A Prospective Study of Iron Status of Exclusively Breastfed Infants Weighing 1800-2499g At Birth and Correlation With Breast Milk Lactoferrin

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Abstract: *Background:* Iron status of exclusively breastfed low birth weight (LBW) infants has not been evaluated sufficiently. We determined the iron status of infants weighing 1800-2499g at birth and correlated it with breast milk lactoferrin levels of their mothers.

Methods: Sixty five exclusively breastfed LBW infants were recruited at 6 weeks and followed-up until 6 months of age. Iron parameters (serum iron, serum ferritin, iron binding capacity, percent transferring saturation) and breastmilk lactoferrin were measured and compared at 6 weeks and 6 months of age. Hemoglobin (Hb) was measured at 6, 10, 14, 18 weeks and 6 months of age. If at any time, Hb of the infant was <10.5g/dl then iron supplementation was started at 3mg/kg/day for a minimum of 2 months or till Hb rose to>10.5g/dl.

Results: Fifty (76.9%) out of 65 infants developed anemia between 10 weeks and 6 months of age. At 6 months of age 32.2% babies showed iron deficiency (serum ferritin level <10ng/mL). There was no correlation between breast milk lactoferrin and infants' serum iron profile either at 6 weeks or at 6 months of age.

Conclusion: About 1/3rd infants developed iron deficiency state at 6 months of age. Three-fourth infants required iron supplementation by 6 months of age. There was no relationship between maternal breast milk lactoferrin with infants' iron profile either at 6 weeks or at 6 months of age.

Keywords: Low birth weight infants, serum ferritin, serum iron, percent transferring saturation, lactoferrin, exclusive breastfeeding.

1. INTRODUCTION

World Health Organization (WHO) recommends exclusive breastfeeding to all infants till 6 months of age [1]. Majority of low birth weight (LBW) infants weighing 1800g to 2499g at birth are able to breastfeed and maintain normal growth and development on exclusive breastfeeding up to six months of age. Iron is essential to many physiological processes, particularly the development of the central nervous system and cognitive functions. During periods of rapid growth, such as infancy, adequate iron levels are essential to maintaining normal development [2]. The iron content of human milk, although low in quantity, has high bioavailability and is considered adequate for the first 6 months of life in term appropriate for gestation infants [3, 4]. It is not known what happens to iron status of LBW and late preterm infants on exclusive breastfeeding. Lactoferrin, an iron binding glycoprotein, is one of the major bioactive components of breastmilk that ensures absorption of iron in the breast milk [5]. It has been reported that breastmilk lactoferrin significantly decreases from onset to 6 months in to lactation and that it has no relationship with the infant's serum iron, serum ferritin, iron binding capacity, and hemoglobin [6]. On the other hand, it has been recently reported that when lactoferrin fortified formula is fed to the breastfed infants it significantly improves serum ferritin and total body iron contents of the infants [7]. Iwai *et al,* [8] conducted a study to assess the iron status of breastfed infants, with birth weight less than 2500g, compared to formula fed babies and found that breastfed LBW infants have higher risk of developing iron deficiency and should receive iron supplementation from 2 months of age.

A Cochrane Database Systematic review in 2012 [9] concluded that preterm LBW infants who received iron supplementation have slightly higher hemoglobin level, improved iron stores and lower risk of developing iron deficiency anemia. But these studies did not ensure exclusive breastfeeding for six months. Another limitation was that most of the studies comprised of heterogeneous group of infants weighing from 1000g to 2499g at birth including very preterm and very low birth weight infants. Late preterm and those weighing 1800-2499g at birth are a special group of infants who have distinct physiological limitations and capabilities

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compared to infants born at <34 weeks gestation and or weighing <1500g at birth. This prospective hospital based study was, therefore, conducted on exclusively breastfed LBW infants weighing 1800-2499g at birth to measure serum ferritin at 6 weeks and 6 months of age and correlate iron profile [serum ferritin (SF), serum iron (SI), total iron binding capacity (TIBC) and percentage saturation of transferring (%TS)] with the breast milk lactoferrin of their mothers.

2. MATERIAL AND METHODS

2.1. Participants

The study was conducted in a teaching hospital. Consecutively born singleton babies weighing 1800-2499g at birth, irrespective of the gestation, and their mothers with hemoglobin ≥11gm/dl at admission for delivery were recruited at 6(+2) weeks of age. These mothers had uncomplicated antenatal course; initiated breastfeeding within one hour, and were exclusively breastfeeding their infants since birth and were willing to do exclusive breastfeeding till six months of age. Infant was classified as term (gestation age 37-41 completed weeks), preterm (gestation age <37 completed week) and post term (gestation age ≥42 completed weeks) according to last menstrual periods Babies weighing between 10th to 90th percentile of birth weight at a gestation were designated as appropriate-for gestation and those below 10th percentile birth weight for gestation were called small-for-gestation [10]. Infants born with perinatal asphyxia, clinical evidence of chromosomal anomalies and major congenital malformations; infants required exchange blood transfusion, treated for sepsis, or initiated on formula feeding in early neonatal period were excluded. Hemoglobin (Hb) was measured at the time of recruitment and infant having Hb<10.5gm/dl was also excluded. All mothers were counseled for practicing exclusive breastfeeding and regular follow-up. At each follow-up visit, the infants were immunized as per State Immunization Schedule and their anthropometric parameters including weight, length, and head circumference were recorded by MM as per standard practice. Hemoglobin was estimated at each follow up visit ostensibly for ethical reasons so that no infant was denied iron supplementation, if Hb fell below 10.5g/dl, at the @ 3mg/Kg for 2 months. The importance of exclusive breastfeeding was reinforced to mothers at each follow-up. Few infants on follow up were found to have received occasional sip of water. Their mothers were counseled to stop giving water and practice exclusive breastfeeding; however, these predominantly breastfed infants [11] were also included in the study as it was assumed that intake of occasional few sips of water would not affect body iron stores. All mothers received calcium, iron and folic acid supplements according to the attending obstetrician.

2.2. Number of Subjects

The sample size was based on a study by Iwai *et al,* [8]. In this study the mean serum ferritin level in low birth weight exclusively breastfed children at 6months of age was 18ng/ml (range 8.3 to 39.1ng/ml). Keeping α and β errors at 5% and 20% (Power being 80%) respectively and assuming precision (absolute) to be 10%, we required 65 mother-infant pairs [12]. As an attrition of 20% was expected on six months follow up finally 80 infant-mother pairs were recruited. The institutional Ethical Committee approved the study.

2.2.1. Sample Collection and Follow-Up

a. Collection of blood samples at the time of recruitment:

I. 0.5ml of venous blood of the infants was collected in EDTA vial for hemoglobin estimation by using automated hematology cell counter MS-9 (Melit Schloesing Laboratories). The automated cell counter was calibrated using standard procedures [13-16]. Infant having Hb≥10.5g was included in the study. Hb level <10.5g/dl was taken as anemia.

II. Three ml blood was collected by venipuncture in an iron free vial. Serum was separated and stored at -20^{0}C for serum iron, serum ferritin, total iron binding capacity (TIBC) and percent transferring saturation (%TS) estimation. The serum samples were thawed to room temperature and serum iron, TIBC and percentage saturation were measured as per the modified guidelines of International Committee for Standardization in Hematology [17]. Serum ferritin was measured using ELISA kit - Microwell Ferritin EIA (DRG Diagnostics EIA 1872, lot no. RN-40499). Serum ferritin level <10ng/ml was taken as iron deficient state.

b. Collection of blood samples on follow-up:

i At 10 (+1), 14 (+1) and 18 (+1) weeks: 0.02ml of blood was taken by heel prick technique and Hb was estimated by cyanmethemogobin method.

ii At six months: Hb and serum iron profile were estimated in a similar manner as described above.

2.2.2. Collection and Analysis of Breast Milk

The breastmilk lactoferrin was measured at 6 (+2) weeks and 6 (+2 weeks) months after delivery. The mother was counseled and demonstrated on a breast model how to manually express the breastmilk. She was requested to collect the breastmilk by manual expression in the morning. After collecting around 10ml of foremilk in a sterile, acid washed and rinsed iron free container, baby was breastfed for about 15 minutes and thereafter, 10ml of hind milk was collected from the same breast. The two samples were mixed and kept frozen at -80°C until analyzed. Before analysis breastmilk samples were thawed and mixed thoroughly. Breast milk Lactoferrin was measured at National Institute of Immunology, New Delhi using a quantitative sandwich enzyme immunoassay technique by Assay Max human lactoferrin ELISA kit (supplied by ASSAYPRO; Catalog No. EL2011-1).

During follow-up at 4 weeks interval if Hb of the infant was found to be <10.5g/dl, iron supplementation was started at 3mg/kg/day in the form of ferrous fumarate drops (elemental iron 5mg/ml). The iron supplementation was continued for a minimum of 2 months or till Hb reached ≥10.5gm/dl.

2.2.3. Statistical Analysis

Data was analyzed using SPSS software package (version 17.0). Mean and standard error (SE) values of serum iron, serum ferritin, total iron binding capacity and percent transferring saturation, and breastmilk lactoferrin at 6 weeks and 6 months in to lactation were compared by paired t-test. A mean standard error (SE) was obtained both for iron supplemented as well as iron unsupplemented babies and compared as well. Change in the Hb values was compared at each interval of follow up. One way ANOVA was applied to test for serum iron parameters for development of anemia over time. A correlation between breast milk lactoferrin levels and serum ferritin of the infants was done by simple regression analysis and a correlation coefficient was obtained.

3. RESULTS

Out of 80infants 65 completed the study successfully. The male and female ratio was 1.3:1.The mean birth weight was 2155g (SE 0.026) and mean gestation age was 37.18 weeks (range 34-41wks). Forty two babies (64.6%) were born at term gestation (37-41 weeks) and 23 infants (35.4%) were late preterm (34-36 weeks). Forty five (69.2%) infants were appropriate for gestational age (AGA).

The mean weight of the babies at 6 weeks was 3.43kg (SE 0.067) and they gained approximately 140.6g weight per week between 6 weeks and 6 months of age which was normal for the exclusively breastfed infants; an exclusively breastfed term normal infant gains a minimum of 125g weight per week [18]. The corresponding mean length [52.31cm(SE 0.27)] and head circumference [34.82cm(SE 0.15)] increased as expected over six months of age.

The mean Hb of the infants at 6, 10, 14, 18 weeks and 6 months of age was 11.46gm/dl (SE 0.11), 11gm/dl (SE 0.11), 10.78gm/dl (SE 0.10), 10.88gm/dl (SE 0.89) and 10.79gm/dl (SE 0.12) respectively and showed a decrease over time with a nadir at 14 weeks of age. The decline in the hemoglobin was significant (p<0.05) at each interval of follow-up (Table **1**).

Of the 65 infants 76.9% (n=50) exclusively breastfed LBW infants required iron supplementation from 10th week of life. The prevalence of anemia in the infants at 10, 14, and 18weeks and 6 months of age was 30.8% (n=20), 43.1% (n=28), 26.2% (n=17) and 38.5% (n=25) respectively (Figure **1**).

Serum ferritin, which could be done in 59 out of 65 infants due to technical reasons, was 128.88ng/ml (SE 5.045) and 25.02ng/ml (SE 2.627) at 6 weeks and 6

Table 1: Mean Hemoglobin and Change in Mean Hemoglobin Levels at 6, 10, 14, 18 Weeks and 6 Months

Age (n=65)	Hemoglobin (g/dl)		Change in Hemoglobin levels (g/dl)	Difference (g/dl) (p-value)
	Mean	SE		
6 wks	11.46	0.115		
10 wks	11.00	0.115	Hb10 wks – Hb 6 wks	-0.46 (<0.05)
14 wks	10.78	0.101	Hb14 wks – Hb 10 wks	-0.22 (<0.05)
18 wks	10.88	0.894	Hb18 wks – Hb14 wks	0.1 (<0.05)
6 months	10.79	0.116	Hb24 wks – Hb18 wks	-0.09 (<0.05)

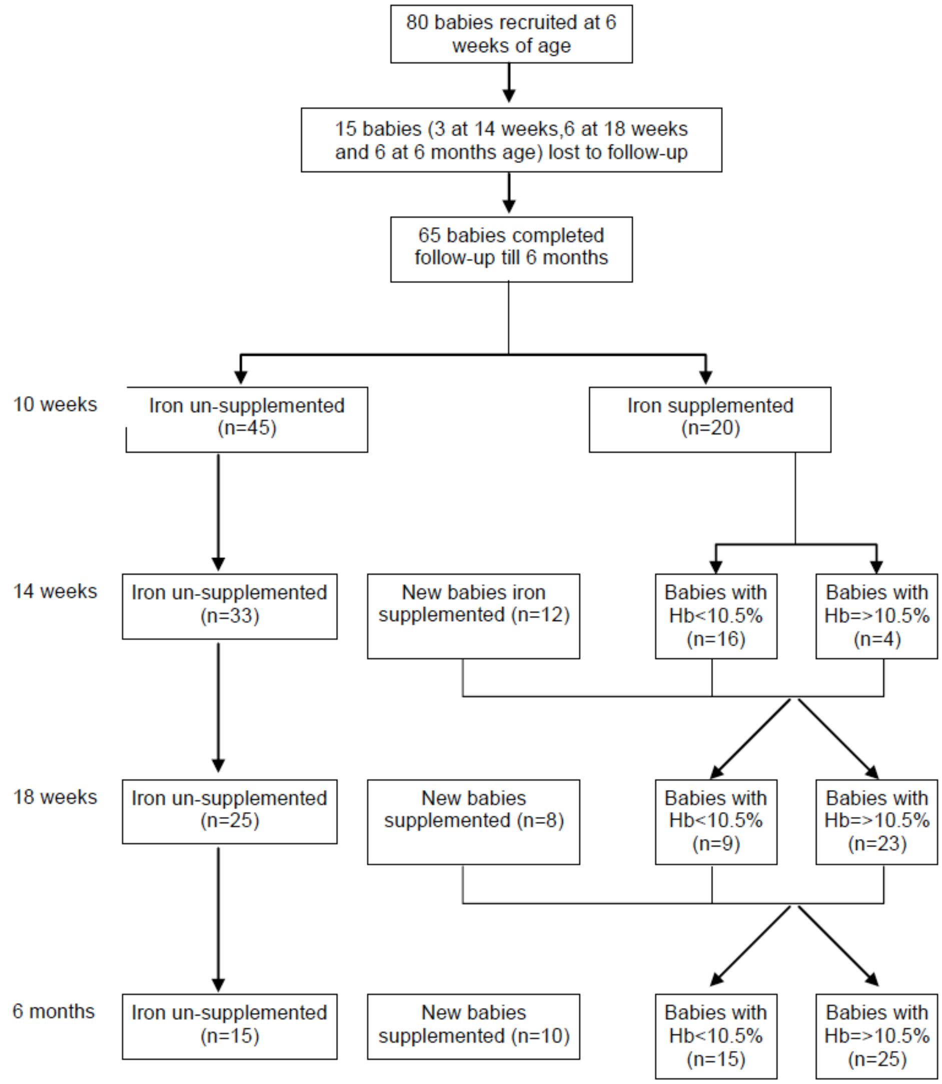

Figure 1: Scheme of follow-up and iron supplementation of infants.

months of age respectively and the fall in the serum ferritin levels was significant (p<0.001). Similarly serum iron levels significantly fell (p <0.001) from 6 weeks of age (106.11µg/dl SE 3.586) to 6 months of age (62.04µg/dl SE 3.707) (Table **2**).

There was no significant difference in the serum ferritin levels in iron supplemented [129.33ng/ml (SE 6.16)] and iron non supplemented infants [127.31ng/ml (SE 7.54)] at 6 weeks of age. There was also no significant difference in the 6-week serum ferritin levels and requirement for iron supplementation at subsequent age intervals (one way ANOVA 0.478; p>0.05). However, serum iron levels and %TS were significantly more (p<0.001) in infants who did not require iron supplementation (Table **3**) and lower the 6-

Table 2: Iron Profile of Infants at 6 Weeks and 6 Months of Age

Iron Parameters n=65	6 Weeks		6 Months		p-Value (2-Tailed)
	Mean	SE	Mean	SE	
S. ferritin (ng/ml) (n=59)	128.88	5.045	25.02	2.627	<0.001
S. iron ((µg /ml)	106.11	3.586	62.04	3.707	<0.001
TIBC (µg/dl)	376.83	5.003	398.95	3.389	<0.001
% TS	28.325	1.008	15.925	0.865	<0.001

Table 3: Iron Parameters in Iron Supplemented and Non Iron Supplemented Babies at 6 Weeks of Age

Time of Iron Supplement or no Iron Supplement	Iron Profile							
	S. Ferritin (ng/ml)		S. Iron (µg/dl)		TIBC (µg/dl)		% TS	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
10 weeks* (n=20)	136.05	9.51	89.05	5.86	371.30	10.72	24.13	1.83
14 week* (n=12)	127.33	11.68	99.25	6.21	390.75	12.05	26.43	1.26
18 weeks* (n=8)	137.88	13.91	106.13	5.95	381.38	14.01	28.49	1.88
6 months* (n=10)	108.78	15.72	109.90	5.51	372.60	10.68	29.61	1.22
Total iron supplement # (n=50)	129.33 (n=46)	6.16	98.40	3.28	377.84	5.97	26.23	0.91
No iron supplement # (n=15)	127.31 (n=13)	7.54	131.80	8.23	373.47	8.89	35.56	2.35
(One way ANOVA between groups*) (P value)	0.478 (>0.05)		0.000 (<0.001)		0.726 (>0.05)		0.000 (<0.001)	

Significance 2 tailed (p value): S. ferritin (0.870 p>0.05); S. iron (0.000 p<0.001); TIBC (0.716 p>0.05); %TS (0.000 p<0.001).

Table 4: Iron Profile of Iron Supplemented and Iron Un-Supplemented Babies at 6 Months of Age

Iron Profile Parameters	Iron Supplemented Babies (n=50)		Iron Un-Supplemented Babies (n=15)		Significance (2 Tailed) (p Value)
	Mean	SE	Mean	SE	
S. ferritin (µg/ml)	23.68 (n=46)	3.01	29.73 (n=13)	5.38	0.344 (>0.05)
S. iron (µg/dl)	59.09	3.29	71.87	11.70	0.148 (>0.05)
TIBC (µg/dl)	399.90	3.78	395.80	7.74	0.614 (>0.05)
% transferring saturation	15.19	0.78	18.37	2.67	0.122 (>0.05)

week serum iron and hemoglobin levels, earlier was the development of anemia and requirement of iron supplementation (one way ANOVA 0.000; p<0.001).

Although serum ferritin levels were similar in iron supplemented and iron unsupplemented infants [23.68ng/dl (SE 3.01) vs 29.73ng/dl (SE 5.38)] at 6 months of age yet 32.2% (n=19/59) infants had serum ferritin level <10ng/mL indicating iron deficiency state in them (Table **4**). The mean serum ferritin levels of iron deficient infants at 6 weeks and 6 months of age were 128.47ng/dl [SE 9.176] and 6.03ng/dl [SE 0.524] respectively. Of them 12 infants had received iron supplementation as per protocol (5 infants at 10 weeks, 4 infants at 14 weeks, and 3 infants at 18 weeks of age). Five of the remaining 7 infants became anemic at 6 months of age and required iron supplement and 2 infants remained non anemic. Serum iron, TIBC and %TS were also not different at 6 months of age between iron supplemented and iron non supplemented infants.

The serum ferritin levels were significantly higher at 6 weeks of age in girl infants [144.05ng/ml (SE 7.179); p=0.03] compared to their male counterparts (121.10ng/ml (SE 6.391)]. Infants were further divided in to two groups according to birth weight. In infants weighing 1800-2000g at birth the serum ferritin level was 146.43ng/ml (SE 9.98) whereas in infants weighing 2001-2499g at birth, it was 123.42ng/ml (SE 37.43) and the difference in the serum ferritin levels was just above the significance level (p=0.052). At 6

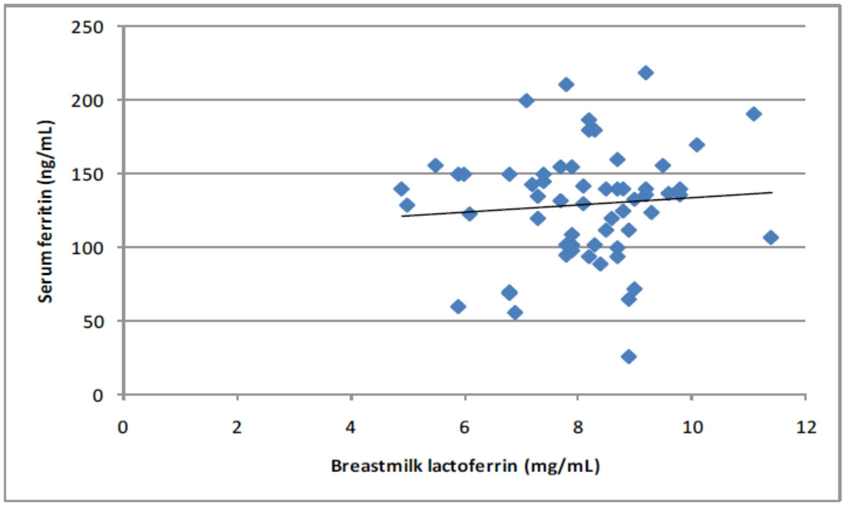

Figure 2: Correlation between breast milk lactoferrin and serum ferritin at 6 weeks (r-value 0.083; p=0.532).

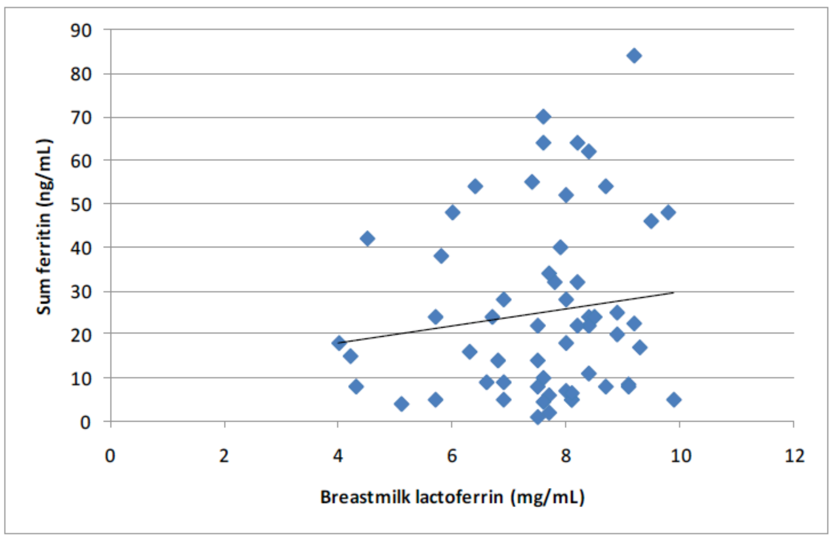

Figure 3: Correlation between breast milk lactoferrin and infants' serum ferritin at 6 months (r-value 0.132; p=0.320).

months of age the serum ferritin levels equaled in girls and boys (p=0.143), and in infants of both weight groups (p=0.131). Gestation of the infants did not affect serum ferritin levels either at 6 weeks (p=0.942) or at 6 months [24.80ng/ml (SE 3.21) vs 25.40ng/ml (SE 4.66) p=0.914] of age.

The mean breast milk lactoferrin levels in our study were 8.15mg/mL (SE 0.16) at 6 weeks of age which significantly (p=0.03) fell down to 7.62mg/mL (SE 0.16) at 6 months of age. But there was no correlation between serum ferritin levels of infants and breastmilk lactoferrin of their mothers at 6 weeks (r=0.083; p=0.532) (Figure **2**) or at 6 months (r=0.132; p=0.320) of age (Figure **3**). Similarly serum iron levels of infants did not correlate with the breastmilk lactoferrin levels of their mothers at any age (At 6 weeks r=-0.053; p=0.677 and at 6 months r=-0.004; p=0.975).

4. DISCUSSION

Infants weighing between 1800g and 2499g at birth or born as late preterm, gestation 34-36 weeks, are a distinct group of infants who have scrapped past the potential risks to life by a whisker yet fall short on all attributes for survival and quality of life. A deficient body iron store of these infants is one such challenge. A term infant is born with 0.5g of total body iron; maximum iron storage takes place in the last trimester [19]. The iron acquired from mother during placental transfer is proportional to body mass and increases as term approaches [20]. Thus, the preterm low birth weight infants have lower iron stores, and require higher iron as compared to term infants [21].It is estimated that Small for Gestational Age (SGA) infants have approximately one-third of the normal stored body iron compared to full term normal weight infants and are more at risk to develop postnatal iron deficiency. Hemoglobin values of neonate change constantly as the newborn adapts to new environment [22]. Despite dropping hemoglobin levels, the ratio of hemoglobin A to hemoglobin F increases and the levels of 2,3-diperosphoglycerate increase. As a result, the oxygen delivery to the tissues actually increases. Thus, this physiological anemia is not functional as oxygen delivery to the tissues is adequate. At 8-12 weeks, hemoglobin reaches its nadir and oxygen delivery to the tissues is impaired. A minimum level of 11.4±0.9g/dL in term infants is achieved by age of 8-12 weeks. Decline in hemoglobin occurs more rapidly in preterm infants (with lowest values at 4 to 8 weeks, as opposed to 10-12 weeks in term infants). When the hemoglobin level falls to a level low enough to limit tissue oxygen delivery, erythropoietin production is stimulated, erythropoiesis resumes, reticulocytosis occur and the hemoglobin mass increases. During this period of active erythropoiesis, iron stores are rapidly depleted. The reticuloendothelial cells have adequate iron for 15-20 weeks in term infants. After this time, the hemoglobin level decreases if iron is not supplemented, either from diet or through supplements [24].

The bioavailability of iron from the breast milk is very high [20]. It has been reported that exclusive breastfeeding is sufficient to maintain body iron stores of term normal weight infants till six months of age [25-27]. These infants maintain hemoglobin ≥10.5g/dl just on exclusive breastfeeding [3, 4,6,28-31]. This is supported by a study by Domellof *et al,* [4] in 2002. They concluded that the absorption of iron from breast milk was higher in unsupplemented group (36.7±18.9%) vs. that of iron-supplemented group (16.9±9.3%). Thus, this change in iron absorption helps in the infant's ability to adapt to a low iron diet and thereby avoid iron deficiency despite low iron intake in early infancy. But will infants born at 34-36 weeks gestation or infants born at term gestation weighing 1800g to 2499g at birth, maintain hemoglobin and iron stores on exclusive breastfeeding till six months of age without iron supplementation? Enough evidence is not available in the published literature and this has prompted us to explore.

Domellof *et al,* [32] have suggested reference values for iron status variables in exclusively breastfed term infants. According to them Hb>10.5g/dl is a normal 2SD cut off value at 4 and 6 months of age. The iron deficient state is suggested by a 2SD cut off value for serum ferriting as <20ng/ml and <9ng/ml at these ages respectively. But for low birth weight infants, weighing 1800-2499g at birth, these values for Hb and serum ferritin at 4 months and 6 months of age are not defined. Therefore, Hb and serum ferritin values for term infants may be considered for discussion. We assumed Hb value of <10.5g/dl suggestive of anemia and took serum ferritin level <10ng/dl as the lower limit for defining iron deficiency state at 6 months of age.

In the present study 76.91% (50/65) LBW infants, weighing 1800-2499g at birth and exclusively breastfed till six months of age, developed anemia at some stage and required iron supplementation from as early as 10 weeks of age. The difference in hemoglobin at 6 months between iron supplemented and unsupplemented babies was 0.75g/dL and was statistically significant (p<0.05). This was in concordance with the study by Siimes *et al,* [30] which was conducted on full term, AGA infants.

Olivares and colleagues [33] after studying 84 LBW infants also concluded that preterm infants, irrespective of adequacy for gestation age, developed anemia by 4 months of age. Lundstrom *et al,* [34] showed that iron un-supplemented infants weighing 1000-2000g at birth and receiving either breast milk or cow's milk, developed iron deficient state between 3 and 5 months of age and required iron supplementation. We could also demonstrate that almost one-third of all exclusively breastfed infants required iron supplementation from 10 weeks of age.

The serum ferritin levels are a marker of body iron stores; in adults 1µg/l of serum ferritin is equivalent to 8–10mg of stored iron [35]. In newborn infants the ratio of serum ferritin to liver non-heme iron concentration is closer to 1:2.7 [36]. As previously explained, the iron stores are utilized during resumption of the erythropoiesis in early infancy after attainment of physiological nadir in the fall of the Hb. Therefore, the serum ferritin levels at 6 months of age are significantly lower compared to that of 6 weeks of age, Brozovic *et al,* [37], studied 47 preterm infants (29-37 weeks) with mean birth weight of 1517gm (920-1870 g) and prescribed iron from 5 weeks of age. Exclusive breastfeeding was not maintained in these infants. The mean serum iron at 6 months was 44µg/dL which was significantly less than serum iron at birth (78µg/dL) and at 2 weeks of age (105µg/dL). Similar results were obtained for TIBC and percentage transferring saturation. In our study also, the difference in iron profile at 6 weeks and at 6 months was statistically significant (p<0.05).

A time may come when body iron stores are exhausted and serum ferritin levels fall to a critical level in maintaining Hb levels as well as fulfilling other physiological requirements of the body. We found that at 6 months of age 19 infants (32.2%)had serum ferritin <10ng/mL indicating iron deficient state. The mean hemoglobin of these babies was within normal range. This can be explained by continuous utilization of iron stores during resumption of erythropoiesis thereby maintaining normal hemoglobin at the cost of depleted iron stores. Siimes *et al,* [38] observed that infants with iron deficiency anemia have normal values for serum ferritin. This is contrary to the fact in adults that iron deficiency anemia appears only after depletion of iron stores. Thus, the pathogenic mechanisms of iron deficiency could be unique in infancy. Hence iron utilization as measured by serum ferritin response is regulated independently from hemoglobin synthesis [39].

Iwai *et al,* [8], studied iron status in 55 LBW infants of gestational age 30-40 weeks and birth weight ranging from 1000-2499 g. Iron deficiency was defined as either serum ferritin <15µg/mL or MCV <70 fl and if baby developed iron deficiency, iron supplementation started at 2mg/kg/day till the end of the study. Serum ferritin was also significantly lower in breastfed group (18.4±8.3 vs 20.7±6.3) and 86% babies developed iron deficiency, 75% of them were weighing between 2000 – 2499 g. In our study, the prevalence of lower serum ferritin values (<10µg/mL) was 29%. The difference can be explained by difference in sample size of exclusively breast fed babies.

Gorton and Cross [20], studied iron status of 145 preterm infants with gestation ranging from 289.9-33.5 weeks and mean birth weight of 1.8kg. Out of which, 69 infants were given formula containing iron (12mg/q) and the other group received formula without iron. Strained foods were started at 3 months. Iron deficiency was defined as hemoglobin (Hb) <9g/dL and haematocrit (Hct) <32% on two successive examinations. At around 6 months, mean Hb in supplemented group was 11g/dL and in control group was 9.8g/dL. Iron deficiency was seen in1/3rd of babies in control group and they were given iron supplementation accordingly.

Supplementation of LBW infants with iron is important to build up their body stores so that when erythropoeisis starts sufficient iron is available to produce Hb [9,20,34,37]. Although timing of iron supplementation may vary it is widely agreed that very low birth weight and very preterm infants should be given therapeutic iron supplementation from 6-8 weeks of life. It may be mentioned here that serum ferritin levels at 6 weeks of age may not predict subsequent development of anemia and iron deficient state in exclusively breastfed LBW infants weighing 1800-2499g at birth. There is no difference in the serum ferritin levels at 6 weeks in iron supplemented and iron non-supplemented infants in this study. Aggarwal *et al,* [40] in a place to be controlled study determined the effect of iron supplementation on predominantly breast fed term LBW infants at 50 days and 80 days of age. They concluded that there was no significant difference in the serum ferritin levels between the two groups after 4 and 8 weeks of iron supplementation, and that iron supplementation resulted in a marginal increase in the hemoglobin after 8 weeks.

Lactoferrin is an important bioactive component and is the second most abundant protein in the human milk. Its concentration in human milk ranges from 7mg/mL in colostrums to <1mg/mL in the mature milk [41]. Breast milk lactoferrin levels depend on ethnicity, race, geographical area, and stage and duration of lactation [42-45]. Lien *et al,* [46] estimated lactoferrin levels in the breast milk of mothers from 9 countries, excluding Indian mothers, and reported that breast milk lactoferrin ranged from 1.37 to 2.12mg/mL. Our values of the breast milk lactoferrin seem to be high but are in conformity with other Indian study both in terms of values and in terms of significant decline in lactoferrin levels by 6 months of age [6]. It is claimed that lactoferrin fortified formula significantly improves serum ferritin and total body iron contents of the breastfed infants [46] but Davidsson *et al,* [47] could not substantiate that lactoferrin facilitates iron absorption from the breast milk. Our study failed to show any correlation between the serum ferritin and serum iron of exclusively breastfed low birth weight infants with breast milk lactoferrin both at 6 weeks and 6 months of age. This observation is in conformity with the scanty published literature on the subject [6, 31, 48]. Exclusively breastfed LBW infants weighing 1800-2499g at birth have high risk of developing iron deficiency state and breast milk alone is not sufficient to meet adequate iron nutrition till six months of age. The limitation of the study is that the subjects included small for gestation infants as well who may behave differently from AGA infants. It is strongly felt that a large cohort study may indicate time, amount and duration of iron supplementation to LBW infants weighing 1800-2499g at birth.

CONCLUSION

We found that 1/3rd exclusively breastfed LBW infants weighing 1800-2499g at birth developed iron deficiency state at 6 months of age. Three-fourth infants required iron supplementation during follow up from as early as 10 weeks of age and breast milk lactoferrin has no correlation with the serum ferritin and serum iron levels of the infant either at 6 weeks or at 6 months of age.

CONTRIBUTION

MM collected data, recorded anthropometry, estimated iron profile, searched literature, prepared first draft; MMAF conceptualized and designed the study, analyzed data, prepared final draft, and will stand guarantor; SS estimated iron profile and critically scrutinized data; OS estimated lactoferrin, searched relevant literature, helped in preparation of manuscript; AKS carried out statistical analysis and provided critical comments on presentation of the data. All authors approved the final draft.

CONFLICT OF INTEREST STATEMENT

There is NO conflict of interest to disclose.

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