RESEARCH PAPER

Iron Profile in Term Small for Gestational Age Infants at 10 Weeks of Age and Correlation With Maternal Iron Profile : *A Prospective Cohort Study*

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Background: Term small for gestational age (SGA) babies are at risk for developing iron deficiency anemia. The association between maternal and infant iron stores is not clear.

Objective: To assess proportion of term SGA neonates developing iron deficiency anemia by 10 weeks of age, and measure correlation between iron profile and hepcidin of babies at birth and at 10 weeks of age with maternal iron profile.

Design: Prospective cohort study conducted from November, 2018 to April, 2020.

Participants: 120 term SGA babies and their mothers.

Intervention: Hemogram, iron profile and serum hepcidin (every fourth case) estimated in mother, cord blood and baby at 10 weeks. Babies developing anemia at 6 weeks detected by hemogram and ferritin were started on iron supplementation and excluded from the study.

Outcome: Proportion of babies developing iron deficiency anemia at 10 weeks of age.

Results: 35 (29.2%) of 120 term SGA babies developed anemia (hemoglobin <9 g/dL) at 6 weeks. Proportion of infants who developed iron deficiency anemia (hemoglobin <9 g/dL and serum ferritin <40 µ/dL) at 6 and 10 weeks of age was 14.2% and 23.3%, respectively. No significant correlation was found bet-ween hemoglobin, iron and hepcidin of the baby in cord blood and at 10 weeks of age with that of mothers. Serum hepcidin in babies at birth (137.5 ng/mL) were higher than maternal values (128 ng/mL).

Conclusion: A significant proportion of term SGA infants developed anemia during early infancy, irrespective of maternal iron status.

Keywords: *Anemia, Ferritin, Hemoglobin, Hepcidin*

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Iron is an essential nutrient during all stages of human development. The requirement of iron increases during periods of rapid growth and differentiation such as pregnancy and infancy [1].

Transfer of iron from the mother to the fetus is an active process that occurs mainly in the third trimester. Most babies born to women with iron deficiency anemia (IDA) have serum iron levels comparable to those born to iron-replete women but with lower serum ferritin levels; thus, suggesting decreased iron stores [2]. Hepcidin operates as a negative feedback regulator of this iron homeostasis across the placenta and ensures appropriate physiological concentration of iron in the fetus. Maternal hepcidin levels gradually decrease in pregnancy from the first to the third trimester to meet the six-fold higher needs for iron absorption and facilitate its placental transfer. Lower hepcidin concentration in the mother increases duodenal iron absorption, allows release of iron from macrophages and hepatic stores to maternal circulation, thereby increasing iron availability to the fetus. On the other hand, higher hepcidin concentration in the placenta than in the mother avoids iron overload in the fetus [3].

Neonates born as preterm, very low birth weight (VLBW) and small for gestational age (SGA) are at risk of developing IDA due to poor iron stores. There are guidelines on iron supplementation to the preterm and VLBW babies but limited studies for term SGA neonates [4-6]. Prevalence of iron deficiency and timing of hemoglobin nadir in this group is not well documented. Existing guidelines do not recommend the time of initiation of iron supplementation in SGA babies. With India contributing to largest number of SGA births worldwide [7], an understanding of prevalence of IDA in SGA during early infancy and its association with maternal iron status will help develop focused preventive strategies. A decrease in hepcidin is considered an early marker of IDA with decreased transferrin saturation and ferritin levels.

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Thus, we planned this study to determine the correlation between iron profile of term SGA neonates and their mothers. The primary objective was to assess proportion of term SGA neonates who develop IDA at 10 weeks of age. The secondary objectives were to correlate serum iron,

total iron binding capacity (TIBC), percent transferrin saturation (TSat), ferritin and hepcidin of SGA babies at birth and at 10 weeks of age with maternal iron profile.

METHODS

This prospective cohort study was conducted in the neonatology unit of Departments of Pediatrics, Obstetrics and Gynecology and Pathology of a tertiary care teaching institute from November, 2018 to April, 2020 after obtaining ethical clearance from institutional ethical committee for human research. Verbal consent was taken from mothers at the time of screening for fetal growth restriction. Written informed consent was taken from the parent/guardian for participation in the study before enrolment.

Pregnant women admitted for delivery in the labor room were screened for suspected fetal growth restriction (FGR) by assessing symphysio-fundal height and abdominal circumference on available antenatal ultra-sound scans by obstetrician. FGR was defined as fetal weight or abdominal circumference less than 10th centile for that gestational age [8]. The neonates born at gestational age of 37- 42 weeks with birth weight less than 10th centile for that gestational age as per the 21st Intergrowth charts and their mothers were then enrolled in the study [9]. Neonates requiring admission in neonatal intensive care unit (NICU) in view of any of the ailments such as sepsis, birth asphyxia, respiratory distress at birth (defined as Downe score of more than 2), seizures or known Rh or ABO incompatibility were excluded from the study. Babies born to mothers who were known cases of hemolytic anemias including thalassemia and sickle cell anemia, chronic kidney disease, chronic liver disease, hemochromatosis, chorioamnionitis, infection with human immunodeficiency virus, hepatitis B, syphilis, toxo-plasmosis, rubella, cytomegalovirus, malaria and obstetric complications like antepartum and postpartum hemorrhage were also excluded from the study. Delayed cord clamping (DCC) was followed at the time of all deliveries as per the standard protocol. Mothers of all the infants were counseled regarding exclusive breastfeeding. Enrolments were done as per the work-shifts of the principal investigator.

Baseline demographic profile of both the mother and the baby with anthropometric parameters (including weight, length, head circumference and ponderal index) of the baby were noted at birth [10]. Three mL each of cord blood and maternal venous blood (within 2 hours of delivery) was collected for hemogram with indices, iron profile, ferritin and transferrin saturation at birth. Babies were discharged within 48 to 72 hours of birth on exclusive breast feeding.

The enrolled babies were followed up at 6 weeks (±1 week) and 10 weeks (±1 week) of age and their growth was monitored. Reinforcement of exclusive breast feeding was done at each visit. All the infants were immunized at each visit as per the National Immunization Schedule [11]. Hemogram with indices and serum ferritin of the baby was done at 6 weeks. Iron deficiency anemia was defined as fall in hemoglobin below 9 g/dL accompanied with serum ferritin <40 µg/L at 6-10 weeks of age as per the European Society for Paediatric Gastroenterology Hepatology and Nutrition (ESPGHAN) committee guidelines [12]. Those who developed anemia were assessed clinically for any features of sepsis, hepatosplenomegaly or blood loss. They were started on iron supplementation (4-6 mg/kg/day) after investigations and were excluded from the analysis [13]. Hemogram with indices, iron profile and serum ferritin were repeated for the rest of the babies at 10 weeks of age.

Samples for serum hepcidin assessment was taken from cord blood, mother (within 2 hours of delivery) and infant at 10 weeks (±1 week) of follow up in every fourth mother-infant pair and were stored at -20 ^{0}C for subsequent analysis.

Hemogram with indices was assessed using auto analyzer (Backmann culter LH 500). Venous sample was collected in iron free tubes for iron studies. Transferrin saturation (TSat), serum iron concentration and total iron binding capacity (TIBC) were done at the department of pathology, as recommended by the Iron Panel of the International Council for Standardisation in Hematology (ICSH) [14]. Serum samples for ferritin and hepcidin levels were stored at -20 ^{0}C until assayed using commercial ELISA kits (Diametra Ferritin ELISA Kit-96 wells Cat No. DKO039 and Human Hepc25 ELISA Kit E-EL-H5497).

In a study by Mukhopadhyaya, et al. [2], proportion of term SGA infants with serum ferritin <40 µg/L at birth was observed to be 34%. Considering absolute precision of 10% with confidence interval of 95% and power of 80%, sample size was calculated to be 87. Assuming that approximately 30% of babies might become anemic by 10 weeks of age and may need iron supplementation before final analysis, we decided to enroll 120 mother-infant pairs at birth.

Statistical analysis: Data were analyzed using SPSS software version 20.0. Proportions were expressed as numbers and percentages. Paired normally distributed variables (hemogram and iron profile) were compared using paired *t* test. Non-normally distributed variables (serum ferritin and serum hepcidin) were compared using Wilcoxon signed rank test/Friedman test. Linear correlation was explored using Pearson correlation (for normally distributed parameters) and Spearman correlation (for non-normally distributed parameters). *P* value <0.05 was considered significant.

RESULTS

The flow of study participants is shown in **Fig. 1**. Thirty-five (29.2%) enrolled babies developed anemia (hemoglobin <9 g/dL) at 6 weeks of age and were started on iron supplementation. Of the remaining 85 infants, another 24 infants (total 49.2%) became anemic at 10 weeks of age (**Fig. 1**). Proportion of term SGA infants who developed iron deficiency anemia (hemoglobin <9 g/dL and serum ferritin <40 µg/dL) at 6 and 10 weeks of age was 14.2% and 23.3%, respectively**.**

Anthropometric parameters of enrolled infants at birth, 6 weeks and 10 weeks are shown in **Table I**. The mean (SD) gestation age was 39 (1.13) weeks (67 boys), and 107 (89.2%) were exclusively breastfed till 10 weeks of age.

The maternal and infant profile of hematological parameters is shown in **Table II**. A significant fall was noted in mean serum iron, percentage transferrin saturation and median serum ferritin levels of infants at 10 weeks of age as compared to that in cord blood. It was also observed that serum iron levels and %transferrin saturation levels of infants in cord blood were more than that of mothers at the time of delivery.

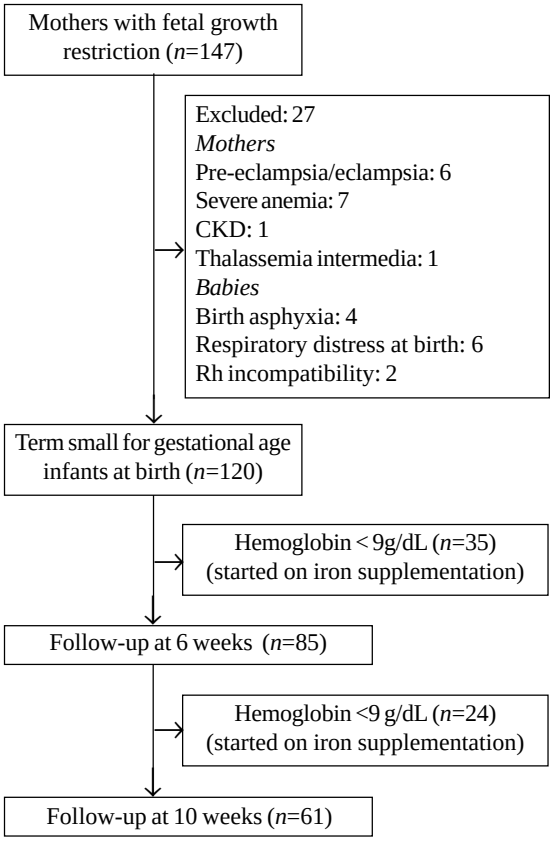

Fig. 1 Flow of participants in the study.

Table I Anthropometric Parameters of Term Small for Gestational Age Infants Enrolled in the Study (*N*=120)

Variables	*At birth, n=120*	*At 6 wk age, n=120*	*At 10 wk age, n=85*
Weight (g)	2431.5 (234.07)	3529.6 (278.10)	4307.8 (310.03)
Length (cm)	48.8 (0.76)	53.1 (2.44)	57.7 (1.30)
HC (cm)	34.5 (0.48)	36.5 (2.16)	38 (0.54)

Data expressed as mean (SD). HC: head circumference.

No significant correlation was found between hemoglobin, iron profile and hepcidin of the baby in cord blood and at 10 weeks of age with maternal profile iron (P>0.05), except a weak positive correlation between maternal serum iron and serum iron of the infant in cord blood (r=0.19, P=0.03). Serum hepcidin in cord blood showed a negative but significant correlation with maternal serum ferritin (r = – 0.39, P=0.033).

DISCUSSION

In our study, it was observed that nearly one-fourth of term SGA infants developed IDA by 10 weeks of age. There was no significant correlation between maternal iron profile with that of babies at birth and at 10 weeks of age. Serum hepcidin of babies at birth negatively correlated with maternal serum ferritin levels.

A higher risk of IDA in term SGA infants could be explained by their poor iron stores at birth with chronic utero-placental insufficiency, higher requirements for catch up growth and higher red cell mass [6]. A previous study [2] lower cord serum ferritin levels in SGA as compared to AGA infants. Cord serum iron levels were also lower in SGA infants than AGA infants suggesting poorer iron stores at the time of birth [15,16]. None of the babies in this study had anemia at birth; though, a significant proportion of these babies developed anemia by 10 weeks of age, the levels below the cutoff of 10 g/dL for physiological anemia of infancy at 8-10 weeks of age [17].

The transfer of iron from mother to fetus is an active process occurring against the concentration gradient via placenta. However, it is uncertain whether the transfer of iron across the placenta is proportional to iron available in the mother or whether it is transferred preferentially as per the requirements of the fetus. The iron status of cord blood in infants correlated weakly with maternal iron status, similar to as seen in earlier studies [18]. A weak positive correlation was reported between maternal iron and cord blood ferritin [19,20], unlike this study where no correlation between maternal hemoglobin and iron status of infants were seen [21,22].

Table II Hematological Parameters and Iron Profile of Mothers and Term Small for Gestational Age Infants in Cord Blood, and at 6 Week and 10 Week of Age

Variable	*Mothers (n=120)*	*Infants*			
		Cord blood (n=120)	*6 week (n=120)*	*10 week (n=85)*	*P value*[b]
Hemoglobin (g/dL)	9.9 (1.53)	15.5 (1.39)	9.8 (1.13)	9.3 (0.77)	<0.001
RBC count ($X10^9$/L)	3.7 (0.60)	4.5 (0.55)	3.47 (0.40)	3.51 (0.40)	<0.001
Hematocrit (%)	31.6 (5.61)	47.8 (6.04)	30.9 (3.67)	29.1 (2.62)	<0.001
Mean corpuscular volume (fL)	86.2 (12.86)	107.1 (7.21)	89.1 (4.83)	83.4 (6.04)	<0.001
MCH (pg/cell)	27.2 (4.10)	34.9 (3.13)	28.4 (2.47)	26.7 (3.19)	<0.001
MCHC (g/dL)	32.4 (9.85)	32.6 (2.82)	31.9 (2.03)	31.9 (2.53)	0.096
Total leukocyte count ($x10^9$/L)	12.1 (4.61)	11.3 (3.71)	8.7 (1.80)	8.9 (1.86)	<0.001
Platelet count ($x10^9$/L)	224 (0.79)	188 (72)	324 (77)	315 (62)	0.019
Serum iron (µg/dL)	88.21 (32.18)	153.23 (46.59)	-	75.16 (30.80)	<0.0001
TIBC (µg/dL)	375.75 (87.83)	249.15 (65.31)	-	242.27 (63.42)	0.901
Transferrin saturation (%)	32.02 (10.81)	63.71 (20.05)	-	25.21 (13.45)	<0.001
Serum ferritin (µg/L)[a]	18.5 (8.00-35.00)	106 (52-153.5)	143 (74.5-11.5)	98 (64-151)	0.032
Serum hepcidin (ng/mL)[a] (*n*=30)	128.0 (112.25-132.75)	137.5 (134.0-141.75)	-	139.5 (134.5-142.0)	0.802

Values expressed as mean (SD) *or* [a]*median (IQR).* [b]*P value for statistical significance between cord blood vs 10 weeks. TIBC: total iron binding capacity; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration.*

Maternal serum hepcidin values were lower than the cord blood hepcidin values, without any correlation between the two. A few studies reported a low positive correlation between the two [23-25]. Lower hepcidin concentration in the mother increases duodenal iron absorption, allows release of iron from macrophages and hepatic stores to increase iron availability to the fetus. On the other hand, higher hepcidin concentration in the placenta than in the mother avoids iron overload in the fetus. A negative correlation between serum hepcidin in cord blood and maternal serum ferritin suggests that hepcidin at birth might be regulated by maternal iron stores.

Being a prospective cohort study, the infants who were anemic at 6 weeks of age were started on iron supplementation and excluded from the study. The improvement in their iron status, was not studied, thus amounting to a limitation of this study. We did not look at the maternal dietary and supplemental iron intake. Moreover, serum hepcidin values were assessed only in 30 infant-mother pairs due to budgetary constraints.

The study concludes that a significant proportion of term SGA neonates develop IDA and need iron supplemen- tation. There are no existing guidelines for prophylactic administration of iron in term SGA neonates. We recommend that iron status of SGA neonates should be evaluated in larger population-based studies. Further, multicentric interventional studies to determine time and dose of iron supplementation should be conducted to formulate guidelines regarding prophylactic iron supplementation to term SGA infants.

Ethics clearance: Institutional Ethics Committee–Human Research, UCMS; No. IEC-HR/2018/36/109, dated Oct 15, 2018.
Contributors: PB, PD: conceived the idea; KS, PB: conceptualized the study and devised its design; BG,PG: provided critical inputs; KS: collected the data; PB,PD,PG,BG: supervised data collection and helped in conduct of study; KS: drafted the manuscript and all authors have critically approved final version of study as submitted, and are willing to be accountable for all aspects of the study.
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WHAT IS ALREADY KNOWN?

- Preterm and very low birth babies are at risk of iron deficiency anemia and need iron supplementation.

WHAT THIS STUDY ADDS?

- Significant proportion of term small for gestational age neonates developed iron deficiency anemia by 10 weeks of age.
- Iron stores of babies at birth were found to be independent of maternal stores.

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