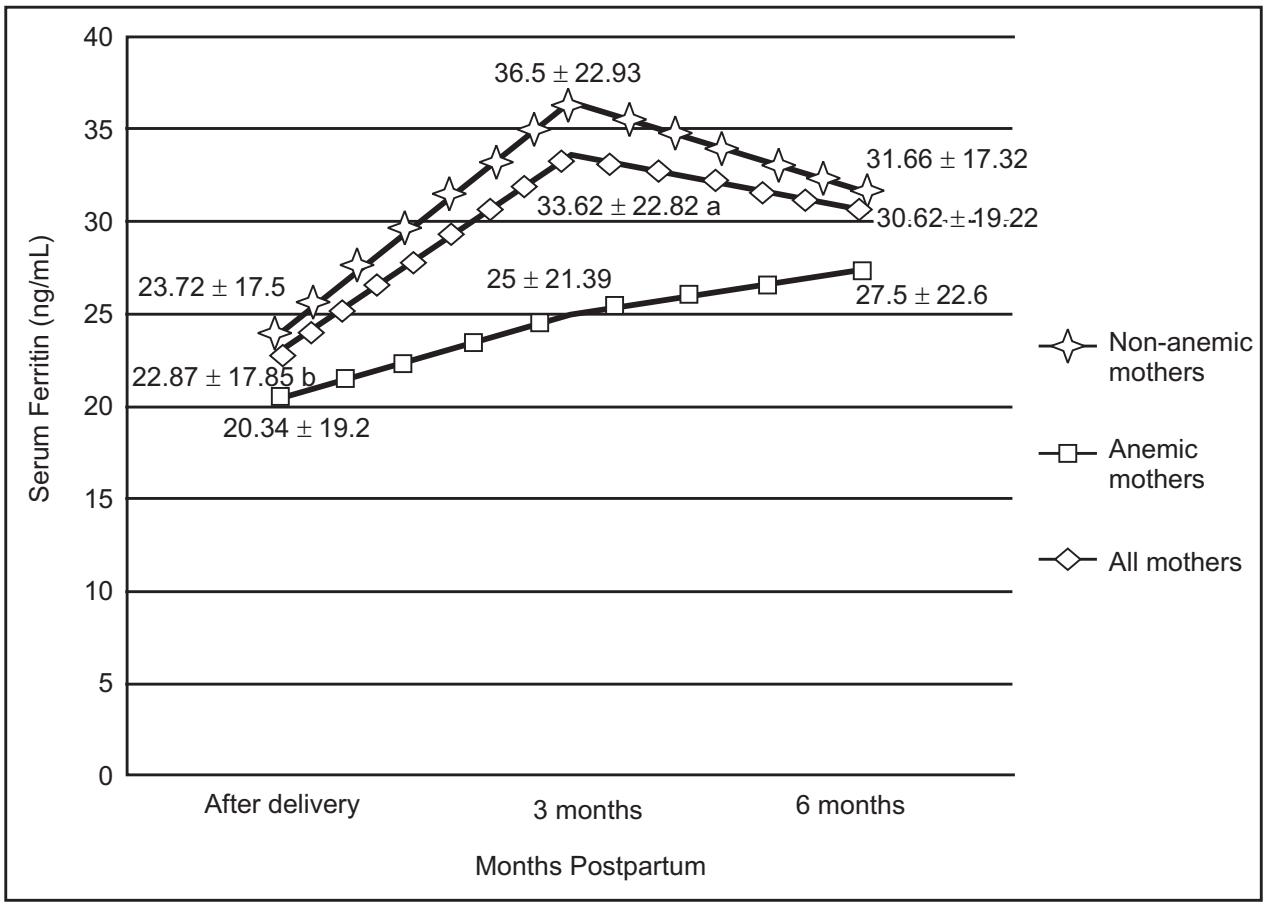

Figure 6: Mean serum ferritin of all mothers, anemic and non-anemic lactating mothers

Note : *a*, *b* : $p = 0.10$

Serum ferritin level. The baseline mean serum ferritin level of all the participants was 22.87 ± 17.85 ng/ml. The baseline serum ferritin of anemic participants ranged from 2 ng/ml to 69 ng/ml with a mean of 20.34 ± 19.2 ng/ml while the non-anemic participants had serum ferritin ranging from 3 ng/ml to 61 ng/ml with a mean of 23.72 ± 17.5 ng/ml (Figure 6). After the 3-month oral iron supplementation, the mean serurm ferritin level of all the participants significantly increased to 33.62 ± 22.82 ng/ml ($P < 0.10$). Among the anemic participants, midline mean serum ferritin increased to 25.0 ± 21.39 ng/ml with an average increase of 4.66 ng/ml. Among the non-anemic participants, the midline mean serum ferritin increased to 36.50 ± 22.93 ng/ml representing an average increase of 12.78 ng/ml. At 6 months,

the mean serum ferritin of all the participants declined slightly to 30.62 ± 19.22 ng/ml. Among the anemic participants, the mean serum ferritin level slightly increased to 27.5 ± 22.60 ng/ml (an increase of 2.5 ng/ml) while the mean serum ferritin of the non-anemic lactating women decreased with a mean of 31. 66 ± 17.32 ng/ml (a decreased of 4.84 ng/ml). These differences however were not statistically significant.

The observed greater average increase in the serum ferritin of the non-anemic participants compared to the anemic participants after 3 months of supplementation could be accounted to the sufficiency of functional iron among the non-anemic participants. Thus, the current iron intake including the iron from the supplements was taken up by the intracellular iron carrier and synthesized by the mucosal cells to the storage form ferritin. Whereas, among the anemic lactating mothers, seemingly, most of the daily iron intake was used to replace basal losses and for the synthesis of hemoglobin, thus lesser amount was stored as ferritin. This was also observed in the significant increases in hemoglobin level of the anemic mothers after 3 months of supplementation. The continued increase in the serum ferritin until 6 months among the anemic lactating women could be attributed to the continued intake of oral iron supplements of the lactating women who remained and became anemic at midline.

Table 1 further shows the iron status of participants on the basis of their serum ferritin concentrations. Based on the classification of the World Health Organization (WHO, 2001), serum ferritin level of <15 ng/ml indicates depleted iron stores. At baseline (after delivery), 60% (6) of the anemic participants had depleted iron stores. Their status improved progressively at 3 months until 6 months where 80% (8) had >15 ng/ml serum ferritin concentration indicating normal iron stores and only 20% (2) still had depleted iron stores. Among the non-anemic participants, those with depleted iron stores with serum ferritin of <15 ng/ml decreased progressively, from 36% (8) to 20% (3) from baseline to 6 months respectively. At 6 months, 80% (20) of all the particitpants had normal iron stores but 20% (5) still had depleted iron stores implying the need for a longer period of supplementation to build on their iron stores.

Table 1

Relative iron stores of participants on the basis of serum ferritin concentration after delivery, after 3 months of supplementation and at 6 months

Participants	*Depleted Iron Stores <15 ng/ml*			*Normal >15 ng/ml*		
	Months Postpartum			**Months Postpartum**		
	At delivery	3 Mos.	6 Mos.	At delivery	3 Mos.	6 Mos.
Anemic	6 (60%)	4(40%)	2 (20%)	4 (40%)	6 (60%)	8 (80%)
Non-anemic	8 (36%)	5 (29%)	3 (20%)	14(64%)	12 (71%)	12 (80%)
Total	14 (44%)	9 (33%)	5 (20%)	18(56%)	18 (67%)	20(80%)

These results in the iron status indices of the anemic and non-anemic lactating women who have taken iron supplements was in accordance to what was reported by Gropper et al. (2005) that the initial effects of oral iron supplements on red cell counts and hemoglobin concentrations take about two weeks whereas iron therapy to build body stores of iron may need six months to one year. Significant improvements in the iron status of the lactating women were observed after 3 months of supplementation. It was observed that the anemic mothers had higher mean incremental change in the hemoglobin concentration compared to the non-anemic mothers. Hemoglobin concentration continued to increase as the anemic lactating mothers continued to take iron supplements until 6 months. Similarly, iron stores as indicated by serum ferritin levels increased in both anemic and non-anemic mothers with higher increases among the non-anemic mothers (12.78 ng/ml vs 4.66 ng/ml) with an average increase of 10.75 ng/ml after 3 months of supplementation.

Three regulators of non-heme iron absorption have been identified in humans (Domeloff et al., 2002). They are known as the stores regulator, the erythropoietic regulator, and the dietary regulator. Many studies have shown that iron absorption is inversely related to iron stores, a mechanism referred to as the

stores regulator. Iron absorption is known to increase in situations when the erythropoietic drive is high in relation to the iron supply called the erythropoietic regulator. This is observed in various types of anemia characterized by iron-deficient erythropoiesis. Iron absorption is also regulated by recent dietary intake, independent of the size of iron stores and rate of erythropoiesis. A bolus dose of iron given enterally was shown to render enterocytes resistant to absorb additional iron for several days, a phenomenon that has been referred to as mucosal block (O'neil-Cutting et al., 1987 cited by Domeloff et al., 2002). In this mucosal block theory, a carrier called intracellular iron carrier (I.I.C) found in the mucosal cell cytoplasm regulates iron absorption from the gut. This intracellular iron carrier is known to deliver a fixed amount of iron to the mitochondria. It also transfers certain amount of Fe^{3+} to apoferritin, which is synthesized by mucosal cells to form the storage form ferritin. This I.I.C holds Fe^{3+} in either protein bound or chelated forms which represent the "carrier-iron-pool" in the intestinal mucosal cells. Presence of sufficient amount of iron in the "carrier-iron pool" keeps the I.I.C nearly or totally saturated and consequently reduces further iron absorption (Chatterjea and Shinde, 2002).

The regulation of absorption is closely tied to the level of the body's iron stores and may range from 10% (for persons with normal iron status) up to 35% (for persons who are iron deficient) (Gropper et al., 2005). Thus, iron absorption can rise to 3 to 6 mg daily when the body is depleted of iron and can fall to 0.5 mg or less daily when iron stores are high. Sites of iron storage and blood forming tissues such as the liver and bone marrow are thought to release compounds that in turn affect intestinal cells, to either increase or decrease iron absorption as needed. One proposed regulator of iron absorption is the protein hepcidin, which is thought to be released from the liver when iron stores are high. Upon release, hepcidin is thought to interact with other proteins, found on the surface of crypt cells in the small intestine. The interaction of hepcidin with these proteins, coupled with cellular uptake of iron into the crypt cells, results in absorptive brush border membrane enterocytes that express fewer brush border membrane transport proteins and fewer other proteins that facilitate iron absorption. Thus, in times of high iron stores, iron absorption will be diminished. Conversely, when iron stores are low, the absence or low levels of hepcidin coupled with low cellular iron uptake would cause crypt cells to evolve into brush border enterocytes that express higher concentrations of transport proteins and enzymes to enhance iron absorption. This hepatic regulator hepcidin acts to prevent iron overload by limiting intestinal uptake and storage in the liver (Nicolas et al., 2002 cited by Kohlmeier, 2003).

Devlin (1997) reported that the major barrier to the absorption of iron is not at the luminal surface of the duodenal mucosal cell. Accordingly, whatever the requirements of the host are, in the face of an adequate delivery of iron to the lumen, a substantial amount of iron will enter the mucosal cell. Regulation of iron transfer occurs between the mucosal cell and the capillary bed. In the normal state, certain processes define the amount of iron that will be transferred. Where there is iron deficiency, the amount of transfer increases; where there is iron overload in the host, the amount transferred is curtailed substantially. One mechanism that has been demonstrated to regulate this transfer of iron across the mucosal-capillary interface is the synthesis of apoferritin by the mucosal cell. In situations in which little iron is required by the host, a large amount of apoferritin is synthesized to trap the iron within the mucosal cell and prevent transfer to the capillary bed. As the cells turn over (within a week), their contents are extruded into the intestinal lumen without absorption occurring. There is increased sequestration by enterocyte ferritin in response to high intracellular iron concentration (Kohlmeier, 2003). The ferritin-bound iron is blocked from proceeding out of the cell and is lost into feces when the cell is shed at the end of its 2-3 day life span. In situations in which there is iron deficiency, virtually no apoferritin is synthesized so as not to compete against the transfer of iron to the deficient host. Iron transferred to the capillaries is trapped by transferrin.

These regulatory mechanisms occurring in the body is believed to have played a vital role in the differences in the effect of the oral iron supplementation among the participants particularly between the anemic and non-anemic lactating mothers whereby there was greater incremental change in the

hemoglobin level of anemic lactating women but minimal increases in the hemoglobin level among the non-anemic lactating women. Whereas, with regards to the storage form ferritin, more iron was taken up by proliferating cells for storage thereby leading to generally higher increases in serum ferritin levels among the non-anemic lactating women.

Iron Status Indices of Anemic and Non-anemic Mothers Supplemented for 3 and 6 Months

The indices of iron status (hemoglobin and serum ferritin) of 10 anemic and 22 non-anemic mothers who took iron supplements for 3 and 6 months were compared to determine possible effects of iron supplementation on a longer duration. Before supplementation, the baseline mean hemoglobin level of the 3-month supplemented anemic mothers was higher (109.5 ± 13.22 g/L) compared to the mothers who have to be supplemented until 6 months (98.5 ± 26.16g/L) (Table 2). Both values however, were significantly lower than that of 3 and 6-month supplemented non-anemic mothers with hemoglobin levels of 140.42 ± 14.32g/L and 137.67 ± 15.95 g/L respectively ($P < 0.05$).

After 3 months of supplementation, the mean hemoglobin of the 3 and 6-month supplemented anemic mothers increased significantly to 140.17 ± 10.11 g/L and 117 ± 4.24g/L respectively ($P < 0.05$) with the 3-month supplemented anemic mothers having significantly higher hemoglobin concentration ($P < 0.05$). The hemoglobin concentration of the 6-month supplemented anemic mothers was still below the cutoff value of 122 g/L hence had to continue iron supplementation for another three months. The mean hemoglobin concentration (140.17 ± 10.11 g/L) of the 3-month supplemented anemic mothers was significantly lower ($P < 0.05$) compared to the hemoglobin level of the 3-month supplemented non-anemic mothers (147.56 ± 10.84g/L). At this point, the hemoglobin level of the 6-month supplemented non-anemic mothers decreased significantly from 137.67 ± 15.95 g/L to 117 ± 6.08 g/L ($P < 0.05$) comparable to the 6-month supplemented anemic mothers. It was at this point that the three non-anemic mothers at baseline were considered to be anemic based on the cut-off value for hemoglobin.

At 6 months, the mean hemoglobin concentration of both the anemic and non-anemic mothers who continued to take iron supplements increased significantly to 145 ± 21.21g/L and 134.67 ± 6.81 g/L, respectively ($P < 0.05$). The mean hemoglobin concentration of the 3-month supplemented anemic mothers slightly increased to 141.5 ± 13.90g/L while the mean hemoglobin concentration of the 3-month supplemented non-anemic mothers slightly declined to 144.80 ± 10.21g/L. The mean hemoglobin level of the 3-month supplemented anemic women (141.5 ± 13.90g/L) was significantly lower ($P < 0.05$) compared to the hemoglobin level of the anemic women (145 ± 21.21g/L) supplemented for 6 months. The hemoglobin levels of the non-anemic mothers supplemented for 3 (144.80 ± 10.21g/L) and 6-months (134.67 ± 6.81g/L) was significantly different ($P < 0.05$). At this time, all of the mothers had normal hemoglobin values ranging from 127 g/L to 170 g/L.

Before supplementation, the baseline serum ferritin of the 3-month supplemented anemic mothers was higher (22.57 ± 33.15 ng/ml) compared to the serum ferritin of the 6-month supplemented anemic mothers (18.25 ± 6.07 ng/ml). The 6-month supplemented non-anemic mothers had higher serum ferritin level (29.0 ± 8.65 ng/ml) compared to the 3-month supplemented non-anemic mothers (22.84 ± 18.40 ng/ml).There was no significant difference observed in the serum ferritin of anemic mothers compared to the non-anemic mothers but the non-anemic mothers had slightly higher serum ferritin levels.

After 3 months of supplementation the mean serum ferritin of the 3-month supplemented anemic mothers increased to 27.50 ± 20.47 ng/ml while the 6-month supplemented anemic mothers decreased to 9.50 ± 4.65 ng/ml, significantly low compared to the former group and to the non-anemic mothers. The mean serum ferritin level of the 3-month supplemented non-anemic mothers increased to 37.47 ± 22.61 ng/ml, higher compared to the 6-month supplemented non-anemic mothers of 30.67 ± 23.0 ng/ml. Among all the lactating mothers, this increase in the serum ferritin levels after 3 months was significant at 10% significance level.

Table 2
Iron status indices of anemic and non-anemic lactating mothers supplemented for 3 months and 6 months

Indices	*Months Postpartum**		
	Baseline (after delivery)	*Midline (after 3 months)*	*Endline (after 6 months)*
Hemoglobin (g/L)			
Anemic (n = 10)			
Supplemented:			
3 months (n = 8)	$109.50 \pm 13.22^{b/B}$	$140.17 \pm 10.11^{b/A/1}$	$141.5 \pm 13.90^{b/1}$
6 months (n = 2)	$98.5 \pm 26.16^{b/B}$	$117 \pm 4.24^{c/A/2}$	$145 \pm 21.21^{a/1}$
Non-anemic (n = 22)			
Supplemented:			
3 months (n = 19)	$140.42 \pm 14.32^{a/A}$	$147.56 \pm 10.48^{a/A/1}$	$144.80 \pm 10.21^{b/1}$
6 months (n = 3)	$137.67 \pm 15.95^{a/A}$	$117 \pm 6.08^{c/B/2}$	$134.67 \pm 6.81^{c/1}$
Serum Ferritin (ng/ml)			
Anemic (n = 10)			
Supplemented:			
3 months (n = 8)	$22.57 \pm 33.15^{a/A}$	$27.50 \pm 20.47^{a/A/1}$	$29.58 \pm 17.80^{b/1}$
6 months (n = 2)	$18.25 \pm 6.07^{a/A}$	$9.50 \pm 4.65^{b/A/1}$	$11.0 \pm 0^{c/1}$
Non-anemic (n = 22)			
Supplemented:			
3 months (n = 19)	$22.84 \pm 18.40^{a/A}$	$37.47 \pm 22.61^{a/A/1}$	$27.72 \pm 17.22^{b/1}$
6 months (n = 3)	$29.0 \pm 8.65^{a/A}$	$30.67 \pm 23.0^{a/A/1}$	$44.67 \pm 29.19^{a/1}$

Values are means ± SD

* Means at baseline, midline and end line with same small letter(s) superscripts in a column are not significantly different at 5% LSD.

* Means at baseline and midline with same big letter(s) superscripts in a row are not significantly different at 5% LSD.

* Means at midline and end line with the same number superscripts in a row are not significantly different at 5% LSD.

At 6 months, the mean serum ferritin of the 3 and 6-month supplemented anemic mothers continued to increase slightly to 29.58 ± 17.80 ng/ml and 11.0 ng/ml, respectively. The serum ferritin of the 3-month supplemented non-anemic mothers decreased to 27.72 ± 17.22 while the 6-month supplemented mothers who continued to take supplements increased to 44.67 ± 29.19 ng/ml. Overall, the interaction between time and group status was highly significant ($P < 0.01$) which means that the increase in the serum ferritin of the supplemented mothers was significant indicating that iron supplementation had improved their serum ferritin levels.

The results showed that generally the anemic women who have very low iron status responded more efficiently to the oral iron supplementation as shown by the significant increases in their hemoglobin levels. However, it was observed that some anemic mothers although had increases in their hemoglobin levels still fall short of achieving normal hemoglobin levels after 3 months of supplementation and thereby needed a longer period of supplementation in order to achieve normal iron status. As observed, anemic women who continued taking iron supplements until 6 months had achieved normal hemoglobin levels by the end of 6-month supplementation. Among the non-anemic mothers, results showed that the modest gains after 3 months supplementation did not extend beyond the period of supplementation as shown by the decreased level of hemoglobin and serum ferritin levels at 6 months. Results of the study also showed that some mothers (5) regardless of their hemoglobin levels (classified as anemic or non-anemic)

at baseline still had depleted iron stores as indicated by their serum ferritin levels (<15 ng/ml) after 3 or 6 months of iron supplementation implying that a longer period of supplementation is necessary for them to build on their iron stores.

Mean Hemoglobin Concentration of Infants Born from Anemic and Non-anemic Mothers

At birth, infants born from anemic mothers had higher mean hemoglobin concentration (182.57 ± 24.02 g/L) than those infants born from non-anemic mothers (179.64 ± 26.95 g/L)(Figure 7). The difference however was not statistically significant. As observed, both groups of infants had high hemoglobin concentration. All hemoglobin values were within normal values. This was expected because of the ample amounts of stored iron in the newborn considering that all infants have normal birth weight. By the end of a full-term pregnancy, a mother should have supplied about 245 mg iron to a singleton fetus (Food and Nutrition Board Institute of Medicine, 2001cited by Kohlmeier, 2003).

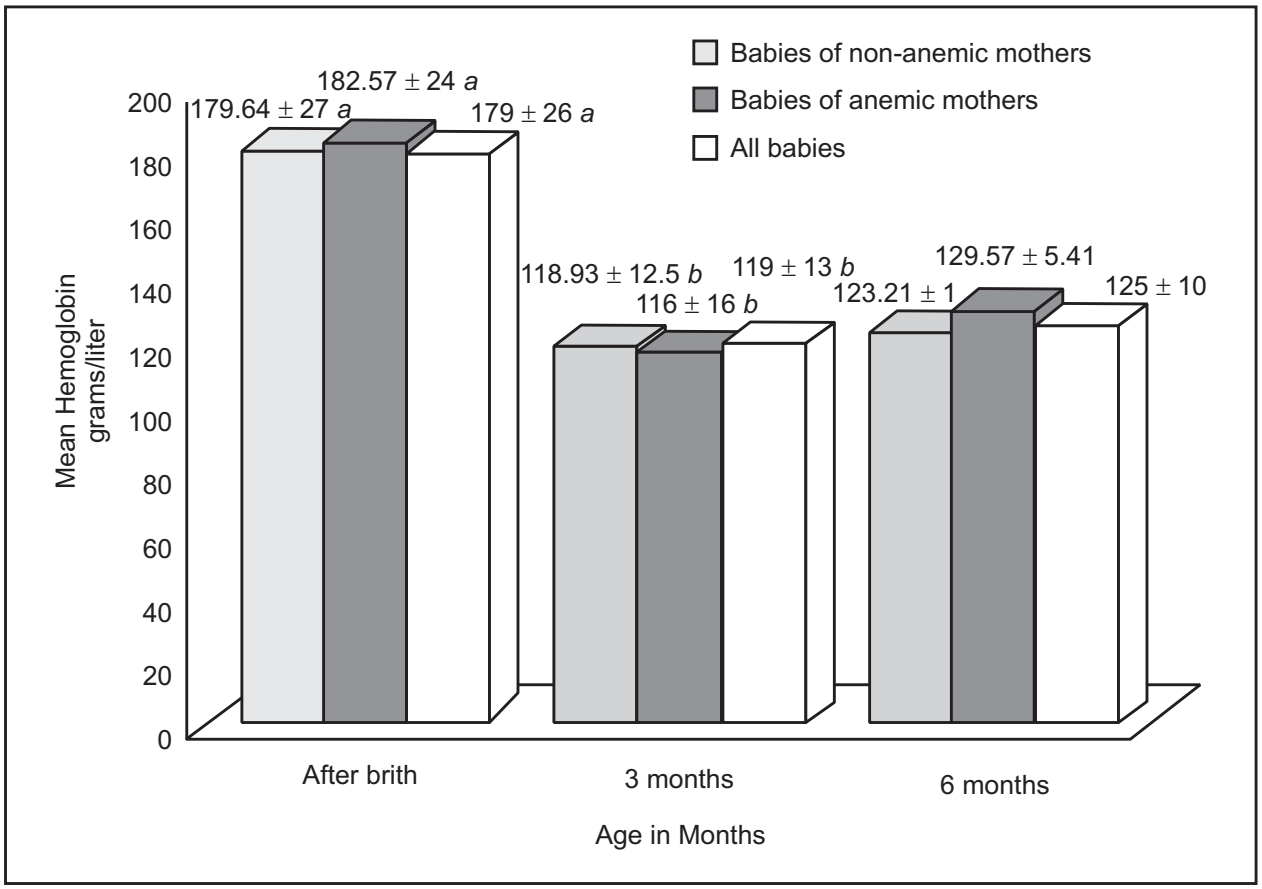

Figure 7: Comparison of the mean hemoglobin concentration of all babies, babies of anemic and non-anemic mothers (*Means after delivery to 3 months postpartum with different superscript letters among groups are significantly different at 1%.)

At 3 months, the mean hemoglobin concentration of all the infants decreased significantly ($P < 0.01$) ranging from 100 g/L to 150g/L with mean of 119 ± 13 g/L .The significant decline was observed in the mean hemoglobin of infants regardless of the iron status of their mothers. However, the mean hemoglobin concentration of babies from non-anemic mothers was shown to be higher, 118.93 ± 12.49 g/L compared to the mean hemoglobin concentration of 116 ± 16.0 g/L of babies born from anemic mothers. Three (12%) of the infants born from anemic mothers had hemoglobin levels below 105 g/L considered to be suffering from iron deficiency anemia (IDA) following the cut off value specified for this study. These infants took 0.3 ml. of iron drops containing 15 mg. elemental iron/0.6 ml. daily until six months of age.

At 6 months, the mean hemoglobin concentration of infants (excluding the iron-supplemented) increased slightly to 125 ± 10 g/L ranging from 101 g/L to 144 g/L. One (4%) infant however, had

hemoglobin level below the cutoff level of 110 g/L thus given iron drops as supplements. The increased on the mean hemoglobin concentration of babies was observed regardless of the iron status of the mother. However, the mean hemoglobin of babies born from anemic mothers, excluding the 3 anemic infants who received iron drops, was 129.57 ± 5.41g/L, higher than the mean hemoglobin of 123 ± 10.88 g/L of the babies born from non-anemic mothers. These differences in the mean hemoglobin concentration of babies born from anemic and non-anemic mothers as well as differences from the baseline to the end line result however was not found to be significant statistically.

The decline in the hemoglobin level of infants at 3 months regardless of whether they are born from anemic or non-anemic mothers was in concordance with previous studies. Hemoglobin declines from the very high level at birth to its lowest level at 6-10 weeks of age (Dallman et al., 1980). This decrease is known as the "physiologic anemia of the newborn" because the iron stores of all young infants, except those born with very low birth weight (<1500 g), are filled as a result of the breakdown of the red blood cells that occurs immediately after birth. Therefore, hemoglobin does not reflect iron stores or supply until –10 week of age (De Pee et al., 2002). Hemoglobin is expected to reach its lowest level at about 2 months of age then it slowly increases again and becomes more or less stable at 6-9 months age, unless iron stores have become depleted.

The lower hemoglobin level at 3 months among infants born from anemic mothers in this present study was similar with the findings of De Pee et al. (2002) among Indonesian infants. Infants of anemic mothers were more likely to have a low hemoglobin concentration. Their study showed that the infants' risk of having a low hemoglobin when birth weight was normal (> 2500g) but the mother was anemic (Hb < 120g/L) was greater than that of infants that had a low birth weight but a non-anemic mother. The finding supports the evidence that children born from anemic mothers have lower iron stores, even when they are born at term and with normal birth weight.

In this present study, at 3 months, the mean hemoglobin concentration decreased significantly with three infants (12%) suffering from iron deficiency anemia. All of these three infants were found to be born from mothers with iron deficiency anemia. As cited by Faqih and Hussain, Stekel (1984) concluded that iron endowment of the newborn may be affected under unusual circumstances of severe iron deficiency in the mother. This is consistent with previous studies conducted. Ziegler et al. (2009) reported the evidence that some normal infants are born with diminished stores. These infants could exhaust their iron endowment and become iron deficient before 6 months of age. Domellof and his co-workers (2001) found the prevalence of IDA among 4 month-old breastfed infants to be about 1% in Sweden and 3% in Honduras. At 6 months of age, the prevalence of IDA in Honduras had increased to 18.8% while it was still 1% in Sweden. Faqih and Hussain (1999) found that 43% of Jordanian infants exclusively breast fed for four months had depleted iron stores and 29 % had iron deficiency anemia. Their study showed that iron deficiency anemia (hemoglobin <10.5g/dl) was significantly higher ($P < 0.05$) in infants exclusively breastfed for four months than those exclusively breastfed for five or six months. The results strongly suggest that in Jordan exclusively breastfeeding for five to six months has a better protective effect against the development of iron-deficiency anemia than a shorter period or four months of exclusive breastfeeding. As noted by the investigators, the high bioavailability of iron in breast milk ranging from 50% to 70% may partly explain these results.

According to Dewey and Chaparro (2007), the iron status of a breastfed infant is strongly influenced by the body iron content at birth, which is determined by factors that operate before birth and at the time of delivery. These factors include maternal iron status before and during pregnancy, infant gestational age and birth weight and the timing of umbilical cord clamping. Delaying the clamping of the umbilical cord for 2 minutes can increase body iron content by approximately 33% (75 mg), and results in greater iron stores at 6 months of age. For exclusively-breast-fed full-term normal-birth-weight infant with delayed umbilical cord clamping, whose mother had adequate iron status during pregnancy, the iron provided from stores and breast milk is sufficient for ≥6 months. The authors concluded that high-risk infants may become iron deficient even before 6 months and that iron supplementation can be beneficial.

Kumar et al. (2008) also documented in their study population characterized by severe anemia with coexisting malnutrition that iron-deficiency anemia during pregnancy could compromise fetal iron status. They found significantly lower levels of hemoglobin, iron, and ferritin in the cord blood of infants born to anemic mothers. Indices of iron nutriture in maternal and cord blood showed significant correlations with each other, suggesting suboptimal iron supply to the fetus in maternal anemia. It is known from previous studies that severe anemia; present from early gestation along with concomitant maternal malnutrition may be associated with reduced placental weight and structural abnormalities of the placenta. The size of the placenta was also shown to be closely related to the surface area of the peripheral villi, which in turn, determines the transport of nutrients across the placenta. In short, the full-term baby has iron stores that are usually sufficient, along with highly bioavailable iron from breast milk, during the first 4-6 months if the mother is healthy. Mild maternal iron deficiency and anemia have few significant repercussions on the iron status of the infant, but severe anemia does (NAS, 1991 cited in RENI, 2002; Goyal, 2015).

In this present study, it was documented that all of the anemic babies were born from anemic mothers who have depleted iron stores. Two of the three anemic babies were born from mothers having the lowest hemoglobin concentration (80g/L and 90g/L) after delivery with one mother exhibiting the least serum ferritin (2ng/ml).Thus, the occurrence of anemia among these three babies born from anemic mothers was likely to be due to lesser iron transferred to them at gestation which resulted to lower iron stores. As such, their iron endowment was exhausted as early as three months compared to the other babies born from non-anemic and mild-to-moderately anemic mothers. The iron provided by breast milk was not sufficient enough to provide for their basal losses as well as for hemoglobin synthesis. This may had been coupled by the observation that one of the anemic babies was above normal (> + 2SD) for his weight following the weight for age (FNRI-DOST IRS Growth Table and Charts) at 3 months, thus, the need for more iron because of the increase in hemoglobin mass and tissue iron. The occurrence of one (1) infant born from a non-anemic mother that had hemoglobin level below the cutoff level of 110 g/L at 6 months was probably because the iron endowment has been mobilized also. In all these cases, we cannot also exclude the possibility that the iron stores of these at risk babies might be lesser since the practice of delayed umbilical cord clamping just recently started in the hospital where these babies were delivered. Hence, these babies were not given the benefit of increasing their iron endowment through this medical procedure.

The increase in the mean hemoglobin concentration with higher mean hemoglobin concentration among the babies born from anemic mothers at 6 months could be attributed to the effect of oral iron supplementation which resulted to the improvement of the iron status of the mother and consequently to their breast milk iron. As observed the increase occurred even if the anemic babies who received iron drops were excluded. It was noted in the correlation analysis that the hemoglobin level of the mothers was significantly correlated to the hemoglobin level of their infant at this point. Results of the study of the group of Preziosi (1997) also showed that at 3 months after delivery, serum ferritin concentrations were significantly higher in infants of women supplemented with iron whose iron status improved significantly.

Considering that the infants were exclusively breast fed, breast milk was their only exogenous source of iron. It was noted that the breast milk iron concentration of the anemic mothers who were given iron supplements remained at 0.045 mg/100g while that of the non-anemic mothers who stopped supplementation decreased significantly to 0.03 mg/100g at 6 months. The mean breast milk iron concentration of 0.045 mg/100g was sufficient to provide the Recommended Nutrient Intake (RNI) for iron of 0.38 mg/day for Filipino infants (RENI, 2002). Assuming a breast milk intake of 850 ml., with the reported mean breast milk production of 750 -850 ml/day during the first 6 months of lactation (FNRI-DOST, 1989 cited in RENI, 2002 and Claudio et al., 2004), this breast milk iron concentration of 0.045 mg/100g was able to provide about 0.34 – 0.38 mg of iron per day to the infant. This amount provided about 89 – 100% of the Recommended Nutrient Intake. Among the infants of non-anemic mothers, the mean milk iron concentration of 0.033 g/100 ml at 6 months provided about 0.25 to 0.28 mg iron representing about 66 – 74% of the Recommended Nutrient Intake. This amount of iron provided was quite comparable to what Kohlmeier (2003) reported that breast feeding women transfer 0.3 to 0.6 mg/day to their infants.

The association of breast milk iron with the iron status of infants has been shown in the correlation analysis that the hemoglobin level of infants at 3 months was significantly correlated to the baseline breast milk iron ($r = 0.374$, $P < 0.10$) as well as the hemoglobin level of the infants at 6 months with the breast milk iron concentration at 3 months ($r = 0.356$, $P < 0.10$) at 10 % significance level.

Breast Milk Iron Concentration

The baseline mean iron concentration in breast milk was 0.044 ± .02 mg/100g among all the participants (Table 3). This value was similar when considered among the anemic and non-anemic participants. Although among the anemic lactating mothers the mean milk iron concentration of 0.038 ± .02 mg/100g was lower than the mean milk iron concentration of 0.044 ± .02 mg/100g among the non-anemic lactating mothers. When the concentration was compared between the 3-month and 6-month supplemented anemic mothers, it showed that 6-month supplemented mothers had a lower mean milk iron concentration of 0.036 ± .04 mg/100g compared to 039 ± .02 mg/100g of the 3-month supplemented mothers. Among the non-anemic mothers, the 3-month supplemented had baseline mean milk iron concentration of 0.044 ± .02 mg/100g quite lower than the 6-month supplemented of 0.049 ± .01mg/100g.

Table 3

Iron concentrations in breast milk for all participants, anemic and non- anemic lactating women supplemented for 3 and 6 months

	Months Postpartum		
	Baseline (2 weeks postpartum)	*Midline (after 3 months)*	*Endline (after 6 months)*
Milk iron (mg/100g) All participants	0.044 ± .02	0.042 ± .02	0.033 ± .01
Anemic (*n* = 10) Supplemented: 3 months (*n* = 8)	0.039 ± .02	0.039 ± .01	0.035 ± .01
6 months (*n* = 2)	0.036 ± .04	0.039 ± .01	0.045 ± .01[a]
Non-anemic (*n* = 22) Supplemented: 3 months (*n* = 19)	0.044 ± .02	0.045 ± .02	0.033 ± .02
6 months (*n* = 3)	0.049 ± .01	0.038 ±.01	0.025 ± .002[b]

All values are means ± SD

* Means with different letters are significantly different, LSD at .05.

After 3 months of iron supplementation, in general, the concentration of breast milk iron remained with a very slight increase (0.036 mg/100g to 0.039 mg/100g) among the 6-month supplemented anemic mothers and a slight decrease (0.049 mg/100g to 0.038 mg/100 g) among the 6-month supplemented non-anemic mothers. At 6 months, a decline was observed among all the participants with a mean milk iron concentration of 0.033 ± .01mg/100g. The milk iron concentration of the 3- and 6-month supplemented non-anemic lactating mothers decreased to 0.033 ± .02 mg/100g and 0.025 ± .002 mg/100g, respectively. However, the iron concentration almost remained constant with a slight increase (0.045 ± .01mg/100g) among the anemic lactating mothers who continued to take iron supplements while the breast milk iron concentration among the 3- month supplemented decreased to 0.035 ± .01 mg/100g. At this point, the difference on the iron concentration between the anemic and non-anemic mothers supplemented for 6 months was statistically significant at 5 % significance level.

The iron concentration in breast milk among the participants in this study was comparable to previous studies in other countries (Feeley et al., 1983, Vouri et al., 1980, Domellof et al., 2004, Dhonushe-Rutteen

et al., 2005 and Kumar et al. 2008). These studies reported milk iron concentrations ranging from 0.10 - 0.57 mg/L .Mastroeni and co-workers (2006) reported a mean iron concentration of 0.12 ± 0.08 mg/100 ml. in colostrum and 0.09 ± 0.05 mg/ml. of mature milk among nursing mothers in Brazil. Parr et al. (1991) as cited by Dorea (2000) reported 0.72 mg/L of milk iron at 3 months lactation in a study conducted in the Philippines. The concentration of iron observed in Filipino milk was higher than for milk samples from other countries. This value reported was also higher compared to the results of this present study. The result however is higher compared to an earlier study of Macapinlac and Guzman, (1984 cited in RENI, 2002) which reported a mean iron content of breast milk of Filipinos to be 0.22 mg/L. Booth and Aukett (1997) reported that iron in breast milk is present in low concentrations ranging from 0.06-.09 mg/100 ml. but is uniquely well absorbed and utilized.

The results of this study also showed that at 6 months lactation, the mean milk iron concentration decreased. A similar trend was observed in other studies during the course of lactation. In a follow-up study of 27 mothers by Vouri et al. (1980), a decrease in the iron concentration of human milk was associated with increasing stage of lactation regardless of the dietary intake of iron. Human milk iron gradually falls from about 0.5 mg/L during the first month of lactation to about 0.3 m/L by 4 to 6 months (Brody, 1994). Raj and co-investigators (2008) reported the mean breast milk iron among non-anemic Indian women to be ranging from 0.89 to 0.26 mg/L and 0.86 to 0.33 mg/L in the anemic mothers from birth to six months. These investigators also noted that there was no significant difference in the breast milk iron between non-anemic and anemic mothers on day 1, 14 weeks and 6 months after delivery.

As regards to the effect of oral iron supplementation during lactation, results of this present study confirms the findings of other studies that the maternal iron status does not seem to play a significant role in breast milk iron concentrations . This present study showed that there was no significant difference in the mean milk iron concentrations after 3 months of iron supplementation. Iron supplementation which increased the hemoglobin level of anemic mothers did not affect milk iron concentrations. It was observed however, that the anemic mothers who continued to take iron supplements until six months had increased breast milk iron concentration at 6 months while it declined among the non-anemic participants.

Domelloff and co-workers (2004) reported that milk iron was not associated with maternal mineral status. Celada et al. (1982) also reported no correlation between the levels of serum ferritin or transferrin saturation on breast milk iron. Murray et al. (1978) found no significant difference in the iron content of breast milk from mothers with deficiency, overload, or normal iron status. Maternal iron deficiency with serum iron concentration of 0.34 mg/L compared with maternal iron concentrations of 2.35 mg/L showed comparable milk iron concentrations.

On the contrary, the study of Fransson (1983) as cited by Dorea (2000) showed that severely anemic Indian mothers (< 80 g/L hemoglobin) had total breast milk iron concentration (1.4 mg/L) higher than those from mothers with higher hemoglobin levels. In another study that the same investigators conducted, they found a significant inverse relationship between iron concentration in milk and maternal hemoglobin levels.

Little is known about the mechanisms and regulation of iron transfer from the mammary gland into milk. However, these results suggest that the iron transport from mother to the milk is irrespective to the maternal iron stores. Indeed, as suggested by Domellof and his co-workers that the transport of iron through the mammary gland epithelium is regulated. This interpretation is in agreement with findings in animal studies. Leong and Lonnerdal (2005) studied the role of intestinal iron transporters, divalent metal transporter 1 (DMT1) and ferroportin 1 (FPN1) in rat mammary gland at different stages of lactation and evaluated the effects of maternal iron status. Their result showed that both DMT 1 and FPN1 are present in rat mammary epithelial cells and that their expression is higher during early lactation and decreases throughout the course of lactation. The decrease in DMT1 and FPN1 correlated with the decline in milk iron during lactation. The investigators believed that both higher milk iron concentrations and higher requirements of iron by the gland may have contributed to the need for expressing more DMT1 and FPN1

during early lactation. Immunostaining also showed that both DMT1 and FPN1 are localized intracellularly in the mammary epithelial cells. This led them to believe that DMT1 may be the iron transporter in the endosomal membrane and that it transports iron out of the endosome to the cytoplasm of the mammary epithelial cells. On the other hand, the intracellular localization of FPN1 suggests the possibility that FPN1 may be involved in the intracellular trafficking of iron between the cytosol and organelles.

These investigators believed that a likely scenario for the metabolic handling of iron in the mammary gland is as follows: iron bound to transferrin in the circulation is taken up by the transferrin receptor at the basal side of the mammary epithelial cell. The transferrin - transferrin receptor is endocytosed into an endosome, where iron is released from transferrin at low pH. Iron is then transported out from the endosome to the cytoplasm via DMT1. The intracellular localization of DMT1 is consistent with its roles in endosomal ferrous transport. Once transported out of the endosome, ferrous iron may be oxidized by a ferroxidase such as ceruloplasmin or hephaestin, which allows iron to be incorporated into ferritin or bound to iron-transport proteins in ferric form. Iron may then be utilized by the oxidative phosphorylation cytochrome system because lactation is an energy-expensive process. Iron in the cytoplasm can also be transported into the Golgi and secretory vesicles by unknown mechanisms. FPN1, which is found to be intracellularly localized, may be involved in this intracellular trafficking of iron between the cytosol and organelles. Iron can then bind to iron-binding proteins, such as casein, lactoferrin and transferrin, which are present in the Golgi and are secreted by exocytosis. Iron in the cytoplasm may also be incorporated into iron-containing enzymes, such as xanthine oxidase, which are secreted with the milk fat globule.

Similar to the results of this present study, results of their study also showed that milk iron was maintained at the same concentration on low-iron rats as in the control rats. This finding implies that there is regulation in the mammary gland that increases the efflux of iron into the milk of low-iron rats as in most human studies that showed that maternal iron status and maternal iron intake do not appear to affect milk iron concentrations. In early lactation (day 10), mammary gland iron was lower in the low-iron rats, which was accompanied by a decrease in mammary gland ferritin protein. However, they observed no increase in DMT1 and FPN1 gene and protein expression, as would be expected in the small intestine during iron deficiency, which suggests different regulatory mechanisms than in the intestine. In addition, no significant change in transferrin receptor protein concentration was found. Interestingly, they observed a smaller size (39-kDa) DMT1 in the low-iron rats, which they believed to be triggered by the lower mammary gland iron. The function of this smaller-size DMT1 protein is not clear but the investigators speculate that it may be responsible for the increase in iron efflux into the milk, thereby maintaining milk iron concentrations in the low-iron rats. They believed that this 39-kDa DMT1 protein may act as an iron chaperone; however, suggested that further studies are needed to confirm the role in increasing iron efflux into the milk during low iron status. During late lactation, the mammary gland iron was not significantly different between the low iron- and control rats. The maintained mammary gland iron in the low-iron rats was attributed to be due to:1) an increase in transferrin receptor in the low-iron rats, which, in turn, increases mammary gland iron, and 2) a lower milk output during late lactation and hence less iron efflux into the milk.

In summary, the presence of DMT1 and FPN1 points to the involvement of these transporters in the transfer of iron from the mammary gland into milk. The response of DMT1 and FPN1 in the mammary gland to low iron status is different from that in the small intestine, which indicates a difference in the regulation of these transporters between tissues. The authors suggest that the mammary gland DMT1 and FPN1 may not simply be up-regulated to increase iron efflux into milk during iron deficiency. Other factors or proteins may be involved in the regulation of iron transfer to milk during iron deficiency.

Thus, this regulatory mechanisms operating in the mammary gland of rats may be the same mechanisms that have been responsible for the maintenance and increases in the milk iron concentrations of the anemic mothers in this present study. The decreased in the milk iron concentration through the course of lactation is possibly due to the decrease in the expression of the iron transporters particularly

divalent metal transporter 1 (DMT1) and ferroportin1 (FPN1). The slight increases and maintenance of the breast milk iron concentration among the anemic mothers is most likely to be due to an expression of a smaller size divalent metal transporter and an increase in the synthesis of transferrin receptors resulting to the increase in the efflux of iron into the breast milk thereby maintaining milk iron concentrations.

Effect of Oral Iron Supplementation on the Iron Status of Anemic Exclusively Breastfed Infants

Hemoglobin Level of Non-anemic and Anemic Infants Supplemented with Oral Iron Drops

The mean hemoglobin level of infants who became anemic at 3 months based on their hemoglobin level was compared to the non-anemic babies from baseline until 6 months to determine the effect of oral iron drops given to the anemic infants. The mean hemoglobin level of non-anemic and anemic infants at baseline was similar, 179.44 ± 26.13 g/L and 176 ± 25.53 g/L respectively (Figure 8).

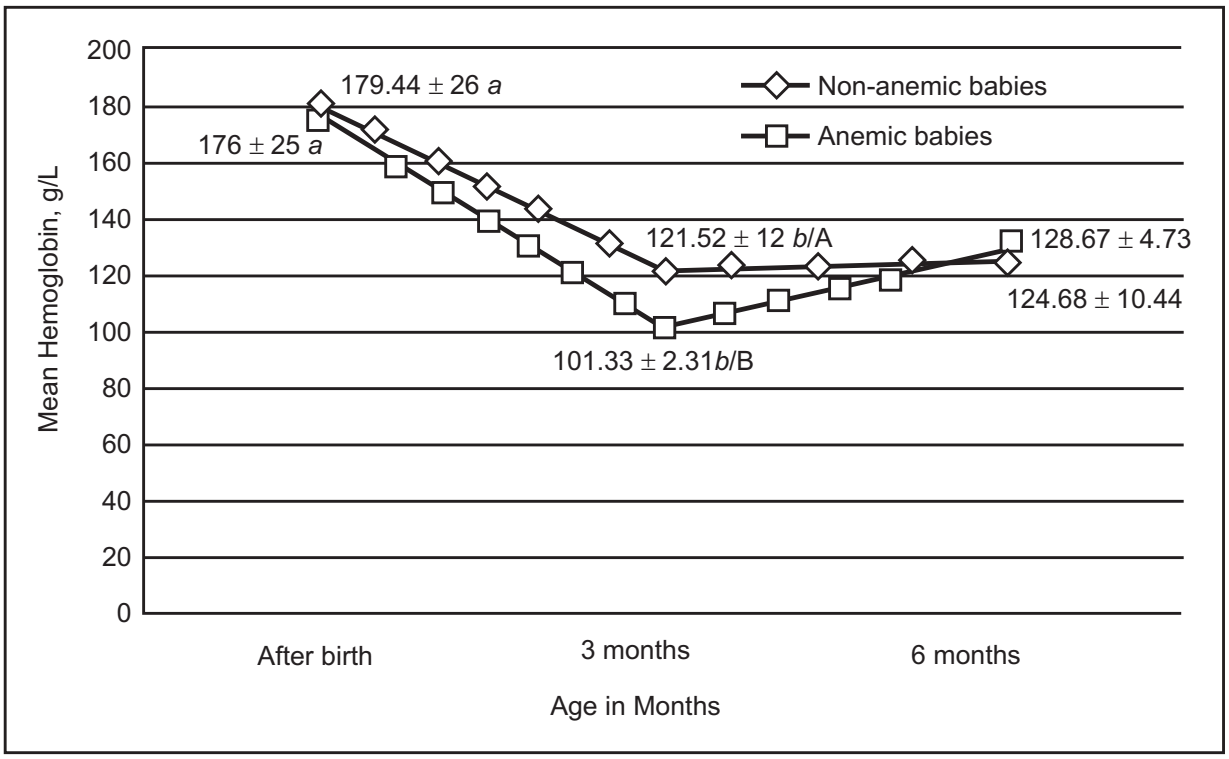

Figure 8: Changes on the hemoglobin level of non-anemic babies and babies who became anemic at 3 months
*** Means after birth to 3 months with different small letters within the same group are significantly different at 1%.**
*** Means at 3 months with different big letters between the anemic and non-anemic babies are significantly different at 5%**

At 3 months of age, there was significant decline in the hemoglobin level of all infants regardless of iron status but the decline was greater among the anemic infants (P<0.01).The difference in their hemoglobin level was statistically significant with the non-anemic infants having a mean hemoglobin of 121.52 ± 12.34 g/L while anemic infants had a mean hemoglobin of 101.33 ± 2.31g/L (P<0.05).

At 6 months of age, after 3 months of taking oral iron drops, the mean hemoglobin of anemic infants increased significantly to 128.67 ± 4.73 g/L (P<0.01). This showed an absolute increase in the mean hemoglobin level of 27 g/L. Among the non-anemic participants (non-supplemented), the mean hemoglobin level also increased to 124.68 ± 10.44 g/L but the mean incremental change of 3 g/L was lesser and was not significant. Results showed that the 0.3 ml. iron drops containing 15 mg. elemental iron/0.6 ml. taken daily was efficient in improving the iron status of anemic infants.

Nguyen et al. (2002) reported that daily iron supplementation for the control of iron deficiency anemia in infants in rural Vietnam resulted in a significant increase in the hemoglobin level of infants by 21.6 ± 12.3 g/L and a decreased in the prevalence of anemia after 3 months of iron supplementation. Domeloff et al. (2002) also reported that iron supplementation had significant effects on iron status of

breastfed infants. Iron-supplemented infants had a higher mean (±SD) plasma ferritin concentrations than did unsupplemented infants at 6 months (116 ± 27 and 69 ± 27 ng/ml, respectively, P=0.002).

Ziegler and co-investigators reported that early iron supplementation of breastfed infants with medicinal iron (ferrous sulfate) in a dose of 7 mg/day from 1 to 5.5 month of age caused some preservation of the iron endowment. Hemoglobin showed the expected decline with age. But the effect was modest and did not extend beyond the period of supplementation. Friel et al. (2003) also found that iron supplementation of breastfed infants had a significant effect on hemoglobin at the end of the supplementation period at 6 months of age.

Relationship Between Indices of Maternal Iron Status and Hemoglobin Level of Infants

The relationship between the indices of the iron status of lactating mothers with the hemoglobin level of infants was determined using Pearson Correlation. Hemoglobin level of the mothers at 3 months was inversely correlated to the infants' hemoglobin level at 6 months (r = 0 – .355; P < 0.10) (Table 4). The hemoglobin level of mothers at 6 months was positively correlated with the infants' hemoglobin level at 6 months (r = 0.374; P0 < .10) at 10 % level of significance. For serum ferritin, at 10% significance level, the baseline serum ferritin of the mothers was also positively correlated to the hemoglobin level of infants at 3 months (r = 0. 342; P < 0.10).

Table 4
Relationship (*r*) between maternal iron status and infant iron status

Material Iron Status Indices	*Hemoglobin Level (Infant)*		
	Baseline	*3 Months*	*6 Months*
Hemoglobin Baseline	.064	.164	–.277
3 Months	.040	.218	–.355*
6 Months	.240	.165	.374*
Serum Ferritin Baseline	–.046	.342*	.038
3 Months	.135	.158	–.248
6 Months	–.282	.205	.049

* significant (P < 0.10)

The inverse relationship of the hemoglobin level of mothers at 3 months with the hemoglobin level of infants at 6 months is seen to be due to the increase in the hemoglobin level of mothers at 3 months which is most likely to be due to the increase in hemoglobin synthesis brought about by the oral iron supplementation. On the other hand, the hemoglobin level of infants tend to decline except for those who took oral supplements because there is minimal dietary absorption from breast milk which is the only exogenous source of iron for the infant. Iron stores are then mobilized to meet the iron requirement. This decline in the iron reserves of the infant may have affected hemoglobin synthesis and breakdown resulting to the decline in the hemoglobin level. Most of the iron in the human body is found in hemoglobin and iron metabolism is largely concerned with the synthesis and breakdown of this protein. Over 65% of body iron is found in hemoglobin (Gropper et al., 2005)

At 6 months, the hemoglobin level of mothers and infants were positively correlated. This reflects that hemoglobin level of infants increased as the hemoglobin level of the mothers increased. In this study, results showed that at this time point, hemoglobin levels of mothers tend to increase except for some of the non-anemic mothers who discontinued taking iron supplements. At the same, the hemoglobin level of infants increased significantly among the anemic infants who took iron supplements while slight increases were observed among the non-supplemented infants.

The significant positive correlation of the mothers' baseline serum ferritin level with the hemoglobin level of infants at 3 months indicate that higher serum ferritin levels representing maternal stores signifies higher hemoglobin levels on the infants at this point. This reflects that if there are enough maternal iron stores, there is likelihood of having adequate hemoglobin concentration until 3 months among their exclusively breastfed infants. The baseline serum ferritn level reflects the maternal iron status at pregnancy. If the pregnant mother has adequate iron intake to provide for the needs of the fetus and placenta as well as her basal needs and expansion of the red cell mass, after delivery, more iron will remain in the body to revert to maternal stores.

Preziosi and his co-workers (1997) reported that pregnant women who took iron supplements had significantly higher hemoglobin, serum iron and serum ferritin values at delivery than those in the placebo group. Consequently, at 3 months after delivery, serum ferritin concentrations were significantly higher in infants of women in the iron-supplemented group. They found no differences according to maternal iron supplementation in serum ferritin, hemoglobin, and other iron indicators in newborns. They did observe, however, that differences in serum ferritin concentrations appeared at 3 months of age and persisted at 6 months. These results indicate the effect of maternal iron status on the offspring.

Turkay and co-investigators (1995) also investigated the influence of maternal iron deficiency anemia on the hemoglobin concentration of the infant. They determined the serum ferritin, iron and hemoglobin values of 27 pregnant women who did not receive oral iron therapy during pregnancy, and the hemoglobin of their normal full-term babies. Mothers were divided into two groups depending on their mean serum levels, the first, consisting of women whose mean serum ferritin was <12 ng/ml and the second group with ferritin levels ≥12 ng/ml at 16 and 34 weeks of pregnancy. The mean serum ferritin levels of mothers at the two time points were compared with the infants' hemoglobin concentration at birth and third month. They found that the difference between these groups were insignificant. In other words, infants' hemoglobin was not affected by maternal ferritin levels. They further concluded that, extreme unusual conditions excluded, maternal iron deficiency does not give rise to iron deficiency in the infant in this period, from birth to the third month postnatal month. The explanation provided is that the placenta and the fetus have a special affinity to iron in the mothers' circulation and iron is transported through the placenta in spite of concentration gradient. This mechanism provides enough iron for fetal erythropoeisis, even if maternal iron storages are depleted. Consequently, hemoglobin concentration of the newborn is not affected by maternal iron depletion or latent iron deficiency anemia.

During pregnancy, the estimated increases in iron requirement are 315 mg for fetal and placental deposition and 500 mg for the increase in erythrocyte mass or a total of 815 mg (FAO/WHO, 1988 cited in RENI, 2002). At delivery, the actual loss of iron in blood, including blood iron entrapped in the placenta, may be in the range of 150-250 mg. Of the 500 mg allowed for erythrocyte mass, about 250-350 mg remains in the body to revert to maternal stores (RENI, 2002).

5. CONCLUSIONS AND RECOMMENDATIONS

Conclusions

The following conclusions were drawn from the results of the study :

- Thirty one per cent of lactating mothers are suffering from iron deficiency anemia in this province. This occurrence of iron deficiency anemia is of moderate magnitude following the Epidemiological Criteria for Assessing Severity and Magnitude of Nutritional Anemia in the Population (FAO/WHO, 1992). At the same time, 44% of the lactating mothers have depleted iron stores having serum ferritin levels <15 ng/ml.
- Moderate iron deficiency anemia exists among exclusively breastfed infants less than 6 months of age. The occurrence of 12 % exclusively breast fed infants (born from anemic mothers) suffering from iron deficiency anemia at 3 months indicate that in deed iron endowment of some infants

is affected by unusual circumstances of iron deficiency in the mother. Infants born from anemic mothers are at risk of becoming anemic because these infants are likely to exhaust their stores and become iron deficient or anemic even before 6 months of age.

- Oral iron supplementation significantly improves maternal iron status. The significant increase in the hemoglobin concentration among the lactating mothers (average incremental change in the hemoglobin level of 10 g/L among all lactating mothers and 27 g/L among the anemic lactating mothers) and the overall increase in the serum ferritin level of 10.75 ng/ml after 3 months of oral iron supplementation proved that taking oral iron supplements containing 60 mg. elemental iron and 400 μg folic acid improves maternal iron status and is more efficient among anemic mothers. However, 20% still had depleted iron stores after 3 or 6 months of iron supplementation implying that a longer period of supplementation is necessary for them to build on their iron stores.
- The significant increase in the hemoglobin level with an average incremental change of 27 g/L among anemic infants after 3 months of iron supplementation proves the efficiency of oral iron supplements in improving the iron status of infants. The giving of 0.3 ml. iron drops containing 15 mg. elemental iron/0.6 ml. daily among anemic infants would certainly improve anemia prevalence among young infants.
- The mean breast milk iron concentration of lactating mothers in Benguet Province of 0.04 mg/100g is comparable to national and international data. Overall, maternal iron status does not seem to play a significant role in breast milk iron concentrations. Oral iron supplementation does not seem to influence breast milk iron. However, the effect may be different in anemic women, as was observed with the very low breast milk iron concentration in women with the lowest hemoglobin levels and in the increases on milk iron concentration of anemic women with improved iron status at 6 months. Breast milk iron concentration generally decreases with increasing stage of lactation but tends to remain constant among iron supplemented anemic women.

6. RECOMMENDATIONS

In the light of the above findings, the following recommendations are hereby proposed:

- The occurrence of moderate magnitude of iron deficiency anemia among the lactating mothers necessitates the continued provision of iron supplements to all lactating mothers for three months postpartum as an immediate intervention. Iron status of lactating mothers should be monitored after three months of iron supplementation (through hemoglobin determination) and mothers found to be anemic should continue taking iron supplements until six months. Selective iron supplementation should continue for anemic lactating mothers until they have attained normal iron status even beyond six months. Strict implementation of iron supplementation among pregnant women is also recommended to prevent repercussions among their infants.
- Alongside with oral iron supplementation as an immediate intervention, long-term sustainable programs towards anemia prevention and control should be intensified. These should include food-based approaches, including the intensification of food fortification, dietary diversification and food product development. Government authorities, academic and research institutions and partnership with the private industries should continue to look for suitable food vehicles that are accessible and affordable to be fortified. Development of iron- rich locally available food products is also seen to be a promising strategy to make iron-rich foods available.
- Iron deficiency among exclusively breastfed infants do occur at 3 to 6 months in full-term, normal birth weight infants. Thus, preventive strategies that would include screening with selective supplementation is recommended as a more suitable approach for the prevention of iron deficiency than universal supplementation during early infancy. Babies born from anemic mothers should be closely monitored because they are at risk for iron deficiency anemia. The 0.3 ml. iron drops given daily containing 15 mg elemental iron /0.6 ml (recommended by the Department of Health for

low birth weight infants) for 3 months was shown to result to an average increase of 27 g/L in the hemoglobin level of anemic infants. Thus, this preparation could be recommended for use among anemic infants < 6 months of age. Policy adjustments and guidelines along this recommendation could be instituted.

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