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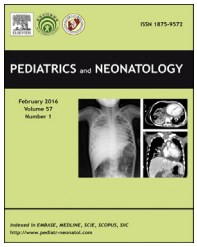

ORIGINAL ARTICLE

Maternal and Cord Blood Hepcidin Concentrations in Severe Iron Deficiency Anemia

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Key Words
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Background: The present study was conducted to assess the maternal and cord blood hepcidin concentrations in severe iron deficiency anemia (IDA) and to find out its correlation with other iron status parameters.
Methods: This prospective observational study was carried out in 30 mothers with severe IDA (hemoglobin < 70 g/L and serum ferritin < 12 μg/L), and 15 healthy nonanemic (hemoglobin ≥ 110 g/L) mothers, who delivered live singleton neonates at term gestation. Mothers and neonates with infection/inflammatory conditions were excluded. Quantitative estimation of complete blood count, serum iron, ferritin, total iron binding capacity (TIBC), and transferrin saturation (Tfsat) was done in maternal and cord blood immediately after delivery by an auto analyzer. Serum hepcidin concentrations were measured by double-antibody sandwich enzyme-linked immunosorbent assay using a Human Hepcidin-25 kit. Data were analyzed by statistical software SPSS 16.0.
Results: The serum iron and ferritin concentrations in severe IDA were 6.7 ± 1.8 μmol/L and 4.1 ± 1.4 μg/L in maternal blood, and 9.5 ± 2.6 μmol/L and 55.4 ± 19.7 μg/L in cord blood, respectively, significantly lower than nonanemic controls ($p < 0.001$). The corresponding serum hepcidin concentrations were 76.6 ± 22.7 μg/L and 110.5 ± 11.8 μg/L, respectively ($p < 0.05$). The proportion of cord blood/maternal blood hepcidin concentration was similar in both anemic (1.4:1) and nonanemic (1.3:1) mothers. Significant correlation was observed among maternal and cord blood hepcidin concentrations and other iron status parameters.
Conclusion: Even in the presence of low serum iron and ferritin, maternal and cord blood

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hepcidin concentrations remained high in severe anemia. Failure of this proportional suppression of hepcidin indicates poor systemic bioavailability of iron to the mother and poor placental transport.

1. Introduction

Iron is an essential micronutrient, necessary for oxygen transport and other vital biological processes of life. Worldwide, iron deficiency anemia (IDA) is the most common nutritional deficiency[1] and one of the major causes of anemia during pregnancy. As per the recent estimates, the worldwide prevalence of anemia in pregnant women is approximately 38%, and in $>$ 50% of them, the cause of anemia is iron deficiency.[2] The rate is even higher in developing countries, and India has the highest prevalence of maternal anemia in the world.[3]

Iron is necessary to support placental and fetal growth. Maternal IDA not only increases the incidence of maternal morbidity and mortality, but it also increases the risk of premature delivery, fetal growth restriction, and perinatal mortality and morbidity.[4–6] Moreover, maternal IDA precludes healthy neurodevelopment of the fetus, since iron is essential for proper neurogenesis, myelination, and neurocognitive maturity.[7,8] Impaired fetal iron transport may have lifelong, irreversible effects on future neurodevelopment.[9]

Iron requirement increases by nearly 10 times during pregnancy (0.8 mg/d in the 1st trimester to 7.5 mg/d in the 3rd trimester),[10] making iron accessibility critical throughout pregnancy. The developing fetus is entirely dependent on its mother for iron accretion, which takes place almost exclusively via the placenta. Placental iron homeostasis results from the tightly coordinated regulation of various proteins involved in iron uptake, transport, intracellular storage, and iron trafficking. The major regulator of systemic iron bioavailability in the human body is hepcidin, a small 25 amino acid amphipathic peptide hormone.[11] Hepcidin has also been documented recently to be one of the key determinants of placental transport of iron and the regulator of maternofetal iron transfer during pregnancy.[12] The fetal hepcidin-placental-ferroportin axis represents an important element in the fetus-dependent control of iron transport through the placenta.[13]

In a developing country like India, most pregnant women are iron-depleted and hardly receive any nutritional or iron supplementation during pregnancy. Although previous authors have shown a significant association among placental transport of iron and maternal hepcidin concentration,[11,12,14–17] no study has documented the state of maternal and fetal hepcidin concentration in the presence of severe maternal IDA. In the present study, we measured serum hepcidin and other iron status parameters, such as complete blood count, serum iron, ferritin, total iron binding capacity (TIBC), and transferrin saturation (Tfsat) in mothers with severe IDA and cord blood of their neonates. We also looked for any correlations among the parameters.

2. Methods

This study was carried out in the Neonatal Unit, Department of Pediatrics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India, after obtaining approval from the Institute Ethics Committee.

2.1. Study participants

Consecutively admitted mothers with severe IDA, who delivered live singleton neonates at term gestation (37–41 weeks), formed the case group. Severe IDA was defined as a hemoglobin $<$ 70 g/L and serum ferritin $<$ 12 μg/L. Mothers with hemolytic anemias including thalassemia and sickle cell anemia, chronic infection/inflammatory diseases, hemochromatosis, chronic kidney disease, chorioamnionitis, infection with human immunodeficiency virus/ hepatitis B/syphilis, Toxoplasma, Rubella, Cytomegalovirus, Herpes (TORCH) infections, malaria, pregnancy induced hypertension, diabetes, and other obstetric complications were excluded from the study. Neonates with perinatal asphyxia, early-onset neonatal sepsis, isoimmune hemolytic anemias, and congenital malformations were also excluded.

Healthy nonanemic mothers with hemoglobin $\geq$ 110 g/L, delivering live singleton neonates at term gestation, served as controls. A written informed consent in the local language was taken from all parents before inclusion in the study.

2.2. Clinical work-up

Demographic details, as well as antenatal and perinatal history including iron and folic acid supplementation received by the mother during pregnancy, were noted. Nutritional status of the mother was determined by measuring weight, height, and body mass index after at least 48 hours of childbirth. Neonates were examined thoroughly after birth to exclude any congenital malformation or other systemic diseases. Birth weight was taken soon after birth. Gestational age was assessed from maternal history and antenatal ultrasound, and clinically corroborated after birth by the New Ballard Score.[18] Neonates were observed for development of any complications during the hospital stay and managed as per our unit protocol.

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2.3. Collection of samples and laboratory analysis

After complete delivery of the neonate, 5 mL of maternal venous blood was collected by aseptic venipuncture of a peripheral vein. Free flowing cord blood (10 mL) was collected from the placental end of the umbilical cord without milking. Quantitative estimation of complete blood count was done by an auto analyzer (Coulter LH 750 Hematology Analyzer, Beckman Coulter, Inc., Brea, CA, USA). For the measurement of serum iron, ferritin, TIBC, and Tfsat, serum was separated immediately by centrifugation (448–700 g/min for 15–20 minutes) and was measured by an auto analyzer (R_X Suzuka Clinical Chemistry Analyzer, Randox, Inc., Crumlin, UK). For the estimation of hepcidin, serum was stored at −20°C until final analysis and measured by double-antibody sandwich enzyme-linked immunosorbent assay using a Human Hepcidin-25 (HEPC25) (Hangzhou Eastbiopharm Co. Ltd. Hangzhou, Zhejiang, China) kit.

2.4. Statistical analysis

The statistical program SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) was used for data entry and analysis. The Mann Whitney U test and Fisher's exact test were used to compare different variables. Spearman correlation was calculated to identify correlation among various parameters. A p value < 0.05 was considered as statistically significant.

3. Results

A convenient sample of 30 mothers with severe IDA and their neonates were included as cases. Fifteen healthy mothers without anemia along with their infants served as controls. The baseline characteristics of the study population are shown in Table 1. Among maternal parameters, body mass index, receipt of antenatal care, dietary habits, and status of iron and folic acid supplementation were significantly better in nonanemic mothers. Among neonatal parameters, mean birth weight of the neonates born to nonanemic mothers was significantly higher than the anemic mothers (3002 ± 428 g vs. 2396 ± 305 g; $p < 0.001$), although no difference was observed in mean gestational age. None of the mothers in any group was obese.

The mean hemoglobin, serum iron, and serum ferritin of anemic mothers were significantly lower than the nonanemic mothers both in maternal and the cord blood ($p < 0.001$). Serum hepcidin concentrations of anemic mothers were also lower in the maternal and the cord blood, but the difference was only modest (76.6 ± 22.7 μg/L vs. 92.4 ± 10.5 μg/L in maternal blood and 110.5 ± 11.8 μg/L vs. 124.0 ± 17.4 μg/L in cord blood, respectively; $p < 0.05$; Table 2).

Table 3 shows the relationship between maternal and cord blood iron status parameters and serum hepcidin. Both in anemic and nonanemic mothers, most of the iron parameters were significantly higher in the cord blood. Maximum difference was observed for serum ferritin and the differences were lower for serum iron and hepcidin.

Table 1 Baseline characteristics of the study population.

Parameter	Nonanemic mothers (maternal Hb ≥ 110 g/L) (n = 15)	Anemic mothers (maternal Hb < 70 g/L) (n = 30)	Mann Whitney U test[a]/Fisher's exact test[b]
Maternal characteristics			
Age (y)	26.3 ± 3.4	25.3 ± 3.7	0.385 (NS)[a]
Body mass index (kg/m^2)	24.3 ± 3.9	20.8 ± 3.2	< 0.01[a]
Gravida	1 (1–3)	1 (1–3)	1.000 (NS)[a]
Parity	0 (0–1)	0 (0–1)	0.146 (NS)[a]
Antenatal care taken	12 (80.0)	4 (13.3)	< 0.001[b]
Vegetarian dietary habit	7 (46.7)	24 (80.0)	< 0.03[b]
Iron & folate supplementation	15 (100.0)	2 (6.7)	< 0.001[b]
Details of delivery			
Mode of delivery			
SVD	12 (80.0)	24 (80.0)	1.000 (NS)[b]
Cesarean section	3 (20.0)	6 (20.0)	
Presentation			
Vertex	15 (100.0)	28 (93.3)	0.545 (NS)[b]
Breech	0 (0.0)	2 (6.7)	
Neonatal characteristics			
Gestational age (wk)	38.8 ± 1.5	38.9 ± 1.3	
	39	39	0.940 (NS)[a]
	(38–40)	(38–40)	
Birth weight (g)	3002 ± 428	2396 ± 305	< 0.001[a]
	3000 (2600–3250)	2460 (2188–2605)	
Apgar score	9 (7–10)	8 (7–9)	1.000 (NS)[a]

Data are presented as n (%), mean ± standard deviation, or median (interquartile range).
Hb = hemoglobin; SVD = spontaneous vaginal delivery.

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Table 2 Comparison of the maternal and cord blood hemoglobin, iron indices, and hepcidin concentration of the study population.

Parameter	Nonanemic mothers (maternal Hb $\geq$ 110 g/L) (n = 15)	Anemic mothers (maternal Hb $<$ 70 g/L) (n = 30)	Mann Whitney U test	Controls/cases ratio
Maternal blood				
Hemoglobin (g/L)	129 $\pm$ 8	55 $\pm$ 14	$<$ 0.001	2.3:1
Serum iron (μmol/L)	22.9 $\pm$ 5.8	6.7 $\pm$ 1.8	$<$ 0.001	3.4:1
Serum ferritin (μg/L)	21.2 $\pm$ 3.6	4.1 $\pm$ 1.4	$<$ 0.001	5.2:1
TIBC (μmol/L)	40.5 $\pm$ 11.5	95.4 $\pm$ 14.0	$<$ 0.001	0.4:1
Transferrin saturation (%)	48.2 $\pm$ 15.7	7.3 $\pm$ 2.4	$<$ 0.001	6.6:1
Serum hepcidin (μg/L)	92.4 $\pm$ 10.5	76.6 $\pm$ 22.7	$<$ 0.05	1.2:1
Median	92.4	84.9		
Percentiles				
5	67.5	33.6		
95	108.3	107.5		
Cord blood				
Hemoglobin (g/L)	163 $\pm$ 16	122 $\pm$ 10	$<$ 0.001	1.3:1
Serum iron (μmol/L)	23.8 $\pm$ 3.2	9.5 $\pm$ 2.6	$<$ 0.001	2.5:1
Serum ferritin (μg/L)	159.7 $\pm$ 26.3	55.4 $\pm$ 19.7	$<$ 0.001	2.9:1
TIBC (μmol/L)	48.8 $\pm$ 6.9	77.6 $\pm$ 11.2	$<$ 0.001	0.6:1
Transferrin saturation (%)	48.2 $\pm$ 15.7	12.5 $\pm$ 4.2	$<$ 0.001	3.8:1
Serum hepcidin (μg/L)	124.0 $\pm$ 17.4	110.5 $\pm$ 11.8	$<$ 0.05	1.12:1
Median	117.2	109.3		
Percentiles				
5	97.6	94.8		
95	151.2	135.6		

Data are presented as mean $\pm$ standard deviation, unless otherwise indicated.
TIBC = total iron binding capacity.

Table 3 The relationship between maternal and cord blood iron status parameters and serum hepcidin in nonanemic and anemic mothers.

Parameter	Cord blood	Maternal blood	Mann Whitney U test	Cord blood/maternal blood ratio
Nonanemic mothers (maternal Hb $\geq$ 110 g/L) (n = 15)				
Hemoglobin (g/L)	163 $\pm$ 16	129 $\pm$ 8	$<$ 0.001	1.3:1
Serum iron (μmol/L)	23.8 $\pm$ 3.2	22.9 $\pm$ 5.8	0.570 (NS)	1.03:1
Serum ferritin (μg/L)	159.7 $\pm$ 26.3	21.2 $\pm$ 3.6	$<$ 0.001	7.5:1
TIBC (μmol/L)	48.8 $\pm$ 6.9	40.5 $\pm$ 11.5	$<$ 0.05	1.2:1
Transferrin saturation (%)	61.8 $\pm$ 14.0	48.2 $\pm$ 15.7	$<$ 0.05	1.3:1
Serum hepcidin (μg/L)	124.0 $\pm$ 17.4	110.5 $\pm$ 11.8	$<$ 0.001	1.3:1
Anemic mothers (Maternal Hb $<$ 70 g/L) (n = 30)				
Hemoglobin (g/L)	122 $\pm$ 10	55 $\pm$ 14	$<$ 0.001	2.2:1
Serum iron (μmol/L)	9.5 $\pm$ 2.6	6.7 $\pm$ 1.8	$<$ 0.01	1.4:1
Serum ferritin (μg/L)	55.4 $\pm$ 19.7	4.1 $\pm$ 1.4	$<$ 0.001	13.5:1
TIBC (μmol/L)	77.6 $\pm$ 11.2	95.4 $\pm$ 14.0	$<$ 0.001	0.8:1
Transferrin saturation (%)	12.5 $\pm$ 4.2	7.3 $\pm$ 2.4	$<$ 0.01	1.7:1
Serum hepcidin (μg/L)	110.5 $\pm$ 11.8	76.6 $\pm$ 22.7	$<$ 0.001	1.4:1

Data are presented as mean $\pm$ standard deviation.
TIBC = total iron binding capacity.

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Table 4 Spearman correlation among maternal and cord blood hemoglobin, iron indices, and hepcidin.

		Maternal iron	Maternal ferritin	Maternal TIBC	Maternal Tfsat	Maternal hepcidin	Cord blood Hb	Cord blood iron	Cord blood ferritin	Cord blood TIBC	Cord blood Tfsat	Cord blood l hepcidin
Maternal Hb	Correlation	0.941*	0.942*	−0.894*	0.903*	0.486*	0.827*	0.884*	0.917*	−0.778*	0.870*	0.556*
	Sig. (2-tailed)	< 0.001	< 0.001	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Maternal iron	Correlation	1	0.945*	−0.886*	0.961*	0.361†	0.807*	0.873*	0.907*	−0.791*	0.866*	0.401*
	Sig. (2-tailed)	_	< 0.001	< 0.001	< 0.001	0.015	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.006
Maternal ferritin	Correlation	_	1	−0.873*	0.926*	0.373†	0.888*	0.907*	0.939*	−0.814*	0.926*	0.439*
	Sig. (2-tailed)	_	_	< 0.001	< 0.001	0.012	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.003
Maternal TIBC	Correlation	_	_	1	−0.923*	−0.417*	−0.838*	−0.750*	−0.842*	0.803*	−0.786*	−0.404*
	Sig. (2-tailed)	_	_	_	< 0.001	0.004	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.006
Maternal Tfsat	Correlation	_	_	_	1	0.330†	0.844*	0.811*	0.896*	−0.800*	0.841*	0.346†
	Sig. (2-tailed)	_	_	_	_	0.027	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.020
Maternal hepcidin	Correlation	_	_	_	_	1	0.386*	0.365†	0.368†	−0.237	0.329†	0.717*
	Sig. (2-tailed)	_	_	_	_	_	0.009	0.014	0.013	0.117	0.027	< 0.001
Cord blood Hb	Correlation	_	_	_	_	_	1	0.798*	0.866*	−0.779*	0.846*	0.388*
	Sig. (2-tailed)	_	_	_	_	_	_	< 0.001	< 0.001	< 0.001	< 0.001	0.008
Cord blood iron	Correlation	_	_	_	_	_	_	1	0.868*	−0.738*	0.965*	0.443*
	Sig. (2-tailed)	_	_	_	_	_	_	_	< 0.001	< 0.001	< 0.001	0.002
Cord blood l ferritin	Correlation	_	_	_	_	_	_	_	1	−0.787*	0.882*	0.457*
	Sig. (2-tailed)	_	_	_	_	_	_	_	_	< 0.001	< 0.001	0.002
Cord blood TIBC	Correlation	_	_	_	_	_	_	_	_	1	−0.828*	−0.291
	Sig. (2-tailed)	_	_	_	_	_	_	_	_	_	< 0.001	0.052
Cord blood Tfsat	Correlation	_	_	_	_	_	_	_	_	_	1	0.358†
	Sig. (2-tailed)	_	_	_	_	_	_	_	_	_	_	0.016

Hb = hemoglobin; TIBC = total iron binding capacity; Tfsat = transferrin saturation.

* Correlation is significant at the 0.01 level (2-tailed).

† Correlation is significant at the 0.05 level (2-tailed).

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The proportion of cord blood/maternal blood hepcidin concentration was similar in both anemic (1.4:1) and non-anemic (1.3:1) mothers.

Spearman correlation among different parameters is shown in Table 4. Significant correlations were observed among all the iron status parameters. Maternal hepcidin was significantly correlated with cord blood hepcidin concentration and other iron status parameters.

4. Discussion

Hepcidin is the major regulatory hormone for iron homeostasis. It inhibits iron absorption or reutilization by binding to ferroportin, the major iron exporter, leading to its internalization and degradation, and thereby decreasing the export of iron into plasma. The most important down-regulator of serum hepcidin is anemia and poor iron status.[19]

The normal serum hepcidin concentration in healthy women volunteers ranges from 17 ng/mL or μg/L to 286 ng/mL or μg/L (5–95%).[20] Serum hepcidin concentrations were reported to decrease with the advancement of pregnancy. Several longitudinal studies observed the lowest hepcidin concentrations in the third trimester.[16,21–23] Increasing need for fetal iron and decreasing maternal iron levels in the third trimester were the probable reasons for the decrease in maternal hepcidin in the third trimester.[24] Hepcidin concentration in pregnant women with lowest iron status was found to be the lowest.[15] In the present study, the serum iron and ferritin concentrations in severe IDA were as low as 6.7 ± 1.8 μmol/L and 4.1 ± 1.4 μg/L in maternal blood and 9.5 ± 2.6 μmol/L and 55.4 ± 19.7 μg/L in cord blood, respectively. The corresponding serum hepcidin concentrations were 76.6 ± 22.7 μg/L and 110.5 ± 11.8 μg/L, respectively.

Ganz et al[20] observed that mean hepcidin concentration in normal term infants was 90.7 ng/mL, similar to that in adults. The observed values of serum hepcidin in studies done in anemic mothers ranged from undetectable[15] to quite low, geometric mean (95% confidence intervals) 2.8 (0.5–13.1) nmol/L[25] to 10.7 (8.5–13.4) ng/mL.[15] In our study, the cord blood hepcidin concentrations were only 1.4 times higher than the maternal levels in severe IDA and the ratio was similar to the mothers without anemia (1.3:1). In the study of Rehu et al[15] the cord blood values were almost six times higher than those in maternal blood. In our study, serum hepcidin concentrations in nonanemic mothers were 92.4 ± 10.5 μg/L. Most studies done in developed countries reported undetectable to very low median values for maternal serum hepcidin concentrations, such as undetectable to 0.25 nmol/L,[16] 9.5 ng/mL in the third trimester,[22] 3.0 nmol/L at 24 hours postpartum,[16] from 2.52 ng/mL to 2.83 ng/mL,[21] and 4.2 (1.6–8.0) nmol/L,[25] whereas one Asian study reported very high maternal serum hepcidin concentrations of 193.637 ± 52.219 μg/L.[26] No study has been done so far from India. Contrary to other studies,[15,27] we have found significant correlation among maternal and cord blood hepcidin concentrations and other iron status parameters.

Although the iron requirement of the fetus is met regardless of maternal iron stores, iron trafficking in fetal organs and tissues through the placenta is tightly regulated, as the developing fetus is extremely vulnerable to iron toxicity and free radical-mediated oxidative stress.[28] Studies have shown that ferroportin is present in the basal membrane of the syncytiotrophoblasts exporting iron from the syncytiotrophoblasts into the fetal circulation.[29] It is not yet known exactly how iron crosses the barrier of the fetal vascular endothelium and enters the fetal circulation from the syncytiotrophoblasts, because ferroportin is not expressed on fetal blood vessels.[29] Hepcidin is pivotal for fetal iron homeostasis. It mediates the regulation of iron efflux from the placenta by controlling the rate of iron absorption and also determining iron mobilization from stores through negative modulation of the function of ferroportin at the basolateral membrane of syncytiotrophoblasts, inhibiting iron release from placental cells.[13] Immunohistochemical demonstration of hepcidin molecules in the villous syncytiotrophoblast and the mesothelial layer of the secondary yolk sac by Evans et al[30] supported this view. Moreover, animal experiments have shown that transferrin receptor 1, expressed on the apical membrane of the syncytiotrophoblasts, another major iron transporter of placenta,[31] is also regulated by hepcidin.[32]

In the present study, mean birth weight of neonates born to mothers with severe IDA was significantly lower. Low birth weight and intrauterine growth restriction (IUGR) are common complications of maternal IDA.[4,5] Briana et al[27] investigated iron homeostasis in full-term IUGR and appropriate-for-gestational-age infants at birth. The authors did not find any difference in hepcidin concentrations between the groups.

In the presence of low serum iron and ferritin, serum hepcidin concentrations should have been reduced further in severe IDA. The mothers were already compromised by their limited access to both heme and non-heme sources of iron, since most of them were vegetarian and did not receive any iron supplementation during pregnancy. Failure of proportional suppression of serum hepcidin further compromised their iron absorption and reutilization, resulting in poor placental transfer and reduced systemic bioavailability of iron to the fetus. The exact reason of high hepcidin concentration is difficult to explain. We tried to exclude the factors which could increase maternal and cord blood hepcidin concentrations, such as infection and inflammatory states, preeclampsia etc.[24] Downregulation of genes responsible for hepcidin expression in mothers with long-standing anemia could be the responsible factor, but this is only a speculation.

Our study was limited by its sample size. Inclusion of mothers with mild-to-moderate anemia in the study group would have been interesting to observe the status of hepcidin in those cases. Secondly, we measured serum hepcidin only after delivery; following up the mothers from the beginning of pregnancy to childbirth would have yielded important information. Moreover, we did not examine the placenta. Immunohistochemistry staining of placenta for hepcidin receptors would have been informative, but it was beyond our study protocol.

To conclude, failure of suppression of maternal and cord blood hepcidin concentrations, even in the presence of poor iron status, is alarming for the mother as well for her

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developing fetus, as it may have long-term consequences. Optimization of the nutritional intake during pregnancy should be ensured. Future longitudinal studies may be carried out to identify the contributing factors for timely implementation of necessary corrective measures.

Conflicts of interest

All authors declare no conflicts of interest.

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