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Original Research Article

Haemoglobin and iron store status in low birth weight babies and effect of early iron supplementation on them - A randomized and open label study

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ABSTRACT

Background: Deficiency of iron affects multiple organ systems, and poor neurodevelopment is the most worrisome effect. Low birth weight neonates are more likely to develop iron deficiency anemia than term counterparts. The guidelines for supplementation of iron in LBW neonates are poorly defined with varying recommendations pertaining to the initiation. Hence this study focuses on the effect of early iron supplementation and iron store status in them.

Materials and Methods: In this randomized open label interventional study, 48 LBW neonates (EI group) were administered iron supplementation (at 2 weeks or earlier when full feeds were achieved), and other 48 were controls. All neonates were assessed for hemoglobin level, RBC count and iron status indicators (Hematocrit, Serum Ferritin) at or after 6 weeks of postnatal age.

Results: The post-intervention values of mean haemoglobin (cases 12.68±1.51g/dl, Controls – 11.38 ± 1.03g/dl), mean serum ferritin (cases: 238.45 ng/ml, CONTROLS- 175.68 ng/ml), mean RBC count (cases: 3.94 million/mm³, and mean hematocrit (cases: 33.16±3.75%, controls 34.04±3.45%) were noted, and the % change at 6 weeks follow up in mean Hb among CASES - 25.19%, CONTROLS 31.45% (*p* value:<0.01); % change in mean ferritin at follow up: cases 15.13%, controls 32.99% (*p* value:<0.001); % change in mean RBC count at follow up: cases 19.21%, CONTROLS 33.82% (*p* value:<0.001)] when compared to pre- intervention values was noted. Though the haematological parameters decreased in both groups, the levels were comparatively higher in the cases compared to the controls, and iron store status was better in early iron-supplemented infants.

Conclusion: Iron supplementation in LBW neonates started as early as 2 weeks postnatally, improves the iron stores and decreases the risk of iron deficiency in these infants.

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1. Introduction

Iron and heme-proteins are pivotal in a wide range of metabolic and synthetic functions that are essential for the normal functioning at cellular level; making iron an indispensable nutrient. Deficiency of iron affects growth and functioning of multiple organ systems, but poor neurodevelopment is the most worrisome effect; leading to poor cognitive, motor and behavioral performance.¹

It is in the first trimester that the placental transfer of iron starts, but significant transfer occurs only in the third trimester.¹ Hence affecting the total body iron content in preterm neonates. In term neonates, 25% of total body iron is in stored form at birth. As haemoglobin level falls after birth with decrease in erythropoiesis, iron is moved back to its stored form. These stores are then used up during the postnatal growth phase. Hence term neonates have very less need of any iron supplementation in first few months of life.

However, low iron stores at birth combined with the rapid postnatal growth phase makes LBW babies (both Term

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SGA and Preterm); more susceptible to iron deficiency compared to term babies. The probability being as high as 77%.² Adding to this, is the higher phlebotomy rates in hospitalized LBW babies.

Prevention of this deficiency in LBW neonates can be achieved by;³

1. Maternal supplementation ensuring that the neonate begins with adequate stores.
2. Minimizing phlebotomy losses that occur during the hospital stays of these neonates.
3. The crucial step of iron supplementation.

However what remains open to controversy is the timing of starting this supplementation.

The American Academy of Paediatrics recommends 2-4 mg/kg/day up to 15mg/day, starting at 2 months of age in preterm neonates. Or when the weight becomes twice the birth weight, and continued throughout the 1st year.⁴

Early iron supplementation, on the other hand, suggests that iron supplementation should begin before iron stores enter a negative balance;⁴ as they get depleted.¹ The strategy here is to start 2-4 mg/kg/day of iron supplementation starting at 1-2 weeks of life. Basis of this being that preterm babies continue absorption of enteral iron even when total iron is sufficient due to the need for erythropoiesis perceived during rapid postnatal growth phase.⁴

As of now, it is ambiguous what the optimal timing is for initiating iron supplementation. One of the major concerns is the role of 'free' iron in oxidative pathogenesis such as NEC, retinopathy of prematurity etc., in preterm neonates. But amongst the studies conducted so far, it has been noted that this was seen with parenteral iron and not enteral forms. Also, unlike parenteral formulations, enteral formulations do not elevate malondialdehyde levels.⁴

As stated previously, LBW infants have an increased risk of developing iron deficiency, and supplementing iron early in the neonatal period can make a notable difference in the incidence, but the guidelines for initiation of iron supplementation are very poorly defined with different countries having different protocols. This study was an attempt to study the effect of early supplementation of iron at 2 weeks of postnatal age.

2. Materials and Methods

This is a randomized open label interventional study in which 106 Low Birth Weight infants delivered at Shri B M Patil Medical College and Hospital, were enrolled; 53 of whom received Early Iron supplementation while the other 53 were enrolled as controls and did not receive any oral iron supplementation. The intervention used was administration of oral iron, at 2mg/kg/dose of elemental iron given twice daily started at 2 weeks of postnatal age or earlier when full feeds are established.

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

2.1. Inclusion criteria

Low Birth Weight infants with birth weights 1.5 -2.5kg and tolerating full feeds at 1-2 weeks of postnatal age were included in the study, delivered via any mode of delivery; irrespective of mother's iron store status.

2.2. Exclusion criteria

Infants with Hb level <12 mg/dl at birth or history of receiving blood transfusions, Infants with gross anomalies and Rh hemolytic disease were excluded.

2.3. Methods

1. Oral and written consent was taken from the subjects prior to the collection of samples.
2. A venous blood sample was collected at or before 2 weeks of postnatal age, when the neonate was able to tolerate full feeds and the hemoglobin level, RBC count, hematocrit and serum ferritin were estimated.
3. The second venous blood sample was drawn at or after 6 weeks of postnatal age for haemoglobin, RBC count, hematocrit and serum ferritin estimation.

2.4. Statistical analysis

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

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

3. Results

3.1. Comparison of mean Hb levels within the study groups

The mean \pm SD of pre-intervention Hb levels and post-intervention Hb levels in cases were 17.36 ± 3.14 g/dl and 12.68 ± 1.51 g/dl respectively. Distribution of mean post-intervention Hb levels is significantly lower compared to mean pre-intervention Hb levels in cases (P-value<0.05).

The mean \pm SD of pre-intervention Hb levels and post-intervention Hb levels in controls were 16.86 ± 2.46 g/dl and 11.38 ± 1.03 g/dl respectively. Distribution of mean post-intervention Hb levels is significantly lower compared to mean pre-intervention Hb levels in controls (P-value<0.05).

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

Table 1: Comparison of mean Hb levels within the study groups and between the study groups

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

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

3.2. Comparison of mean Hb levels between cases and controls

The mean \pm SD of pre-intervention Hb levels in cases and controls were 17.36 ± 3.14 g/dl and 16.86 ± 2.46 g/dl respectively.

The mean pre-intervention Hb levels were not significantly different between cases and controls (P-value>0.05).

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

The mean % change in Hb levels in cases and controls at the post-intervention follow-up were 25.19% and 31.45% respectively

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

3.3. Comparison of median Serum Ferritin levels within study groups

The median pre-intervention serum Ferritin levels and post-intervention serum Ferritin levels in cases were 277.91 ng/ml and 238.45 ng/ml respectively. Distribution of median post-intervention serum Ferritin levels is significantly lower compared to mean pre-intervention serum Ferritin levels in cases (P-value<0.05).

The median pre-intervention serum Ferritin levels and post-intervention serum Ferritin levels in controls were 263.06 ng/ml and 175.68 ng/ml respectively. The median post-intervention serum Ferritin levels is significantly lower compared to mean pre-intervention serum Ferritin levels in controls (P-value<0.05).

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

3.4. Comparison of median Serum Ferritin levels between cases and controls

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

The median post-intervention serum Ferritin levels in cases and controls was 238.45 ng/ml and 175.68 ng/ml respectively. The median post-intervention serum Ferritin level is significantly higher in cases compared to controls (P-value<0.05).

The median % change in serum Ferritin levels in cases and controls at the post-intervention follow-up was 15.13% and 32.99% respectively. The median post-intervention % change in serum Ferritin levels is significantly lower in cases compared to controls (P-value<0.05) indicating a comparatively lesser drop in serum ferritin in iron supplemented neonates; and hence a better iron store status in them at 6 weeks.

3.4.1. Comparison of mean RBC count within the study groups

The mean \pm SD of pre-intervention RBC count and post-intervention RBC count in cases were 4.91 ± 0.85 million/mm³ and 3.94 ± 0.86 million/mm³ respectively. The mean post-intervention RBC count was significantly lower compared to mean pre-intervention RBC count in cases (P-value<0.05).

The mean \pm SD of pre-intervention RBC count and post-intervention RBC count in controls was 4.79 ± 0.67 million/mm³ and 3.16 ± 0.51 million/mm³ respectively. The mean post-intervention RBC count was significantly lower compared to mean pre-intervention RBC count in controls (P-value<0.05).

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

Table 2: Comparison of median Serum Ferritin levels within and between the two study groups.

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

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

Table 3: Comparison of mean RBC count within and between study groups

RBC count (million/mm ³)	Cases (n=48)		Controls (n=48)		P-value [Inter-group]
	Mean	SD	Mean	SD	
Pre (0 – 2 weeks)	4.91	0.85	4.79	0.67	0.473 ^{NS}
Post (6 weeks)	3.94	0.86	3.16	0.51	0.001***
% Change at 6-weeks	19.21%	–	33.82%	–	0.001***
P-value [Intra-group]					
Pre vs Post	0.001***		0.001***		

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

3.5. Comparison of mean RBC count between cases and controls

The mean ± SD of pre-intervention RBC count levels in cases and controls were 4.91 ± 0.85 million/mm³ and 4.79 ± 0.67 million/mm³ respectively. Distribution of mean pre-intervention RBC count was not significantly different between the cases and controls (P-value>0.05).

The mean ± SD of post-intervention RBC count in cases and controls was 3.94 ± 0.86 million/mm³ and 3.16 ± 0.51 million/mm³ respectively. Distribution of mean post-intervention RBC count is significantly higher in cases compared to controls. (P-value<0.05).

The mean % change in RBC count in cases and controls at the post-intervention follow-up was 19.21% and 33.82% respectively. The mean post-intervention % change in RBC count is significantly lower in cases compared to controls (P-value<0.05). Hence the fall in the RBC count at the follow up among cases is significantly lower than the fall in RBC count among the controls at follow up.

3.6. Comparison of mean Hematocrit levels within the study groups

The mean ± SD of pre-intervention Hematocrit levels and post-intervention Hematocrit levels in cases were 52.03 ± 9.60 % and 33.16 ± 3.75 % respectively. Distribution of mean post-intervention Hematocrit levels are significantly lower compared to mean pre-intervention Hematocrit levels among cases (P-value<0.05).

The mean ± SD of pre-intervention Hematocrit levels and post-intervention Hematocrit levels in controls were 51.11 ± 7.99 % and 34.04 ± 3.45 % respectively. The mean post-intervention Hematocrit levels is significantly lower compared to mean pre-intervention Hematocrit levels among controls (P-value<0.05).

3.7. Comparison of mean Hematocrit levels between cases and controls

The mean ± SD of pre-intervention Hematocrit levels in cases and controls was 52.03 ± 9.60 % and 51.11 ± 7.99% respectively. There was no statistically significant difference between the pre-intervention mean hematocrit values of the two study groups (P-value>0.05).

The mean ± SD of post-intervention Hematocrit levels in cases and controls was 33.16 ± 3.75 % and 34.04 ± 3.45 % respectively. There was no statistically significant difference between the post-intervention mean hematocrit values of the two study groups (P-value>0.05).

The mean % change in Hematocrit levels in cases and controls at the post-intervention follow-up was 34.32% and 32.43% respectively. The mean post-intervention % change in Hematocrit levels was also not significantly different between the two study groups (P-value>0.05).

4. Discussion

In the present study, LBW infants were administered 2 mg/kg/dose BD of oral iron. The pre-intervention values of mean haemoglobin, mean serum ferritin, mean RBC count,

Table 4: Comparison of mean Hematocrit levels within and between cases and controls

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

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

and mean hematocrit did not differ significantly between cases and controls.

The post-intervention values of mean haemoglobin (CASES Hb: 12.68, mean serum ferritin (CASES: 238.45 ng/ml, CONTROLS- 175.68 ng/ml), mean RBC count (CASES: 3.94 million/mm³, controls 3.16±0.51 million/mm³, and mean hematocrit (CASES: 33.16±3.75%, controls 34.04±3.45%), were considerably lower than the pre-intervention values (mean Hb: CASES 17.36±3.14g/dl, controls 16.86±2.46g/dl; mean sr. ferritin cases: 277.91ng/ml, controls 263.06ng/ml; mean RBC count: cases 4.91 ±0.85 million/mm³, controls 4.79±0.67 million/mm³, mean haematocrit cases 52.03±9.60%, controls 51.11±7.99%) in both cases and control groups, indicating a drop in their levels regardless of supplementation.

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

Our results were similar to a study conducted by Joy R et al in 2013, (5) which evaluated “the effect of early iron supplementation vs late iron supplementation in low birth weight infants”. Though our study did not include

comparison with a late iron supplementation group, the results of early iron supplementation followed up at ≥6 weeks were similar to the results noted in the EI (early iron) group of this study.

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

Joy R et al noted that in spite of the drop in ferritin levels at 12 weeks (82 ± 5 ng/ml) and drop in hemoglobin at 12 weeks (10.1± 0.4 g/dl), these levels were still better when compared to LI (Late Iron supplementation) group values of serum ferritin (63 ± 3 ng/ml) and hemoglobin (9.2 ± 0.4 g/dl) at 12 weeks, indicating a statistically improved iron store status in the early iron supplementation group. Similar findings of improved iron store status at 6 weeks [238.45 (54.8 – 630.5) ng/ml] and improved hemoglobin levels (12.68 ± 1.51 g/dl) were noted among the cases in our study when compared to controls at 6 weeks [ferritin 175.68(39.8-443.6) ng/ml; hemoglobin- 11.38 ± 1.03 g/dl].

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

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

Whereas a randomized controlled trial on early iron supplementation in VLBW infants by Mari Jeeva Sankar, Renu Saxena, Kalaivani Mani et al. concluded that there was no statistically significant difference in serum ferritin and hematocrit values between early iron supplemented cases

Table 5:

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

and the control group when levels were estimated at 60 days of postnatal age. The fall in these iron status indicators from their baseline values was also not significantly different between the groups.⁷

A meta-analysis conducted based on PubMed and Cochrane databases by Hong-Xing Jin et al assessed the effects of early and late Iron supplementation amongst 246 babies (121 for EI and 125 for LI group). Early supplementation led to significantly lower decrease in serum Ferritin and Haemoglobin levels; along with lower rates of blood transfusion requirements compared to late supplementation.⁸

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

5. Conclusion

In our study, early iron supplementation at 2 mg/kg given twice daily in LBW neonates, improved iron store status, resulting in a decrease in incidence of iron deficiency anaemia among preterm and LBW infants. No adverse effects of supplementation were observed. Thus reinforcing that iron supplementation as early as 2 weeks of postnatal age in LBW babies (started at 2 weeks of age or when baby is tolerating full feeds) helps to improve the iron store status and reduces the incidence of iron deficiency in LBW neonates.

6. Disclaimers

The authors certify that they have obtained all appropriate patient consent forms. In the forms the patient's attenders have/has given their consent for the clinical information to be reported. The patient attenders understand that their names and initials will not be published & due efforts will be made so as to conceal their identity.

7. Shortcomings

Larger studies are necessary to strengthen the position. Our study compares the outcomes of early iron supplemented

babies to those of unsupplemented babies; further studies comparing the outcomes of early and late iron supplementation would provide a clearer understanding of the effects of iron supplementation. Further the effect of iron supplementation on neurodevelopment, cognitive function, or growth was not followed up, which might have given better support for early iron supplementation of LBW babies.

8. Source of Funding

None.

9. Conflict of Interest

None.


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