

# Cord Blood and Breast Milk Iron Status in Maternal Anemia

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## ABSTRACT

**OBJECTIVES.** The purpose of this work was to assess the effect of severe maternal iron-deficiency anemia and nutritional status on cord blood and breast milk iron status.

**METHODS.** We conducted a prospective observational study over a 6-month period in a teaching hospital in central India. The study population consisted of 55 anemic (hemoglobin: <110 g/L) and 20 healthy nonanemic (hemoglobin: ≥110 g/L) pregnant women who delivered singleton live births at term gestation. We measured hemoglobin, iron, and ferritin levels in paired maternal and cord blood and iron levels in early (day 3 ± 1) and late (day 15 ± 3) transitional milk. Maternal anthropometry, including weight, height, midarm circumference, triceps skinfold thickness, and placental weight, were recorded. The main outcome measure of the study was to find out the relationship of maternal hemoglobin, iron, ferritin, and anthropometry with hemoglobin, iron, and ferritin in cord blood and iron levels in breast milk.

**RESULTS.** Concentrations of hemoglobin, iron, and ferritin were significantly lower in the cord blood of anemic mothers and showed linear relationships with maternal hemoglobin and ferritin levels. Breast milk iron content was significantly reduced in severely anemic mothers but not in those with mild-to-moderate anemia. Breast milk iron level correlated with maternal hemoglobin and iron levels but not with ferritin levels. Maternal anthropometry had significant correlations with indices of iron nutriture in maternal and cord blood but showed no relationship with breast milk iron content. Placental weight was comparable between anemic and nonanemic mothers.

**CONCLUSIONS.** Maternal anemia, particularly the severe type, adversely affects cord blood and breast milk iron status. Maternal nutritional status exerts a significant influence on fetal iron status but has little influence on breast milk iron content.

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### Key Words

breast milk, cord blood, iron status, maternal anemia, newborn

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**A**NEMIA REMAINS WIDELY prevalent in India and other developing countries. Studies from India indicate that 75% of young children, 90% of adolescent girls, and 85% of pregnant women suffer from anemia, mostly because of iron deficiency.<sup>1,2</sup> Approximately 33.6% of pregnant women have hemoglobin levels between 70 and 90 g/L, and 13% have hemoglobin levels of <70 g/L.<sup>3,4</sup> Indeed, a majority of women in the reproductive age group in developing countries are anemic even before conception; pregnancy only tends to intensify it further. It follows that the nature of pregnancy anemia in developing countries is different from that in the developed world. It is widely prevalent and more severe in degree, frequently coexists with maternal malnutrition, and is of long duration, present since the beginning of pregnancy or antedating it in many subjects. Under these circumstances, the competing demands of mother and fetus may disturb the normal maternal-fetal iron homeostasis.

The relationship between maternal and fetal iron status is still disputed. Studies report that iron transfer to the fetus may depend on<sup>5-11</sup> or be independent of<sup>12-17</sup> maternal iron status. A similar uncertainty exists with respect to the influence of maternal anemia on breast milk iron status.<sup>18-23</sup> One possible reason for this discrepancy could be the noninclusion of an adequate number of pregnant women with severe anemia in earlier studies. It is conceivable that in women with mild-to-moderate maternal anemia, biological adaptive mechanisms ensure adequate iron transfer

to the fetus and breast milk. But in women with severe maternal anemia, these protective mechanisms might fail, leading to insufficient iron supply to the fetus and breast milk.<sup>24</sup> Moreover, earlier studies also did not examine the influence of concomitant maternal malnutrition in maternal anemia on fetal iron nutrition in anemic women. Maternal malnutrition, by producing morphologic changes in placenta, may affect nutrient transfer from mother to fetus.<sup>25</sup> The purpose of the present study was to assess the effect of severe maternal iron-deficiency anemia and nutritional status on cord blood and breast milk iron status.

## METHODS

### Participants

The study was conducted on consecutively selected 55 anemic (hemoglobin: <110 g/L) and 20 healthy nonanemic pregnant women (hemoglobin: ≥110 g/L) delivering singleton live births at term gestation (37–41 weeks) in a university hospital. The subjects were divided into 4 groups: group 1 (hemoglobin: ≤60 g/L; *n* = 21); group 2 (hemoglobin: 61–85 g/L; *n* = 16); group 3 (hemoglobin: 86–109 g/L; *n* = 18); and group 4 (hemoglobin: ≥110 g/L; *n* = 20). Exclusion criteria were prolonged rupture of membranes (>24 hours), fever, foul-smelling liquor, antepartum hemorrhage, pregnancy-induced hypertension, eclampsia, diabetes mellitus, liver or kidney disorders, and any other systemic illness. Gestational age was calculated from the first day of the last menstrual period and confirmed by the New Ballard score.<sup>26</sup> All of the anemic mothers belonged to lower socioeconomic status and received no regular antenatal care or iron supplementation during pregnancy. Informed consent was taken from mothers, and the study protocol was approved by the institute ethics committee.

### Collection of Samples and Laboratory Analysis

Paired maternal and cord blood samples were collected in iron-free polyethylene tubes from the mother's ante-cubital vein during the first stage of labor and from the placental end of the umbilical cord without milking just after the second stage for the estimation of hemoglobin level by using the cyanmethemoglobin method,<sup>27</sup> serum iron by atomic absorption spectrophotometry (model 2380; Perkin Elmer, Waltham, MA), and serum ferritin by the enzyme-linked immunosorbent assay method.<sup>28</sup> Samples were stored at -20°C until analyzed. Breast milk samples were collected after delivery by manual expression on day 3 ± 1 (early transitional milk) and on day 15 ± 3 (late transitional milk). Mothers were instructed to first clean their breasts with a gauge piece soaked with plain water, then allowing the area to air dry. After discarding an initial 4 to 5 mL of milk, an aliquot of 10 mL was collected in deionized glass jars and finally transferred to iron-free polyethylene tubes. Milk expression was done from 1 breast only, between 9:00 AM to 12:00 PM, ~1.5 to 2.0 hours after the last breastfeeding. Milk samples were preserved at -20°C until they were analyzed for iron content by atomic absorption spectrophotometry as per Moser and Reyn-

olds.<sup>29</sup> None of the mothers had mastitis or febrile illness during the period of study. All of the mothers received explicit instructions regarding the handling of a glass jar to avoid contamination. Milk expression was done under the supervision of nurses or resident doctors. All of the mothers practiced exclusive breastfeeding.

### Measurement of Maternal Anthropometry and Placental Weight

Maternal anthropometry was recorded using standard methods.<sup>30</sup> Weight was recorded on the third day after delivery to the nearest 100 g. Standing height was measured with the help of stadiometer to the nearest 1 cm. Midarm circumference was measured on the left arm to the nearest 0.1 cm. Triceps skinfold thickness was measured with a large skinfold caliper (Cambridge Scientific Industries, Inc, Cambridge, MD) to the nearest 1 mm at the midpoint of the left arm on the triceps muscle. The skinfold was raised and caliper applied for 2 to 3 seconds, and then readings were taken. The measurements were made in triplicate, and their mean was taken as the final value of triceps skinfold thickness. The placental weight was recorded on beam balance to the nearest 20 g. Before taking weight, the cord was cut at a level with placental surface and the membranes trimmed off. Blood clots were removed from the placenta, and subchorionic vessels were drained of blood by gentle pressure.

### Statistical Analysis

Statistical analysis was performed by using statistical software SPSS 10 (SPSS Inc, Chicago, IL). One-way analysis of variance (analysis of variance F) and Student-Newman-Kuel tests were used for normally distributed variables. Serum ferritin values, being skewed, were analyzed by Kruskal-Wallis H test and the Mann-Whitney U test. Other tests included calculation of correlation coefficients (*r<sub>s</sub>*) and Spearman's rank correlation coefficients (*r<sub>s</sub>*).

## RESULTS

Different study groups were comparable with respect to maternal age, median parity, gestation, height, triceps skinfold thickness, and placental weight. In comparison with control mothers (group 4), all of the anemic women had lower weight, whereas midarm circumference was less only in the severely anemic group (group 1). Concentrations of hemoglobin, iron, and ferritin were significantly lower in the cord blood of anemic mothers and showed linear relationships with maternal hemoglobin levels (Table 1). Iron content was significantly reduced in the breast milk of severely anemic women. On the other hand, breast milk iron content in mild-to-moderate maternal anemia was comparable with the control group. Low serum ferritin values in anemic women indicate iron deficiency as a cause of anemia in these cases. Maternal ferritin levels had significant correlations with hemoglobin levels (*r<sub>s</sub>* = 0.488; *P* < .001), iron (*r<sub>s</sub>* = 0.445; *P* < .001), and ferritin (*r<sub>s</sub>* = 0.440; *P* < .001) in cord blood. However, maternal ferritin levels showed an insignificant correlation with

**TABLE 1** Indices of Iron Nutriture (Mean  $\pm$  SD) in Maternal and Cord Blood and Breast Milk of Anemic Mothers

Study Group	Range of Maternal Hemoglobin, g/L		Hemoglobin, g/L		Iron, $\mu$ mol/L			Ferritin, $\mu$ g/L	
	Maternal Blood	Cord Blood	Maternal Blood	Cord Blood	Maternal Blood	Early Transitional Milk	Late Transitional Milk	Maternal Blood	Cord Blood
1 (n = 21)	52 $\pm$ 7 <sup>a</sup>	156 $\pm$ 6 <sup>a</sup>	4,885 $\pm$ 1,461 <sup>a</sup>	24,428 $\pm$ 3,388 <sup>a</sup>	11,543 $\pm$ 3,921 <sup>a</sup>	12,293 $\pm$ 3,064 <sup>a</sup>	1.11 $\pm$ 1.48 <sup>a</sup>	38.05 $\pm$ 9.22 <sup>a</sup>	
2 (n = 16)	71 $\pm$ 6 <sup>a</sup>	158 $\pm$ 11 <sup>a</sup>	9,571 $\pm$ 2,261 <sup>a</sup>	27,745 $\pm$ 4,689 <sup>a</sup>	13,842 $\pm$ 3,361 <sup>a</sup>	13,213 $\pm$ 3,592 <sup>a</sup>	2.60 $\pm$ 2.11 <sup>a</sup>	56.46 $\pm$ 8.41 <sup>a</sup>	
3 (n = 18)	86–109	166 $\pm$ 8 <sup>a</sup>	14,488 $\pm$ 1,965 <sup>a</sup>	30,530 $\pm$ 5,382 <sup>a</sup>	14,482 $\pm$ 2,966 <sup>a</sup>	14,061 $\pm$ 2,223 <sup>a</sup>	7.55 $\pm$ 3.21 <sup>a</sup>	263.61 $\pm$ 189.39 <sup>a</sup>	
4 (n = 20)	$\geq$ 110	172 $\pm$ 7 <sup>a</sup>	18,292 $\pm$ 1,452 <sup>a</sup>	34,147 $\pm$ 2,327 <sup>a</sup>	15,231 $\pm$ 2,767 <sup>a</sup>	14,901 $\pm$ 2,412 <sup>a</sup>	39.35 $\pm$ 27.91 <sup>a</sup>	180.98 $\pm$ 164.84 <sup>a</sup>	
Analysis of variance	F = 452.01 <sup>b</sup>	F = 14.52 <sup>c</sup>	F = 215.76 <sup>b</sup>	F = 18.81 <sup>b</sup>	F = 4.523 <sup>c</sup>	F = 5.686 <sup>c</sup>	H = 62.24 <sup>b,d</sup>	H = 56.58 <sup>b,d</sup>	
Student-Newman-Keul test									
1 vs 4	49.86 <sup>b</sup>	8.42 <sup>b</sup>	34.05 <sup>b</sup>	10.39 <sup>b</sup>	4.92 <sup>c</sup>	5.57 <sup>b</sup>	9.37 <sup>b,e</sup>	9.55 <sup>b,e</sup>	
2 vs 4	33.87 <sup>b</sup>	7.0 <sup>b</sup>	20.64 <sup>b</sup>	6.31 <sup>b</sup>	2.01 (NS)	2.42 (NS)	13.05 <sup>b,e</sup>	11.16 <sup>b,e</sup>	
3 vs 4	20.73 <sup>b</sup>	3.0 <sup>f</sup>	9.38 <sup>f</sup>	3.68 <sup>f</sup>	0.96 (NS)	1.25 (NS)	10.42 <sup>b,e</sup>	1.17 (NS) <sup>e</sup>	
Correlation coefficient	—	+0.616 <sup>b</sup>	+0.937 <sup>b</sup>	+0.643 <sup>b</sup>	+0.382 <sup>b</sup>	+0.383 <sup>b</sup>	+0.636 <sup>b,g</sup>	+0.458 <sup>b,g</sup>	

NS indicates not significant; —, data are not applicable.

<sup>a</sup> Data represent the mean  $\pm$  SD.

<sup>b</sup> The P value is <.001.

<sup>c</sup> The P value is <.01.

<sup>d</sup> Data are from the Kruskal-Wallis test.

<sup>e</sup> Data are from the Mann-Whitney U test.

<sup>f</sup> The P value is <.05.

<sup>g</sup> Data are from the Spearman rank correlation coefficient.

early transitional milk iron ( $r_s = 0.226$ ;  $P > .05$ ) and a weak correlation with late transitional milk iron ( $r_s = 0.251$ ;  $P < .05$ ). Maternal iron levels had significant correlations with iron in cord blood ( $r = 0.764$ ;  $P < .001$ ), early transitional milk ( $r = 0.439$ ;  $P < .001$ ), and late transitional milk ( $r = 0.451$ ;  $P < .001$ ). Table 2 shows that indices of iron nutriture in maternal and cord blood had significant correlations with maternal weight, midarm circumference, and triceps skinfold thickness but had only a slight correlation with maternal height. In contrast, maternal anthropometry showed no relationship with iron content in breast milk.

## DISCUSSION

Our study population was characterized by severe anemia with coexisting malnutrition. The serum ferritin levels were low in all of the anemic women in the study group, confirming the etiology of anemia as iron deficiency. In the present study, it was documented that iron-deficiency anemia during pregnancy could compromise fetal iron status. Significantly lower levels of hemoglobin, iron, and ferritin were found in the cord blood of infants born to anemic mothers. Moreover, indices of iron nutriture in maternal and cord blood showed significant correlations with each other, suggesting suboptimal iron supply to the fetus in maternal anemia. Similar findings were documented in previous studies, which showed the negative influence of maternal anemia on fetal iron accumulation.<sup>5–11</sup> However, some investigators could not find any relationship between maternal and fetal iron nutriture, thereby concluding that the fetus continues to extract iron efficiently from the mother regardless of her iron balance.<sup>12–17</sup> However, these studies did not include a sufficient number of pregnant women with severe anemia. Moreover, these studies also did not examine the effect of simultaneously occurring maternal malnutrition on fetal iron status. Both of these conditions could affect iron supply to the fetus by inducing placental changes. It is known that severe anemia, present from early gestation along with concomitant maternal malnutrition, may be associated with reduced placental weight<sup>31,32</sup> and structural abnormalities of the placenta.<sup>25</sup> Placental size is closely related to the surface area of peripheral villi,<sup>33</sup> which, in turn, determines the transport of nutrients across the placenta.<sup>34</sup> In the present study there was no significant difference in the placental size between anemic and nonanemic control mothers. It was likely that coexisting maternal malnutrition prevented the enlargement of the placenta in anemic women.

Iron is actively transported from mother to the fetus.<sup>35</sup> In the iron-deficiency state, there is upregulation of iron transport proteins in the placenta,<sup>24</sup> which ensures adequate iron supply to the growing fetus. This explains higher iron and ferritin levels in cord blood compared with the maternal blood, even in cases of severe maternal anemia. However, the linear relationships of cord blood iron and ferritin levels with maternal hemoglobin and ferritin levels in this study show that the fetus accrues iron in direct proportion to the levels available in mother. Thus, it seems that placental iron transport

**TABLE 2** Relationship Between Maternal Anthropometry and Indices of Iron Nutriture in Maternal and Cord Blood and Breast Milk

Maternal Characteristic	Maternal Blood			Cord Blood			Breast Milk Iron	
	Hemoglobin, <i>r</i>	Ferritin, <i>r<sub>s</sub></i>	Iron, <i>r</i>	Hemoglobin, <i>r</i>	Ferritin, <i>r<sub>s</sub></i>	Iron, <i>r</i>	Early Transitional Milk, <i>r</i>	Late Transitional Milk, <i>r</i>
Weight	0.339 <sup>a</sup>	0.343 <sup>a</sup>	0.306 <sup>a</sup>	0.267 <sup>b</sup>	0.023 (NS)	0.231 <sup>b</sup>	0.103 (NS)	0.115 (NS)
Height	0.226 (NS)	0.279 <sup>b</sup>	0.215 (NS)	0.237 <sup>b</sup>	0.097 (NS)	0.235 <sup>b</sup>	-0.025 (NS)	-0.010 (NS)
Midarm circumference	0.437 <sup>c</sup>	0.423 <sup>c</sup>	0.43 <sup>c</sup>	0.463 <sup>c</sup>	0.166 (NS)	0.531 <sup>c</sup>	0.024 (NS)	0.035 (NS)
Triceps skinfold thickness	0.378 <sup>c</sup>	0.366 <sup>a</sup>	0.323 <sup>a</sup>	0.285 <sup>b</sup>	0.228 (NS)	0.428 <sup>c</sup>	-0.073 (NS)	-0.068 (NS)

NS indicates not significant.

<sup>a</sup> The *P* value is <.01.

<sup>b</sup> The *P* value is <.05.

<sup>c</sup> The *P* value is <.001.

mechanisms are not inviolate, and in severe maternal anemia these mechanisms might fail, leading to an insufficient iron supply to the fetus.

The present study demonstrated that breast milk iron content is impaired in iron-deficiency anemia. We observed a significant reduction of iron levels in early and late transitional milk samples of severely anemic mothers. The linear relationship of maternal hemoglobin and iron levels with iron levels in early and late transitional milk samples further supported this observation. On the other hand, maternal serum ferritin levels showed no correlation with early transitional milk iron and only weak correlation with late transitional milk iron levels. In contrast, other studies have found no relation between maternal and breast milk iron status.<sup>18-23</sup> However, many of these studies did not include cases of severe anemia,<sup>18,21,22</sup> which might have affected their results. Because breast milk is the only source of iron in exclusively breastfed young infants in the first few months of life, the adequacy of iron content is important. This study showed that severely anemic women produce milk of low iron content, which may not fulfill the iron requirements of these infants. In contrast to severe anemia, breast milk iron levels were maintained in mild-to-moderate maternal anemia.

The mechanisms of iron transport from the mammary gland into milk are not well understood. Divalent metal transporter 1 and ferroprotein 1 may be involved in transporting iron into milk.<sup>36</sup> Studies show that the expression of divalent metal transporter 1 protein is up-regulated in iron deficiency, resulting in an increased efficiency of iron transport into milk.<sup>36</sup> This may explain why breast milk iron levels remained unaffected in mild-to-moderate anemia and why iron levels were higher in breast milk than in maternal blood, even in cases of severe anemia in this study. However, the regulatory mechanisms are not inexhaustible. The linear relationship of maternal hemoglobin and iron levels with breast milk iron content shows that iron is transported into milk in direct proportion with the levels found in maternal circulation.

The present study shows that maternal nutritional status wields a significant influence on maternal and fetal iron nutriture but has little influence on breast milk iron content. Anthropometric parameters such as weight, midarm circumference, and triceps skinfold thickness exerted greater influence on maternal and fetal iron status than did

height. The former measurements are indicators of recent nutritional experience and reflect the adequacy of diet in current pregnancy. Height, on the other hand, represents past nutritional status. Thus, it seems that nutritional status in current pregnancy may be an important determinant of maternal and fetal iron status. Improving nutrition in current pregnancy may have a favorable influence on fetal iron status.

The present study had the limitation of not assessing maternal iron and nutritional status from early gestation, which would have been more meaningful to see its effect on fetal iron nutrition. However, it was not possible to do so, because none of the study participants were registered in the first trimester and attended hospital for the first time only during delivery. Moreover, it would have been unethical to study the impact of maternal anemia from early pregnancy without supplementing them with iron.

Serum ferritin levels reflect body iron stores.<sup>37</sup> The significantly lower ferritin values in the cord blood of neonates born to anemic mothers showed diminished iron stores in these infants. The consumption of breast milk with low iron content may further deplete the iron stores that are already low at birth. This could have serious consequences for the young infant at a time when iron demands are high. Iron deficiency in early life may have long-term adverse effects on cognitive development<sup>38,39</sup> and may also impair cellular immunity.<sup>40</sup> Thus, the deleterious effects of maternal anemia extend far beyond pregnancy and early infancy. Effective strategies are urgently needed to control maternal anemia in the developing world. Improving the nutritional status and iron status of pregnant and lactating women will have a favorable impact on maternal, fetal, and infant iron nutriture. Another approach to improve the iron status of young infant is to delay the clamping of the umbilical cord after birth.<sup>41</sup> In conclusion, the data of the present study indicate that severe maternal iron-deficiency anemia adversely affects cord blood and breast milk iron status. Furthermore, maternal nutritional status exerts a significant influence on fetal iron nutriture but has little influence on breast milk iron content.

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