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Early versus Late Enteral Prophylactic Iron Supplementation in Preterm Infants: A Randomized Controlled Trial

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ABSTRACT

Background: *Iron deficiency is commonly encountered in preterm infants and there is controversy about its timing of supplementation. This study was aimed to evaluate the effect of early versus late iron supplementation on iron status and anthropometry of admitted preterm newborns in the neonatal intensive unit (NICU) of a tertiary care hospital.*

Methodology: *This Randomized Controlled Trial was conducted in the Department of Neonatology, Bangabandhu Sheikh Mujib Medical University, Dhaka. Twenty-one preterm in early group and nineteen in late group were included in the study. Iron supplementation was introduced at 2 weeks of age in early intervention (EI) group and 6 weeks of age in lateintervention (LI) group. Sample for CBC, red cell indices, S. ferritin and C-reactive protein was pre-intervention sample at 2 weeks in EI group and at 6 weeks in LI group and follow up sampling was done at 12 weeks in both groups. The intervention was used that ferric hydroxide polymaltose at a dose of 2 mg/kg/day of elemental iron per oral once daily mixed with expressed breast milk. Same drugs of same manufacture company with same batch number were provided to the babies. Weight, length and head circumference were measured at birth, 2 weeks and 12 weeks of age in early group and at birth,6weeks and 12 weeks in late group.*

Result: *Mean gestational age and mean birth weight (gm) was 33.52±1.91 and 33.48±1.46, weeks and 1622.86±438.73 g and 1753.16±401.76 g in early and late group respectively. Female outnumber the male in early group &male outnumber the female in late group, p value was statistically significant. Comparison of red cells parameters and S. ferritin between early and late group neonates at 12 weeks showed that mean S. Ferritin (ng/ml) was significantly higher in early group (92.75±47.33 ng/mL) as compared with late group (56.95±35.06 ng/mL), (p value 0.01). At 12 weeks of age, anthropometry showed mean weight (p=0.695), mean length (p=0.291) and mean OFC(p=0.983) in Early and Late groups were not statistically significant.*

***Conclusion**: Early iron supplementation at 2 weeks of age in preterm newborn result in significantly higher serum ferritin level than late iron supplementation group but hemoglobin, red cell indices, anthropometry did not differ significantly in between early & late iron supplementation groups.*

Keywords
Premature newborn, Newborn nutrition, Iron supplementation.

Introduction
Prematurity is one of the important causes of neonatal morbidity and mortality globally as well as in Bangladesh. In south central Asia, the highest incidence for low birth weight is in Bangladesh 22.6 percent [1]. The most common micronutrient deficiency in the world is iron deficiency (ID) and results in approximately one billion cases of anemia worldwide [2].

The Preterm infants are more susceptible to develop iron deficiency during their first 6 postnatal months due to lower iron stores at birth and rapid growth.

During the third trimester of pregnancy, significant fetal iron accretion occurs [2]. The infant's gestational age and birth weight are strongly influenced by the size of the infant's iron (Fe) store at birth. Possible factors for developing iron deficiency in premature infants include: small iron stores at birth, rapidly increasing red cell mass, maternal conditions like diabetes mellitus, hypertension etc. increased hemolysis, decrease red blood cell life span, low level of circulating erythropoietin levels, frequent blood sampling and loss of blood due to surgery [2].

Iron is an essential micronutrient. It plays a significant role in critical cellular functions in all organ systems in all species. Iron is particularly vital for early brain growth and function in humans since it supports neuronal and glial energy metabolism, neurotransmitter synthesis and myelination.

Deficiency of iron in infancy is associated with neurodevelopmental deficits, poor physical growth, gastrointestinal disturbances, thyroid dysfunction, altered immunity and temperature instability, delayed maturation of the auditory brainstem response and abnormalities of memory and behavior [3].

Anemia typically is a late sign and suggests significant depletion of iron stores. In the setting of iron deficiency anemia, subsequent iron supplementation corrects the anemia but does not overcome the neurocognitive deficits [4].

The timing of prophylactic enteral iron supplementation in preterm babies has been a matter of great controversy. In fact, the different international associations recommend different timings for initiation of iron supplementation for these babies. American Academy of Pediatrics (AAP) and the Nutrition Committee of Canadian Pediatric Society have recommended delayed iron supplementation in preterm very low birth weight infants at 4 weeks and 6–8 weeks postnatal age, respectively. The European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommend initiation over a wide range of 2–6 weeks of age [5].

In Bangladesh, a common practice is to start the supplementation from 6 weeks of postnatal age.
No data is available regarding impact of early & late initiation of iron therapy among the premature newborns. Therefore, a RCT was conducted to compare iron storage, red cells parameters and anthropometry status in between early supplementation group with late supplementation group.

Materials and Methods
This Randomized Controlled Trial was performed in the Department of Neonatology, BSMMU, Dhaka city over a twelve- month period from August 2019 to September 2020 after approval by IRB. A written informed consent was taken from parents. Admitted inborn neonate preterm < 37 weeks of gestation & who reached enteral feeds of >120 mL/kg/day by 2 weeks postnatal age were included in this study. Babies with multiple gestation, major congenital anomaly, ABO or Rh hemolytic disease were excluded. Newborns were randomized at 2 weeks of age when reach feeds >120 mL/kg/day or on exclusive breast feeding. The enrolled neonates were randomly assigned with computer-generated random number tables via software named 'Random Allocation Software, to receive either of the intervention. Any babies who reach feeds>120 mL/kg/day or on exclusive breast feeding got discharge before 2 weeks of age then randomization was done at 2 weeks of age &contact with parents over phone about pre-intervention blood samplings. Babies were brought by parents for pre-intervention blood sampling. After blood sampling, parents were advised to give oral iron supplementation. In babies, whose iron supplementation was started at 2 weeks of postnatal age considered as early initiation (EI) group. In those iron supplementation was started at 6 weeks of postnatal age considered as late initiation (LI) group.

Details of maternal and perinatal medical history and anthropometry were recorded for each neonate. During admission venous blood sampling was done and it was taken as baseline investigation. 2 ml blood sample was taken into 2 tubes: EDTA (ethylene diamine tetra acetic acid) tube for complete blood count (CBC) [Hb as g/dl, packed cell volume (PCV) as %, mean corpuscular volume (MCV) as fl, mean corpuscular hemoglobin concentration (MCHC) as g/dl and redcell distribution width (RDW) as %], and plain tube for S. ferritin & CRP. Red cell parameters were assessed using fully automated blood cell counter (Sysmex, XT-4000i model, Japan) and different parameters including Hb, RBCs count, hematocrit (Hct), mean corpuscular hemoglobin (MCH), Mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration(MCHC) were assessed in clinical pathology department of BSMMU. Serum ferritin was measured as ng/ml by fully automated bidirectional interfacedchemo-luminescent immune assay and CRP was measured automatically as mg/L (Siemens Dimension R×L Max' machine(USA)in Biochemistry

department of BSMMU.

The intervention was used that ferric hydroxide polymaltose at a dose of 2 mg/kg/day of elemental iron per oral once daily mixed with expressed breast milk when they met eligibility criteria. When babies were eligible for early group in hospital stay, iron supplementation was given. But when babies were at home and eligible for early and late group supplementationthen parents or guardian were communicated over phone and they brought their babies for blood sampling. The intervention medication, ferric hydroxide poly maltose, was manufactured by Incepta Pharmaceutical Company, Bangladesh, marketed as the name 'Drop Compiron'. Same batch number (19011) of drug was supplied to all the participants. The parents were taught regarding the dosage of iron and advised to maintain a chart of ensuring the daily dose which was also supplied by the researcher. All the used-up bottles of supplemental iron was advised to bring on follow up so that the drug administration could be ensured.

Venous blood sampling was done at 2 weeks of postnatal age in case of early initiation group and at 6 weeks of age in case of late initiation group before starting of iron supplementation. Like baseline investigation, 2 ml venous blood sample was measured for S. ferritin, Hemoglobin, red cell indices and CRP. At 12 weeks of age venous blood sampling was done in both groups and blood sample was measured for S. ferritin, hemoglobin, red cell indices and CRP.

Anthropometry like weight, length and head circumference were measured on at birth,2,6 and 12 weeks of age. In Early group it was taken at birth,2 weeks and 12 weeks of age. In late group it was taken at birth,6weeks and 12 weeks of age. The newborns weights were taken without clothing soon after birth on an electronic scale with a precision of 10 g [Model 914, SALTER]. Those babies whose birth could not be attended weight were taken within 12 hours of birth. The length was measured by infantometer in centimeters. Head circumference or OFC (occipital frontal circumference) was measured over the most prominent part on the back of the head (occiput) and just above the eyebrows (supraorbital ridges) by using a measuring tape.

When babies were brought for follow up at 12 weeks weight was taken without any cloth before feeding and after voiding by the same weighing machine with a precision of 10 gm [Model 914, SALTER]. Length and OFC were taken in the same procedure when babies were brought for follow up at 12 weeks. Reference values were taken from textbook of neonatology, Roberton, 5th edition [6].

Sample size was calculated by using the statistical formula:

$$n = \frac{\sigma 2 \times (u + v)^2}{(\mu - \mu 0)^2}$$

μ = mean difference of serum ferritin level from a previous literature (82-63)=19

μ0 = hypothesized value, we considered mean difference (17 is sufficient to obtain power of the study).

σ = mean of SD obtained from a previous literature = 2

v = 1.96for 5 % of level of significance

u = 0.842 (from Z table) at 80 % power

So, $n = \frac{4 \times (0.842 + 1.96)2}{19-17} = 31$

Data were analyzed using the Statistical Package for Social Science (SPSS) version 20 and a P value <0.05 was considered significant. Mean and standard deviation and 95% confidence intervals were used to summarize data that were normally distributed. Chi square test was used to test for associations between categorical variables. Independent sample t-test was used to test for differences between two group means.

Results

Among 145 admitted preterm neonates, 72 babies were eligible for the study. Till completion of the study, 40 babies completed follow up at 12 weeks, among them 21 babies were in early initiation (EI) group and 19 babies were in late initiation (LI) group as shown in Figure 1.

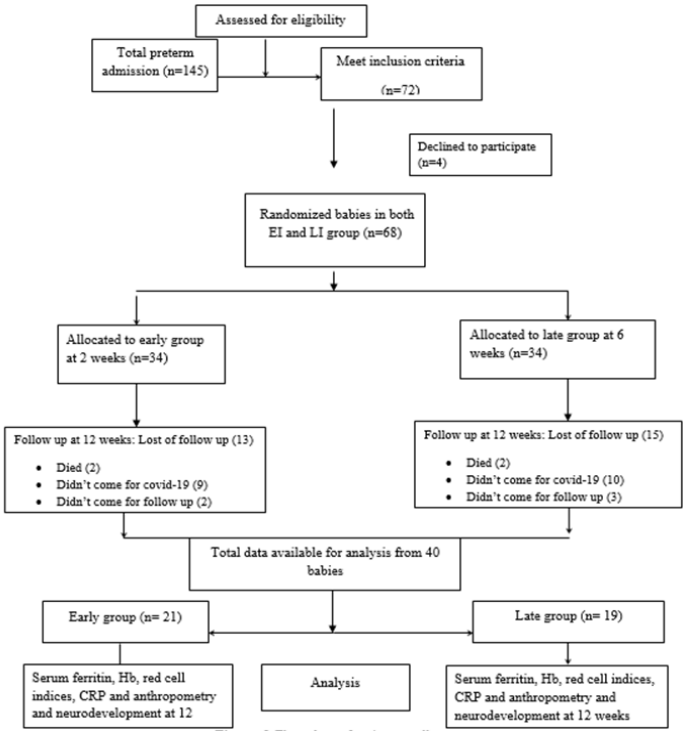

Figure 1: Flow chart of patient enrollment.

In Table 1, baseline characteristics of mothers and neonates were observed. Mean gestational age, mean birth weight and most of the characters were similar in both groups. In Early group, slight female predominance (61.9%) and in late group, slight male (78.9%) predominance was observed.

In Table 2, Baseline Mean Serum ferritin, hemoglobin, Hematocrit, RBC count, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, CRP were also similar in both groups.

Table 1: Baseline characteristics of mothers and neonates in between two groups at birth.

Parameter	EI group (n=21)	LI group (n=19)	p-value
Birth weight (gm)	1622.86 ± 438.73	1753.16 ± 401.76	0.335^{ns}
Gestational age (weeks) n (%)	33.52 ± 1.91	33.48 ± 1.46	0.935^{ns}
Male/Female	8 (38.1)/ 13 (61.9)	15 (78.9)/ 4 (21.1)	0.009^{s}
Small for gestational age	9 (42.86)	5 (26.32)	0.273^{ns}
Transfusion before 12 weeks	8 (38.1)	7 (36.84)	0.935^{ns}
Bloodletting >8% before 12 weeks	8 (38.1)	7 (36.84)	0.935^{ns}
Length at birth	41.17 ± 2.93	40.882.61	0.477^{ns}
OFC at birth	29.712.43	29.70 ± 1.55	0.147^{ns}
Antenatal complications			
Gestational Diabetes Mellitus	4 (19.04)	6 (31.57)	0.361^{ns}
Pregnancy-induced hypertension	15 (71.4)	11 (57.89)	0.370^{ns}
Maternal UTI	3 (14.29)	2 (10.53)	0.720^{ns}
Premature rupture of membrane	3 (14.29)	5 (26.32)	0.342^{ns}
APH	3(14.29)	2(10.53)	0.72^{ns}
Mode of delivery			
Vaginal delivery	5 (23.81)	4 (21.05)	
Caesarean section	16 (76.19)	15 (78.95)	

Continuous data are presented as mean ± SD and categorical data as number and percentage Statistical test: Chi square test, Independent Sample t test. S, significant; ns, not significant.

Table 2: Baseline Serum Ferritin, Red cell indices and CRP of two groups at admission.

Parameter	Early group (n=21)	Late group (n=19)	p-value
Serum Ferritin (ng/mL)	204.03±102.09	225.79±131.32	0.560^{ns}
Hemoglobin (g/dL)	17.80±2.40	17.90±2.23	0.893^{ns}
Hematocrit (40-54 %)	50.66±6.82	51.87±6.95	0.582^{ns}
RBC count (4.5-5.5*10^12/ L)	4.85±0.56834	4.81±0.67529	0.838^{ns}
Mean corpuscular volume (80-96 fL)	103.66±6.09	107.65±8.67	0.098^{ns}
Mean corpuscular hemoglobin (27-32 pg)	25.96±2.34	25.50±2.21196	0.531^{ns}
Mean corpuscular hemoglobin concentration (31-35 g/dl)	34.88±1.14	35.63±5.05	0.508^{ns}
RDW(%)	19.12±2.15	19.12±1.80	0.977^{ns}
s-CRP (mg/L)	1.32±1.57	1.55±2.55	0.730^{ns}

Data are presented as mean ± SD and number (percentage) unless otherwise indicated.

In Table 3, When red cell parameters and iron status were compared at 12 weeks, in table-3, mean S. Ferritin (ng/ml) was significantly high in EI group (p value 0.01).

Table 3: Comparison of Ferritin and hematological parameters in early and late group neonates at 12 weeks.

Parameters	Early group(n=21)	Late group(n=19)	p-value
S.Ferritin(ng/ml)	92.75±47.33	56.95±35.06	0.01^{s}
Hb(g/dl)	10.10±1.29	9.92±1.76	0.715^{ns}
Hct(%)	29.58±3.27	28.88±5.15	0.609^{ns}
RBC count (*106/L)	3.96±0.645	3.90±0.89	0.837^{ns}
MCV(fl)	75.85±6.98	74.80±6.17	0.098^{ns}
MCH(pg)	25.96±2.34	25.50±2.21	0.531^{ns}
MCHC(g/dl	34.20±1.10	34±1.44	0.608^{ns}
RDW(%)	15.09±1.81	15.70±3.79	0.511^{ns}
s-CRP (mg/L)	2.16±2.68	1.35±1.58	0.262^{ns}

All values were given as mean ± SD. Statistical test: Independent Sample t test. S, significant; ns,

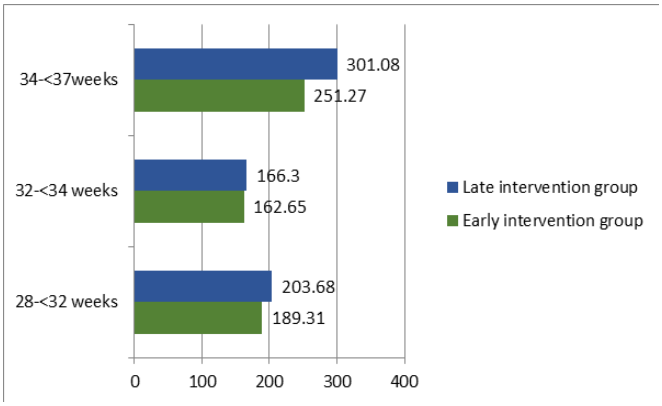

Figure 2: Comparison of S. Ferritin level (ng/ml) in early & late group in different gestational age at admission.

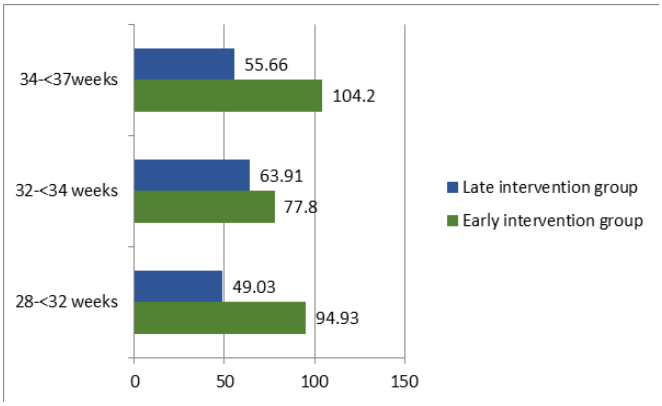

Figure 3: Comparison of Serum Ferritin in early & late group in different gestational age at 12 weeks of age. With increasing gestational age group serum ferritin was increased but in same gestational age groups S. Ferritin was more in early group.

Table 4: Comparison of anthropometry at 12 weeks of age.

Parameters	Early group(n=21) (mean± SD)	Late group(n=19) (mean± SD)	p-value
Weight(gm)	4140.95±582.71	4058.42±734.97	0.695 ^{ns}
Length(cm)	53.83±2.60	54.73±2.89	0.291 ^{ns}
OFC(cm)	37.05±1.48	37.31±1.39	0.983 ^{ns}

Statistical test: Independent t-test.

Table 4 is showing the comparison assessment of anthropometry at 12 weeks of age. The values were not statistically significant.

Discussion

In this study, we used oral iron polymaltose complex at a dose of 2 mg/kg/day according to the American Academy of Pediatrics (AAP) and European Society of Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) recommendations [5]. The two time periods were chosen for the two groups (2 weeks and 6 weeks, respectively). It was based on the ESPGHAN recommendations and on the evidence that supplemental iron gets well incorporated into red blood cells when the onset of erythropoiesis is starts at 6-8weeks [7]. In this study ferric hydroxide (iron polymaltose complex) was used for its better absorption with minimal gastric irritation. It is easily converted to soluble form by the action of gastric acid and reduced to ferrous form in the small intestine.

In this study serum ferritin was used as an indicator of iron stores and each ferritin value was correlated with the CRP value to detect the effect of inflammation. Baseline characteristics of the studied neonates were presented in mean birth weight (gm) which was 1622.86 ± 438.73 in early group & 1753.16±401.76 in late group which was almost similar in both the groups. Mean gestational age was 33.52±1.91 and 33.48±1.46weeks of early group and late group respectively.

Serum ferritin (ng/mL) and hemoglobin (g/dL) was 204.03±102.09 vs. 225.79± 131.32 and 17.80±2.40 vs. 17.90± 2.23 in EI and LI groups respectively. There was no statistically significant difference in the gestational age, anthropometric parameters, baseline ferritin & hemoglobin values between the two groups, these results were similar to the previous observations by Joy R et al. [7].

In this study serum ferritin level on follow-up at 12 weeks in the Early Iron supplementation (EI) group was significantly higher than the Late iron supplementation group (LI). In Early supplementation (EI) group serum ferritin was 92.75±47.33 and the Late iron supplementation group (LI) was 56.95±35.06, p=0.01. Joy R et al. and Arnon S et al. observed similar findings in their study [9,10]. In our study, we observed that a delayed initiation of iron supplementation led to a decrease in serum ferritin level. Similarly, early initiation of iron supplementation led to an increase in iron stores within the margin of safety (25–200ng/mL). Similar, findings were found in Joy R et al. so thereby justifying early initiation of iron supplementation in preterm VLBW babies [9].

In contrast to this study results Franz A, et al., did not detect any significant differences in serum ferritin levels, presumably due to the greater blood transfusion requirements in the LI group [11]. Sankar M, et al. found no difference in serum ferritin in EI & LI group because of small sample size [12]. This difference may be due to heterogeneity of study populations, with different gestational ages, birth weights and timing of iron initiation.

At the end of the study, at 12 weeks of age, all the growth parameters (weight, length and head circumference) of the participants were found comparatively more in EI group, though were not statistically significant. These findings go similarly with previous other studies [9,11,13].

Developmental advancements were evaluated using the Bayley scale III at 12 weeks of age. Mean cognitive score was 74.90±15.59 vs. 74.73±15.04; mean motor score 71.61±13.76 vs. 72.36±12.63; mean language score was in 83.38 ±12.18 vs. 85.94±12.27 in EI and LI group respectively. In all three parameters p value were not statistically significant.

Elaine K. et al., in their systematic review, has also reported no statistically significant long-term effects on cognition, motor skills, visual impairment, or behavioral outcomes. However, they noted a trend suggesting that earlier intervention may be more beneficial than delayed initiation [14].

Joy et al. observed short-term neurological improvement at 12 weeks in the early intervention group, though it was not statistically significant. The study did not include long-term follow-up [9].

Friel et al. observed no long-term differences in mental development between infants given high versus normal iron supplements. However, they used Griffiths Mental Development Assessment tool [13].

Berglund et al. reported no significant long-term differences between treatment groups based on Wechsler Intelligence Scale scores [15].

Bora et al. observed that newborns who received supplemental iron early demonstrated age-appropriate motor development compared to those who received it later [16].

Limitation

This was a single-center study with a small sample size, primarily due to constraints during the COVID-19 pandemic. Additionally, long-term follow-up was not conducted, which could have provided valuable insights into outcomes beyond 12 weeks of age.

Conclusion

Preterm newborns who received iron at 2 weeks had significantly higher serum ferritin levels than those who received it later, but hemoglobin and red cell indices were similar between the groups.

Recommendation

A study conducted on a larger population, with additional assessments of maternal nutritional status, hematological profile, and infant feeding practices, may uncover further findings that could contribute to improving infant hematological outcomes. In the present study, neurodevelopmental assessments—including cognitive, motor, and language skills—did not show statistically significant differences between groups. A blinded study with long-term follow-up is recommended in similar demographic settings, as it may reveal more definitive and promising results.

Author Contribution

Dr. Mohtarama Mostari and Dr. Md. Morshed Monzur Kabir had conceptualized the study idea, gone through the literature reviews and constructed the study framework. Dr. Abdullahel Amaan, Dr. Azmery Saima, Dr. Mohammad Ala Uddin and Dr. Md Gias Uddin had verified the study procedure and analytical methods and actively participated in communicating with the caregivers. Professor Dr. Md. Abdul Mannan encouraged and supervised the study. All authors discussed the results and contributed to the final manuscript.

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