Study of maternal influences on fetal iron status at term using cord blood transferrin receptors

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Abstract

Aims—To determine effects of maternal iron depletion and smoking on iron status of term babies using serum transferrin receptors (STfR) and their ratio to ferritin (TfR-F index) in cord blood.

Methods—Iron, ferritin, STfR, and haemoglobin (Hb) concentration were measured and TfR-F index calculated in 67 cord /maternal blood pairs. Twenty six mothers were iron depleted (ferritin <10 µg/l) and 28 were smokers.

Results—Maternal iron depletion was associated with decreased cord ferritin (113 *v* 171 µg/l) and Hb (156 *v* 168 g/l) but no change in STfR or TfR-F index. Smoking was associated with increased cord Hb (168 *v* 157 g/l) and TfR-F index (4.1 *v* 3.4), and decreased ferritin (123 *v* 190 µg/l). Cord TfR-F index and Hb were positively correlated (*r* = 0.48).

Conclusions—Maternal iron depletion is associated with reduced fetal iron stores but no change in free iron availability. Smoking is associated with increased fetal iron requirements for erythropoiesis.

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Keywords: iron status; pregnancy; smoking; transferrin receptors

Iron status is difficult to assess during the neonatal period. Rapid red cell turnover in the first few weeks of life leads to increased serum iron, transferrin saturation, and circulating ferritin concentration, irrespective of iron availability.[1] In preterm babies iron stores are depleted and together with inadequate erythropoiesis this may lead to the anaemia of prematurity.[2][3] In such cases the relative contribution of iron deficiency to this anaemia is unclear.[4][5] A reliable test of iron status in the newborn would enable paediatricians to prescribe the appropriate iron supplementation for individual infants.

In the blood iron is bound to transferrin and intracellular iron uptake is mediated via transferrin receptors on the cell membrane.[6] Serum transferrin receptor (STfR) is a truncated form of the membrane receptor and the concentration of circulating STfR is proportional to the quantity of membrane bound receptor.[7] Measurement of STfR has been proposed as a means of determining iron status. STfR concentrations are increased in iron deficiency anaemia in adults and can help to differentiate this disorder from other causes of anaemia.[8–11] The concentrations of STfR are higher in neonates than in children[12] and adults,[13][14] and they become further increased by active

haemolysis.[15] STfR concentrations increase in neonates during treatment with recombinant DNA derived erythropoietin[16] and cord blood STfR concentrations are thought to indicate fetal iron status.[17][18]

Recently Klausner and his colleagues[19][20] have reported that transferrin receptor (TfR) and ferritin synthesis are coordinately controlled at the RNA level by intracellular binding proteins whose conformations are iron responsive and which bind cognate sites on the respective transcripts. Thus in iron deficiency the proteins bind 5' sites on the ferritin mRNA, preventing its translation, and 3' sites on the TfR mRNA, thereby prolonging its half life and ensuring increased production of TfR protein. In contrast, when intracellular iron is plentiful, the conformation of the proteins prevents them from binding to the mRNAs, so ferritin production is increased and TfR production is decreased. Thus it is reasonable to assume that both serum ferritin and TfR might be useful indicators of iron status. Indeed it has been proposed that the ratio of STfR to the log of the ferritin concentration (TfR-F index) may give the single most accurate indication of tissue iron status.[10][21]

The aims of this study were to utilise STfR and TfR-F index to assess the effects of maternal iron depletion, iron supplementation, and cigarette smoking on fetal iron status at birth.

Subjects and methods

This was a prospective study of 67 mothers and their babies born at term (37–42 weeks) in the Royal Maternity Hospital, Belfast. The Research Ethics Committee of the Queen's University of Belfast approved the study. Samples were collected from consecutive deliveries when a research fellow was available. When booking in this hospital all pregnant women are advised to start oral iron supplements (ferrous fumarate 305 mg daily) at 18 weeks' gestation. Information about smoking and oral iron supplementation during pregnancy was obtained from the mother at delivery. Mothers who reported that they complied with the regimen for iron supplementation throughout pregnancy were categorised as being on iron supplements. Mothers who reported smoking cigarettes throughout pregnancy were taken to be smokers. Following delivery, and after maternal consent, umbilical venous blood (2–3 ml) was taken from the placenta and a simultaneous venous sample (5 ml) taken from each mother's antecubital vein. Babies with known haemolytic disease were excluded. The mothers were defined as iron depleted if their serum ferritin was less than 10 µg/l.

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Table 1 Correlations between STfR and TfR-F index and other measures of iron status in the mother

Maternal blood	STfR		TfR-F index	
	r	p	r	p
Iron	−0.36	0.0032	−0.35	0.0039
Ferritin	−0.51	<0.0001	—	—
TIBC	+0.27	0.032	+0.34	0.0057
Hb	−0.45	0.0003	−0.46	0.0002
MCV	−0.58	<0.0001	−0.58	<0.0001

LABORATORY MEASUREMENTS

Each sample was divided into two aliquots. An EDTA sample (0.6 ml) was sent immediately for measurement of Hb and mean cell volume (MCV, Sysmex SE 9000 series analyser). The remainder was left to clot at room temperature. After centrifugation the serum was separated and stored at −70°C for subsequent analysis. The concentrations of iron, ferritin, and STfR and the total iron binding capacity (TIBC) were measured in both maternal and cord blood sera. Iron was measured by the tripyridyltriazine (TPTZ) method on a Technicon AAII AutoAnalyzer. For determination of TIBC, the serum sample was saturated by the addition of ammonium ferrous sulphate, the excess iron was removed utilising an alumina column, and the bound iron was measured by the addition of TPTZ. An immunoradiometric assay using mouse antibody (ICN Pharmaceuticals) was used to measure ferritin. STfR was measured using an immunoenzymometric assay (Orion Diagnostica). Fifteen of the EDTA samples clotted and were therefore unsuitable for measurement of haemoglobin concentration.

STATISTICAL ANALYSIS

Spearman rank correlation coefficients were used to quantify relations between indices reflecting iron status and were considered significant at p < 0.05. The population was then examined in groups according to mother's iron status, iron supplementation, and smoking. Comparisons between the groups were made using the Student's unpaired t test or Mann–Whitney U test where appropriate and differences were considered significant at p < 0.05. Statistics were calculated using STATVIEW for Windows, version 4.57, 1996 (Abacus Concepts Inc., Berkeley, California, USA).

Results

Sixty seven paired maternal/cord blood samples were studied. Forty one mothers had been taking oral iron supplements. Maternal Hb was slightly lower at antenatal booking (122 v 128 g/l; p = 0.08) and significantly higher at delivery (121 v 113 g/l; p = 0.019) in those who had taken iron supplements. The groups were similar in terms of age but those taking iron were more likely to be primigravida. Twenty six mothers were iron depleted and 28 (41%) had smoked during the pregnancy. The smokers were younger than non-smokers (26.2 v 31.5 years; p = 0.0025) but were of similar parity, and there were no differences in booking Hb, Apgar scores, or any measure of iron status at delivery. Fifteen of the cord blood EDTA samples clotted and were unsuitable for measurement. The babies were all well and none was admitted to the neonatal unit. Their mean (SD) gestation was 39.0 (1.3) weeks and mean (SD) birth weight was 3337 (516) g.

Overall, the STfR concentrations (median; IQR) were more than twice as high in cord blood (8.5; 6.4–10.6 mg/l) as in maternal blood (3.5; 2.3–5.0 mg/l). The ferritin concentrations were also higher in cord blood (132; 93–238 µg/l) than maternal blood (14; 7–29 µg/l) and as a result the TfR-F index was similar in cord and maternal blood (3.8; 3.0–4.8 and 3.5; 1.8–6.2 respectively).

The STfR and TfR-F indices in maternal blood were significantly correlated with all other measures of maternal iron status and both indices were increased in mothers with iron deficiency (table 1). In contrast there were no significant correlations between maternal iron status and any of these measures in cord blood (results not shown).

Maternal iron depletion, defined as serum ferritin less than 10 µg/l, was associated with significantly lower cord blood Hb and ferritin concentrations, but the STfR concentration and TfR-F index were unaffected, suggesting no significant decrease in tissue iron concentrations (table 2). Oral iron supplementation was associated with improved maternal iron status, as reflected by a lower maternal TfR-F index

Table 2 Effect of maternal iron depletion (ferritin <10 µg/l) on fetal iron status assessed in cord blood at term

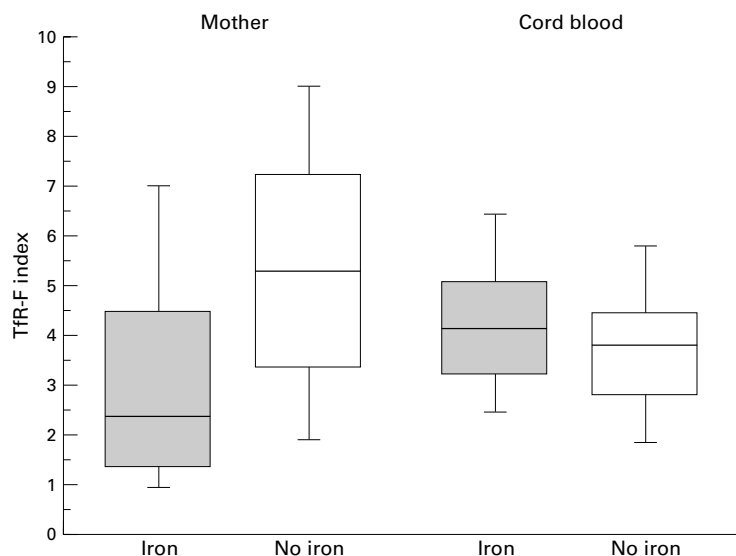| Cord blood	Iron replete (n = 41)	Iron depleted (n = 26)	Significance (p)
Hb (g/l)	168 (156–176) (n = 30)	156 (144–168) (n = 22)	0.048
Iron (µmol/l)	26.5 (23.0–30.5)	26.5 (22.0–29.0)	0.78
TIBC (µmol/l)	39.0 (34.0–48.0)	45.0 (39.0–48.7)	0.45
Ferritin (µg/l)	171 (121–259)	113 (71–191)	0.026
STfR (mg/l)	9.4 (6.7–10.8)	7.5 (6.1–9.4)	0.12
TfR-F index	3.8 (3.1–5.1)	3.7 (2.8–4.4)	0.42
Gender (male:female)	23:18	10:16	0.25
Gestational age (wk)	39 (38–40)	39 (38–40)	0.61
Birthweight (g)	3374 (3090–3680)	3282 (3030–3600)	0.48

Results shown as median (interquartile range).

Figure 1 Effect of iron supplements on maternal and cord blood TfR-F index. The box represents the median, 25th, and 75th percentiles and the whiskers extend to the full range.

Table 3   Effect of maternal smoking on fetal iron status assessed in cord blood at term

Cord blood	Maternal smoker (n = 28)	Non-smoker (n = 39)	Significance (p)
Hb (g/l)	168 (159–175) (n = 27)	157 (139–171) (n = 25)	0.024
Iron (µmol/l)	24.5 (19.0–27.5)	27.0 (24.0–31.0)	0.022
TIBC (µmol/l)	45.0 (37.5–51.0)	39.0 (33.0–47.0)	0.066
Ferritin (µg/l)	123 (80–171)	190 (113–262)	0.02
STfR (mg/l)	9.3 (7.5–10.6)	7.6 (5.6–10.6)	0.06
TfR-F index	4.1 (3.7–5.3)	3.4 (2.6–4.5)	0.0082
Gender (M:F)	11:17	22:17	0.26
Gestational age (wk)	39 (38.5–40)	39 (38–39)	0.07
Birthweight (g)	3318 (3182–3607)	3350 (2977–3630)	0.48

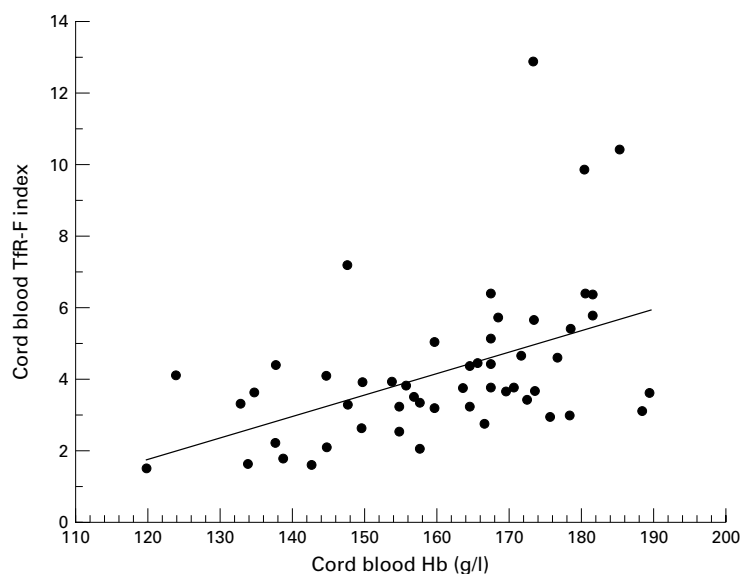Results shown as median (interquartile range).

Figure 2   Correlation between cord blood Hb and TfR-F index. The TfR-F index in cord blood was positively correlated with Hb.

(2.36 *v* 5.29; p = 0.002), but there was no effect on fetal iron status (4.11 *v* 3.77; p = 0.38) (fig 1).

Maternal smoking was associated with higher cord blood Hb as well as a reduction in ferritin and a trend towards increased STfR concentrations. This shift towards a more iron depleted picture is reflected in the increased cord blood TfR-F ratio (table 3). The cord blood TfR-F index was positively correlated with cord blood Hb (*r* = 0.48; p = 0.0006; fig 2). This suggests that rather than measuring iron deficiency, the TfR-F index in cord blood measures the iron requirements for erythrocyte production.

Discussion

The transferrin receptor mediates the uptake of iron from transferrin. The expression of TfRs is regulated post-transcriptionally by intracellular iron by a mechanism that involves iron-responsive elements (IREs) in the 3' untranslated region of TfR mRNA.[19 20 22] Cytoplasmic iron regulatory proteins (IRPs) recognise and bind the IREs when iron is scarce. The bound proteins cause a prolongation of the TfR mRNA half life that results in an increase in TfR synthesis. Simultaneously, in a highly coordinated process IRPs bind to the 5' end of the ferritin gene and prevent its transcription when iron is scarce. Together these mechanisms ensure that iron is available for essential processes such as Hb production in erythroid precursor cells and that it is not sequestered into ferritin. On this basis the TfR-F index would be expected to give a good indication of iron status so that when iron is scarce the TfR-F index will be high. Conversely, when iron is plentiful the coordinated mechanism involving IRPs will cause a decrease in the TfR-F index.

STfR concentrations are also increased during increased erythropoiesis, possibly because of the increased number of erythroid progenitor cells that express high numbers of transferrin receptors.[23] In a longitudinal study of very low birthweight infants, the STfR was shown to increase during treatment with recombinant human erythropoietin.[16] This raises the question as to whether or not iron availability affects the number of TfRs during periods of increased erythropoiesis. The rate of erythropoiesis in the neonate is 1.5 to 4 times higher than in normal adults, based on reticulocyte counts in cord blood.[24] This suggests that STfR concentrations in cord blood should be 1.5 to 4 times the maternal concentration if both mother and fetus have adequate available iron for erythropoiesis, but that the fetal:maternal ratio would be lower if the mother is deficient in iron. The fetal:maternal ratio of 2.2 (8.4/3.8) found in the present study is consistent with the notion that iron availability was not a limiting factor in erythropoiesis. This idea is also supported by the mean TfR-F indices in the mothers and babies.

Severe, prolonged iron deficiency anaemia in pregnancy, often found in developing countries, can lead to a reduction in fetal iron stores.[25] Our study, from a healthy western European population, shows that mild to moderate maternal iron depletion occurring during pregnancy has very little effect on the iron status of the fetus. Only 17 mothers in this study had a haemoglobin concentration less than 110 g/l and only six had one less than 100 g/l. We found that maternal anaemia in this population also had no effect on the cord blood STfR and TfR-F index.

Maternal smoking increases the fetal red cell mass, probably via increased erythropoeitin induced by hypoxia.[26 27] Iron stores are mobilised for erythrocyte production, thereby explaining the reduction in ferritin concentration. The slight increase in cord blood STfR may be a result of free iron in the cytosol of erythroid progenitor cells being incorporated into haemoglobin, leading to a reduction in concentration of intracellular iron. It is reasonable to assume that any cause of increased fetal red cell mass, such as placental insufficiency or maternal diabetes mellitus might also reduce ferritin and increase cord blood StfR.[28] This could explain why this study is at odds with the series reported by Rusia *et al* showing increased cord blood STfR concentrations in babies born to mothers with iron deficiency anaemia.[18] In their study, the babies from iron deficient mothers were also more growth retarded and had higher cord blood Hb concentrations.

TfR-F index has not previously been evaluated in neonates. It gives a measure of iron requirements in relation to iron availability. However the interpretation of the TfR-F index in the neonate and in cord blood may be limited by the fact that there is no "gold standard" for measuring iron status in the newborn. Data from this study suggest that in the iron replete fetus, the TfR-F index gives a measure of iron requirements for erythropoeisis and may reflect red cell turnover.

Further work is needed to define the normal range of STfR and TfR-F index in the preterm population, and to discover how they vary in relation to iron status and rate of erythropoeisis. In future STfR concentrations may provide insight into the relative contribution of iron deficiency and inadequate erythropoiesis to the development of anaemia of prematurity. We conclude that cord blood STfR concentrations and TfR-F index are unaffected by mild maternal iron deficiency, but may be increased by factors that increase the fetal production of haemoglobin.

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