ORIGINAL RESEARCH

An observational study to determine the adequacy of prophylactic iron supplementation in preterm neonates born in a tertiary care hospital

[1]Dr. Harprasad M, [2]Dr. Kushvanth Kolibailu, [3]Dr Soumyashree G, [4]Dr. Ashish S. Mallige

[1]Clinical Fellow PICU, Brihmingam Children's Hospital, U.K
[2]Assistant Professor, Pediatris, Kodagu Institute of Medical Sciences, Madikeri, Karnataka, India
[3,4]Assistant Professor (Pediatrics), BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India

Corresponding Author
Dr. Ashish S. Mallige
Assistant Professor(Pediatrics), BGS Global Institute of Medical Sciences, Bengaluru, Karnataka, India

Received: 02Sept, 2023 Accepted: 25Sept, 2023

ABSTRACT
Iron deficiency in infancy is widely related to several clinical and developmental problems such as delayed auditory brainstem response maturation, memory and behavioral abnormalities and several other neurodevelopmental deficits. This was a hospital based cross sectional study conducted with prior clearance from the Institutional Ethics committee (IEC) of this tertiary care hospital. All children who met the inclusion criteria during the study period after due consent from parents underwent the following: Hospital records were studies to for further information regarding morbidity, treatment details, nutrition during hospital stay, amount of blood drawn for investigations during NICU etc. Among the population evaluated mean of Hb was 11.03 gm/dL (max 13.4 and min 8.3) and serum ferritin was 43.94 µg/L (max 181.3 and min 10.3). As per postnatal age and weight defined criteria 20% (26 out of 131) developed anaemia while on prophylactic iron supplementation. However, 65% of the iron supplemented study population (85 out of 131) still had adequate ferritin levels (20 mcg/dL) at 3 months corrected age.

Key words: Prophylactic iron supplementation, preterm neonates born, ferritin levels

INTRODUCTION

Preterm birth is a major cause of morbidity and mortality in newborn and survivors have significant risks of long-term neurodevelopmental sequelae and functional impairments.

Among the various complications associated with prematurity, anaemia of prematurity is one of the most common complication associated with adverse impact on growth and development.

Anaemia is a common laboratory and clinical finding in the new born period and carries a broad differential diagnosis [1]. The diagnosis and interpretation of anaemia in the new born period are complex and require careful consideration of the gestational age and general health status of the infant, details of the perinatal course and delivery and maternal health through pregnancy, delivery and postpartum period[1].

Iron deficiency in infancy is widely related to several clinical and developmental problems such as delayed auditory brainstem response maturation, memory and behavioural abnormalities and several other neurodevelopmental deficits[2, 3].

Iron deficiency anaemia in preterm infants impair cell differentiation and thereby alter normal neurodevelopmental process. Iron deficiency has been associated with impaired immunity, poor physical growth and temperature instability [4].

A decline in circulating RBCs is experienced by all neonates in the first few weeks of life due to multiple physiologic factors [5]. Over the first few weeks of postnatal life,increased oxygen in the environment reduces the erythropoietic drive, and this normal developmental and physiologic process results in a slow decrease in haematocrit and haemoglobin concentration according to postnatal age for both term/post term and preterm (29-34 week) gestation infants. The oxygen deliver eventually becomes limiting enough to stimulate new active.

RBC underproduction is common in neonates, particularly among preterm infants because of the enough oxygen delivery to tissues during the 1st week

of postnatal life facilitated by relative polycythaemia and physiologic right shift in the oxyhaemoglobin dissociation curve, leading to limited erythropoietic drive. erythropoiesis and the haemoglobin concentration begins to rise.

This physiological nadir usually occurs between 6 and 10 weeks of life for term infants, while preterm infants reach their nadir earlier at 4-8 weeks of age[1].

Erythropoiesis is stimulated at this nadir thereby using up the iron stores. There is a rapid depletion in already low preterm iron stores than term infants[6].The haemoglobin concentrations fall rapidly in preterm infants to approximately 8g/dL with birth weight of 1.0 to 1.5 kg and to 7g/dL in infants with <1kg birth weight[1-5].

This physiologic underproduction of erythrocytes appears to be prolonged resulting in a steeper physiologic nadirin preterm infants, called as anaemia of prematurity.

In preterm infants who are sick this is exaggerated by additional factors such as phlebotomy losses for lab investigation, low plasma erythropoietin which in turn may be due to reduced production and rapid growth due to accelerated catabolism and concomitant increase in red cell mass/volume and other co-morbidities, which in turn leads to early attainment of haemoglobin nadir [7, 8].

Even though there is no increase in nadir of haemoglobin prior to the age of 10 to 14 weeks does not increase the nadir of haemoglobin level due to iron administration, it is stored for later use [6].

Some of the risk factors studied in preterm neonates causing iron depletion were low gestational age, low birth weight, small for gestational age, frequent blood draws, anaemia in mother, low serum ferritin levels at birth and low iron intake during hospitalisation[4].

METHODOLOGY

STUDY DESIGN: Observational study.

STUDY SETTING: TertiaryCarehospitalwithlevel-IIINICU.

STUDY POPULATION: Preterm babies < 37 week born in our centre over one and ahalf years.

SAMPLE SIZE

1. Population size of preterm babies <37 completed weeks in our hospital over previous one and a half years was 120.
2. Incidence of anemia in preterm babies 25%.
3. To detect 25% anemia with 80% power and with Confidence level of 95%.
4. Sample size was calculated by using Epi-info software ver7 (CDC Atlanta) and the worked-out sample size is 92.

SAMPLING TECHNIQUE

Consecutive type of non-probability sampling was followed for selecting subjects. All the preterm babies born in the hospital during the study period were included in the study once they fulfilled the inclusion criteria and were started on iron supplements.

SELECTION OF STUDY SUBJECTS

INCLUSION CRITERIA

a) All pre-term babies born in a tertiary care hospital who were started on prophylactic oral iron supplementation between 2 to 3 weeks of postnatal age on achieving full oral feeds.

EXCLUSION CRITERIA

a) Babies who have received blood transfusion.
b) Babies with Hemoglobinopathies/congenital blood disorders.

This was a hospital based cross sectional study conducted with prior clearance from the Institutional Ethics committee (IEC) of this tertiary care hospital.

All children who met the inclusion criteria during the study period after due consent from parents underwent the following:

Hospital records were studies to for further information regarding morbidity, treatment details, nutrition during hospital stay, amount of blood drawn for investigations during NICU etc.

Written informed consent as per WHO format regarding participation in this study was taken from the parents. Those willing to participate were included in the study.

RESULTS

Table1: Serum Ferritin at 3 months corrected age incomparison with gestational age of study population

Gestational age in weeks	N	Mean	S. D	Minimum	Maximum	R-value	P-value
< 28 weeks	5	26.83	10.06	10.54	37.80	0.429	0.0005**
28-34weeks	49	33.56	16.00	10.30	70.34		
34-36weeks	77	51.88	25.80	16.40	182.30		
**** Highly Significant at P < 0.05 level**							

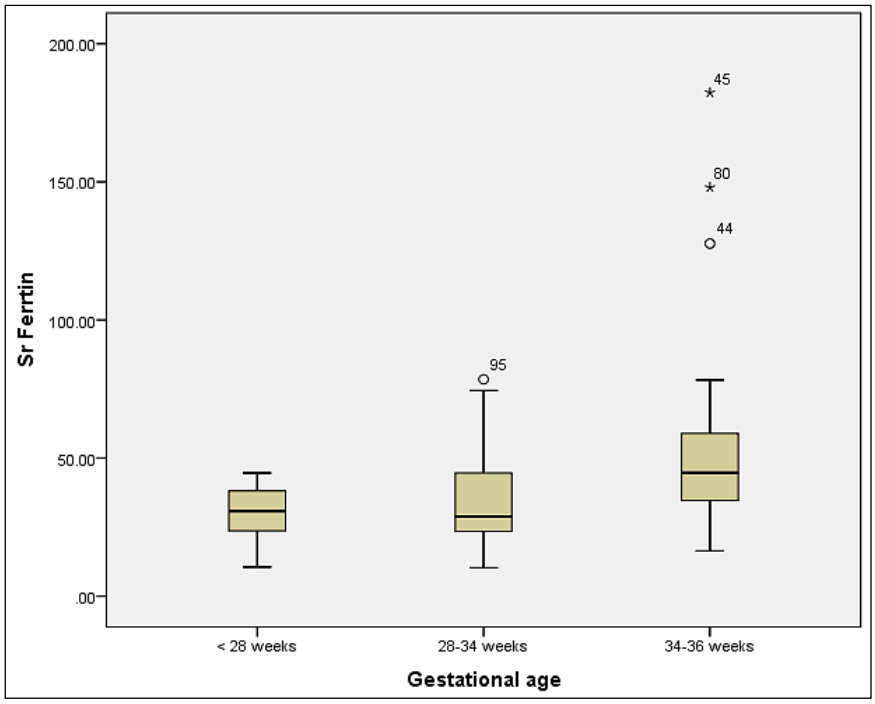

Fig 1: Serum ferritin values at 3 months CA in comparison with gestational age of study population

Mean Serum ferritin values among study population is lowest among those with gestational age <28 weeks (26.83 µg/L) while highest is found among those belonging to gestational age 34-36 weeks (51.88 µg/L). This shows a significant association (P <0.01) of decline in serum ferritin values among population with decreasing gesational age.

Table 2:Hb at 3 months corrected age in comparisonwith gestational age

Gestational Age	N	Mean	Std Deviation	StdError Mean	R value	P value
<28 weeks	5	9.60	1.342	.600	0.515	<0.0001**
28-34 weeks	49	10.14	1.155	.165		
34-36 weeks	77	11.39	.989	.113		
**** Highly significant at P <0.05 level**						

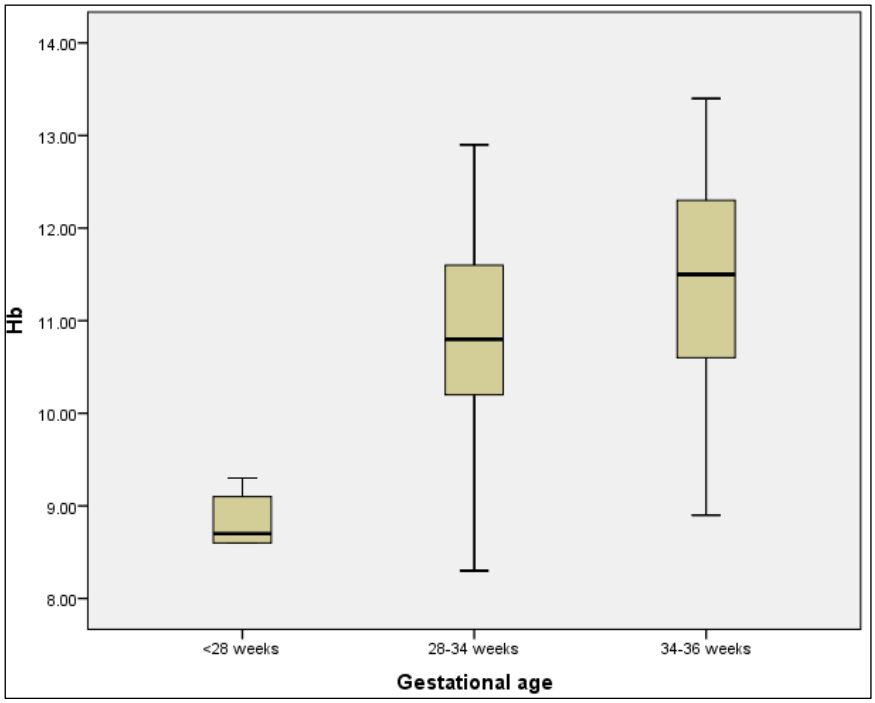

Fig 2: Hb at 3 months corrected age in comparison withgestational age

Mean Hb among study population was lowest among <28 weeks (9.60±1.342) with highest among 34-36 weeks (11.39 ± 0/98). Hb values shows sgnificant increase(P>0.01) with increase in gestationl age.

Table3:Hb Values at 3 months corrected age incomparison with birth weight

Birth Weight	N	Mean	Std.Deviation	Std. Error	95% Confidence Interval for Mean		R value	P-Value
					Lower Bound	Upper Bound		
Normal	25	11.64	1.036	.207	11.21	12.07	-0.519	<0.0001**
LBW	75	11.05	1.051	.121	10.81	11.30		
VLBW	27	9.89	1.086	.209	9.46	10.32		
ELBW	4	8.75	.500	.250	7.95	9.55		
Total	131	10.85	1.241	.108	10.64	11.07		
** Highly significant at P <0.05 level								

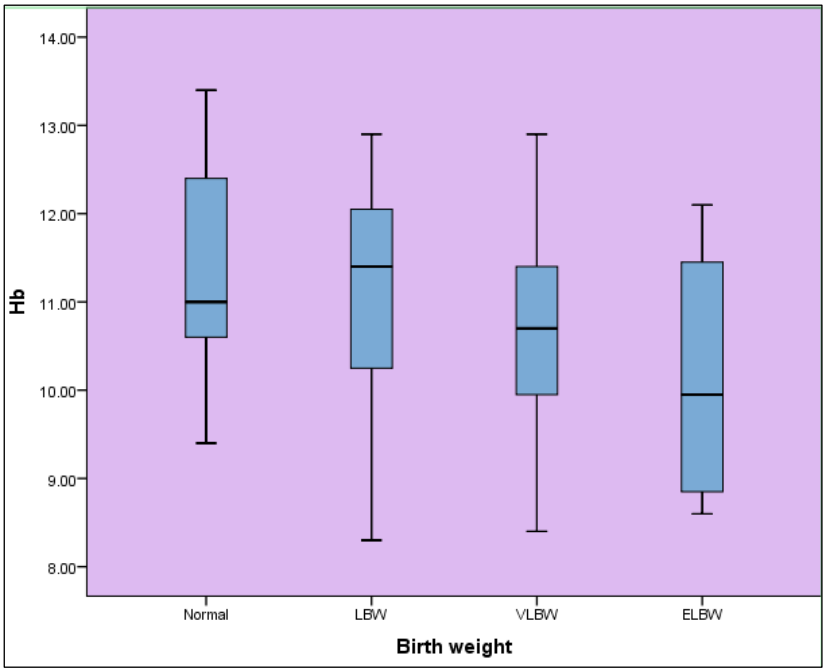

Fig 3: Hb values at 3 months corrected age in comparisonwith birth weight

Mean Hb among study population with various weight categories shows that lowest mean Hb is seen among extreme low birth weight population (mean 8.75 ± 0.5) followed by VLBW,LBW and highest mean Hb seen in Normal birth weigth (mean Hb – 11.64 ± 1.03).

There is a significant negative (P <0.01; R -0.519) among birth weight and Hb at 3 months of corrected age.

Table 4: Serum ferritin values in comparison with birthweight

Birth Weight	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		R value	P-Value
					Lower Bound	Upper Bound		
Normal	25	54.00	15.54	3.11	47.58	60.42	-0.369	0.0001**
LBW	75	45.89	27.19	3.14	39.64	52.15		
VLBW	27	29.85	14.92	2.87	23.95	35.76		
ELBW	4	17.00	3.55	1.78	11.34	22.66		
Total	131	43.25	24.39	2.13	39.04	47.47		
**** Highly significant at P <0.05 level**								

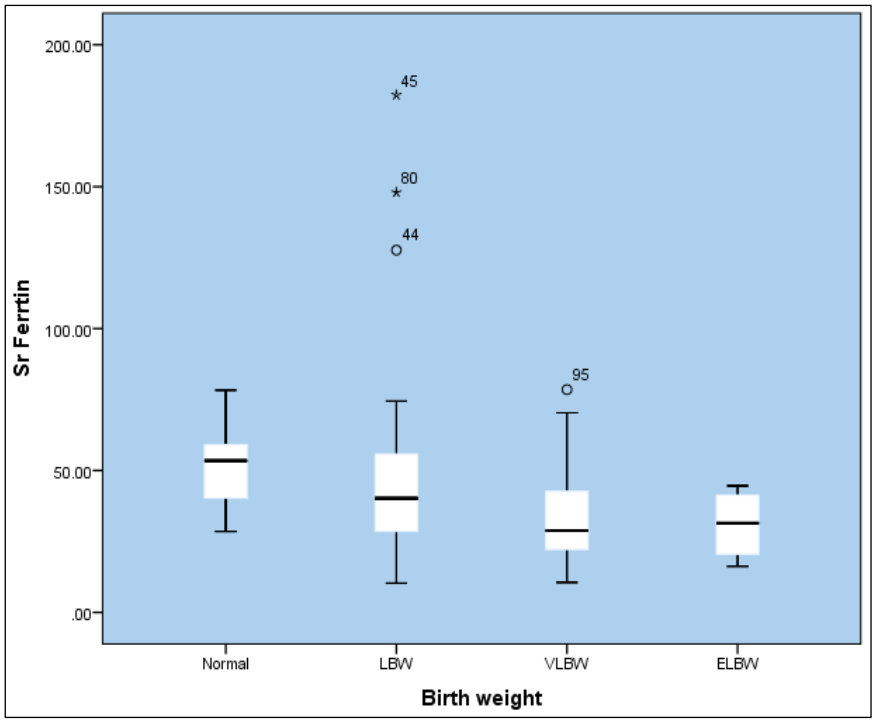

Fig 4:Serum ferrtin values a 3months CA in comparisonwith birth weight

Mean ferritin values among study population was lowest (17 ± 3.5) among ELBW with highest among normal birth weight (54.0 ± 15.54) with intermediate values among LBW and VLBW population. There exists a significant association (p<0.01) among serum ferritin values and birth weight.

Table 5:Serum ferritin among study populationreceivingformula milk vs predominant breast feeding

PN Formula milk		N	Mean	S. D	R-value	Sig
Sr Ferritin	Yes	20	39.9	30.68	0.59	0.507 #
	No	111	43.85	23.19		
# No Statistical Significance at P>0.05 level						

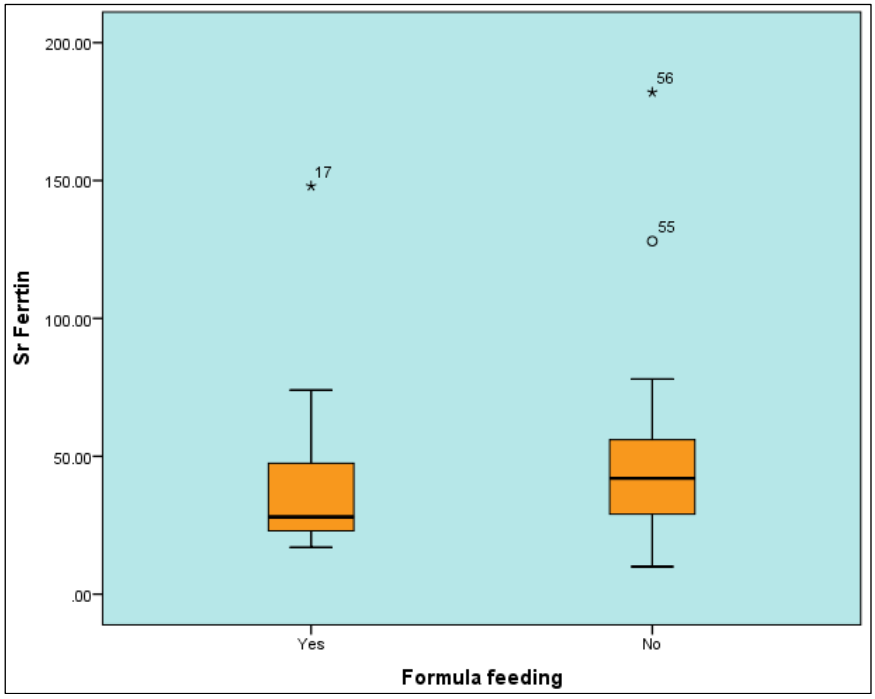

Fig 5: Serum ferritin among population receivingpredominant breast feeds vs formula feeds

Table 6:Comparison of Hb levels among studypopulation receiving predominant breast milk vs formulamilk

PN Formula milk		N	Mean	Std. Deviation	Std. ErrorMean	R-value	Sig
Hb	Yes	20	10.0	0.85	0.85	0.294	0.001**
	No	111	11.0	1.2	1.23		
** Statistically significant at $p<0.05$ level # No Statistical Significance at P>0.05 level							

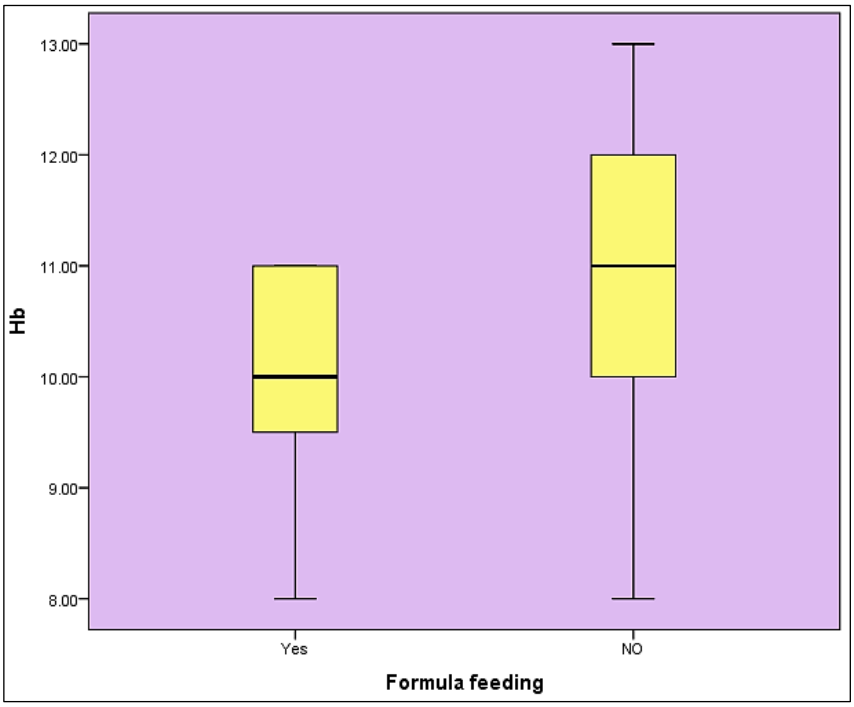

Fig 6: Comparison of Hb levels among study populationreceiving predominant breast milk and formula milk

There was no significant difference (p>0.01) noted in serum ferritin values among population receiving formula milk or breast milk; while a significant association is seen between Hb values and feeding characteristics.

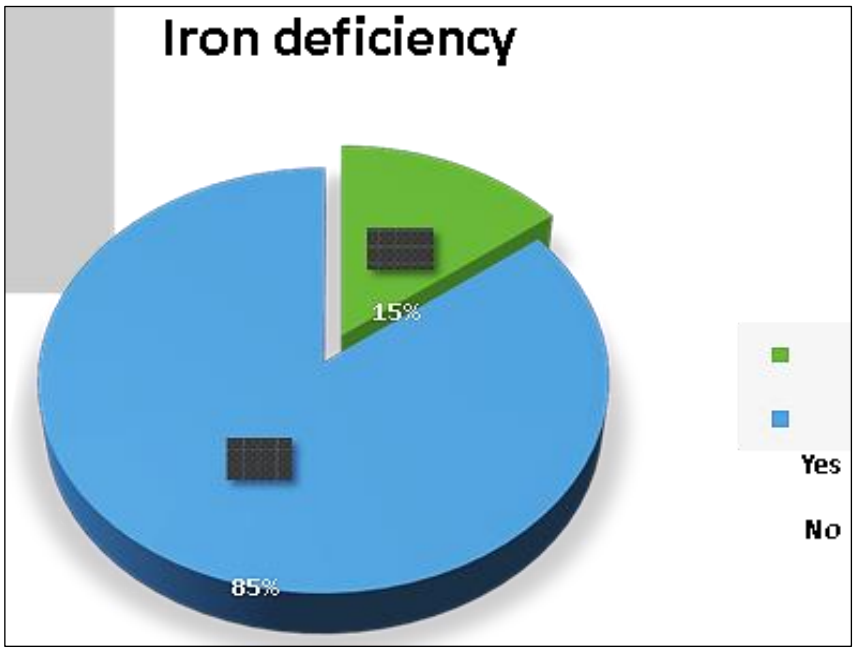

Fig 7: Prevalence of iron deficiency among studypopulation

Among the population evaluated mean of Hb was 11.03 gm/dL (max 13.4 and min 8.3) and serum ferritin was 43.94 µg/L (max 181.3 and min 10.3). As per postnatal age and weight defined criteria 20% (26 out of 131) developed anaemia while on prophylactic iron supplementation.

However, 65% of the iron supplemented study population (85 out of 131) still had adequate ferritin levels (20 mcg/dL) at 3 months corrected age.

Table 7: Correlation between Hb and serum ferritin valuesof study population

		Sr Ferritin	Hb
Sr Ferritin	**Pearson Correlation**	1	.272**
	Sig. (2-tailed)		.002
	N	131	131
Hb	**Pearson Correlation**	.272**	1
	Sig. (2-tailed)	.002	
	N	131	131
****. Correlation is significant at the 0.01 level (2-tailed).**			

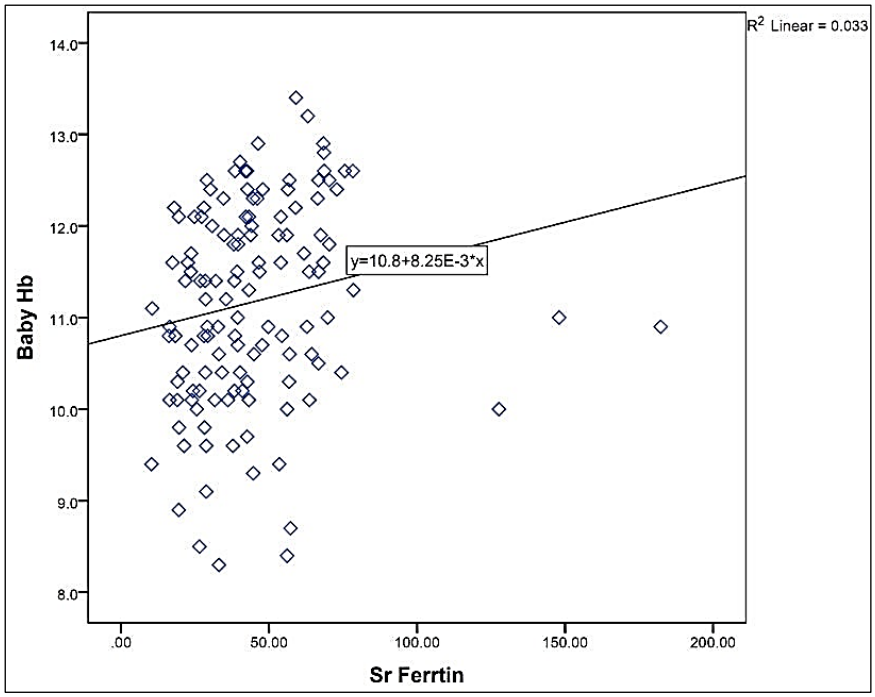

Fig 8: Scatter plot: Hb vs Serum ferritin of studypopulation

There exists a significant correlation (p<0.01) between serum ferritinand Hb of study population at 3 months of corrected age.

DISCUSSION

Among various groups based on birth weight higher Hb levels were found among preterm babies with normal weight (mean Hb of 11.6 gm/dL) while a decrease of Hb was noted with a decrease in birth weight.

Higher serum ferritin values were foundamong preterm babies with normal weight (mean 54±15.5 µg/L) while low birth weight babies had lower ferritin concentrations at three months corrected age. A significant association was noted among Hb and Serum ferritin (p<0.05) at 3 months corrected age in comparison with birth weight.

Association of higher birth weight with Hb and ferritin at 3 months signifies composite effect of possibly better foetal nutrition and nutrient accretionprenatally. However, serum ferritin has a wide range of normal values and affected by many factors, hence, external validity of this observation needs to be considered cautiously.

A study by Olivers *et al.* showed a significantly higher cord SF in preterm AGA when compared to preterm SGA group with 55% preterm SGA neonates having abnormally low cord SF levels (< 60 µg/L) in comparison with preterm AGA neonates (20%)[9].

A significant difference in Hb concentration was notices among various groups of infants at four months of postnatal age who did not receive iron supplementation. Among those who had received iron supplementation Hb values were higher in comparison to the non-supplemented infants atfour months of age. There was no significant difference in transferrin saturation, total iron binding capacity (TIBC) or

serum iron concentrations among the various groups at 4 months postnatal age.[9]

Akkermans *et al.* in a study involving 200 preterm neonates (Gestational age:32-35 weeks) noticed a significant association between birth weight and iron deficiency anaemia with multiple blood draws for investigations and frequent parenteral feeding during hospital stay serving as an independent risk factor at a later stagefor iron depletion.[10]

Our study conforms with the previous studies in establishing a significant positive association between birth weight and iron status at follow up.

In our study it was observed that the mean Hb at three months corrected age of those who had undergone blood investigation during NICU stay was 10.4 gm/dL with lowest (9.08 gm/dL) among those who had undergone a cumulative blood loss of >10 ml during NICU stay.

Similarly, the mean serum ferritin at 3 months corrected age among the population who underwent blood investigation was 35.57 µg/L with lowest values found among those with a cumulative blood loss of >10 ml during NICU stay.

A significant association (p<0.05) was seen between phlebotomy loss attributed to blood investigation with Hb and serum ferritin levels at 3 months corrected age. Also, the greater the blood loss lesser was the Hb and serum ferritin.

In a study by Mukhopadhya*et al*., the mean phlebotomy loss in NICU among term small for gestational age infants was 1.7± 3 ml(106). Similarnegative correlation between phlebotomy loss and iron status in first few weeks of life has been noted in few other studies too.[11]

Widness*et al*. in his study found that requirement for RBC transfusion was significantly lower in those who underwent mini-blood investigation by an in-line umbilical artery catheter analyser in comparison with those who underwent regular laboratory exam in their first postnatal week.[11]

This observation highlights the need for rational approach towards limiting investigations to bare minimum to ensure minimum blood loss during the NICU stay. Also, limiting phlebotomy loss by using micro-technique advances may decrease the prevalence of iron-deficiency at later postnatal age.

In the present study, most of the preterm infants had received various combination and proportion of breast feeds and formula feeds. However, after discharge majority (111 out 131; 84.7%) of the preterm infants were predominantly on breast feeds with formula feeds contributing <10% of the total daily intake. Fifteen percent (20 out of 131) were predominantly on formula feeds. The formula feeding group comprised mainly of VLBW and ELBW category, twins and few late preterm, where maternal lactation failure was a reason.

The mean Hb among the population receiving predominant formula milk was 10.25 gm/dL while those receiving predominant breast milk was11.39 gm/dL. The mean ferritin values were 42.85 µg/L among those receiving formula milk and 45.14 µg/L among those receiving predominant breast feeds. This observation is not surprising as the formula feeding has inherent disadvantages and time frame required to attain full tolerancealso is longer. These observations can be used to impress upon exclusive breast feeding by the mothers.

In a prospective study by Girffin*et al.* no significant difference in plasma ferritin and haemoglobin or incidence of iron deficiency was found among randomly assigned groups of healthy low birth weight infants who were assigned into three groups with one receiving iron fortified preterm formula, one receiving iron fortified term formula and the third receiving both for 6 months corrected age [12]. However, this study was in a controlled setting with scope for periodic monitoring of the babies which had an advantage of feed supervision and built in advantage of repeated assessments and advice.

In the present study only 2 out of 131 from the breast-fed group had ferritin values <12µg/L which is taken as cut-off for iron deficiency in previous studies. All the infants in formula fed group had serum ferritin levels >12 µg/L. in contrast to the previous studies. Hb among formula fed infants were lower than breast fed group, which could be explained by the fact that most infants in formula fed group had greater morbidity and longer NICU stay than breast fed group.

In the present study there was a significant correlation between serum ferritin values and Hb values at 3 months corrected age (p<0.01).

In a study by Schiza*et al.* it was found that among preterm infants (32-36 weeks) of postnatal age between 3 and 12 months of life, there was no difference in Hb concentration between those with high serum ferritin and those with low serum ferritin at birth. [13]

Yamada *et al.* in 2014, found that,though ferritin is a reference marker ofiron storage it was hardly affected by the depletion of iron stores initially but eventually depleted by two months of age.[14]

As the nadir in preterm neonates is reached earlier i.e. 5-10 weeks, adequateserumferritinconcentrationscontributedbyearl yironsupplementation by 3-4 weeks postnatal age could improve the Hb values in comparison with non-supplemented infants.

Hence, this observation may help to strengthen the recommendation to screen only haemoglobin at ambulatory care settings with estimation of ferritin restricted to select high risk cases.

CONCLUSION

In our study despite being on prophylactic oral iron supplementation at 2mg/kg/day few healthy preterm infants had iron deficiency (15%) and iron deficiency anaemia (21%) at the age of 3 months corrected age.

Gestational age and birth weight were found to have a negative correlation with postnatal iron status in early infancy. Increased phlebotomy loss in NICU was associated with reduced iron status in early infancy. Serum ferritin values were sufficient and had a positive correlation with Hb when iron supplementation is started in early infancy.

REFERENCES

1. Kliegman RM. Nelson textbook of pediatrics. 21st edition. Philadelphia, MO: Elsevier; 2019.
2. Rao R, Georgieff MK. Iron in fetal and neonatal nutrition. Seminars in Fetal and Neonatal Medicine. 2007 Feb;12(1):54–63.
3. Makrides M, Anderson A, Gibson RA, Collins CT. Improving the Neurodevelopmental Outcomes of Low-Birthweight Infants. In: Bhatia J, Bhutta ZA, Kalhan SC, editors. Nestlé Nutrition Institute Workshop Series [Internet]. Basel: S. KARGER AG; 2013 [cited 2019 Nov 17]. p. 211–21.
4. Ohls RK. Neurodevelopmental Outcome and Growth at 18 to 22 Months' Corrected Age in Extremely Low Birth Weight Infants Treated with Early Erythropoietin and Iron. PEDIATRICS. 2004 Nov 1;114(5):1287–91.
5. Wardrop CA, Holland BM, Veale KE, Jones JG, Gray OP. Non-physiological anaemia of prematurity. Arch Dis Child. 1978 Nov;53(11):855–60.
6. Eichenwald EC, Hansen AR, Martin C, Stark AR. Cloherty andStark's manual of neonatal care [Internet]. 2017
7. Strauss RG. Anaemia of Prematurity: Pathophysiology & Treatment. Blood Rev. 2010 Nov;24(6):221–5.
8. Wang Y, Wu Y, Li T, Wang X, Zhu C. Iron Metabolism and Brain Development in Premature Infants. Front Physiol. 2019 Apr 25;10:463.
9. Olivares M, Llaguno S, Marin V, Hertrampf E, Mena P, Milad M. Iron status in low-birth-weight infants, small and appropriate for gestational age. A follow-up study. Acta Paediatrica. 1992 Oct;81(10):824–8.
10. Akkermans MD, Uijterschout L, Abbink M, Vos P, Rövekamp-Abels L, Boersma B, *et al*.Predictive factors of iron depletion in late preterm infants at the postnatal age of 6 weeks. Eur J Clin Nutr. 2016 Aug;70(8):941–6.
11. Widness JA. Pathophysiology, Diagnosis, and Prevention of Neonatal Anemia. NeoReviews. 2000 Apr 1;1(4):61e–68.
12. Griffin *et al*.Iron nutritional status in preterm infants fed formulas fortified with iron | ADC Fetal & Neonatal Edition.BMJ volume 81,issur
13. Schiza V, Giapros V, Pantou K, Theocharis P, Challa A, Andronikou
14. S. Serum transferrin receptor, ferritin, and reticulocyte maturity indices during the first year of life in 'large' preterm infants. Eur J Haematol. 2007 Nov;79(5):439–46.
15. Yamada RT, Leone CR. Hematological and iron content evolution in exclusively breastfed late-preterm newborns. Clinics (Sao Paulo). 2014 Dec;69(12):792–8.