Published in final edited form as:
J Pediatr Hematol Oncol. 2013 August ; 35(6): 473–477. doi:10.1097/MPH.0b013e3182707f2e.

Impact of Multiple Prenatal Risk Factors on Newborn Iron Status at Delivery

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Abstract

Background—Maternal anemia and several complications of pregnancy can affect fetal iron acquisition.

Aim—Because it is unknown whether the effects of demographic and maternal risk factors are summative, we examined cord iron status in newborns with multiple risk factors for acquiring iron deficiency (ID).

Methods—Cord blood indices from healthy control newborns with and without risk factors for newborn or infant ID were studied.

Results—Newborns with greater risk factors had poorer erythrocyte and storage iron status. Poorest status was seen if mothers with comorbid obesity and diabetes delivered large-for-gestation newborns. Findings highlight the importance of identifying risk factors.

Keywords

diabetes; minority; obesity; ferritin; iron deficiency

Introduction

Iron deficiency (ID) is the most common nutrient deficiency worldwide.[1] ID and ID with anemia (IDA) in infancy are common in nonindustrialized countries and while occurring in only 2-3% of infants overall in the US, is still very common among certain groups of infants in the US.[1,2] Inadequate fetal iron allotment increases the risk for IDA as an infant, because approximately half the iron needed for infant growth should normally be obtained before birth.[3,4] In the US, recognized risk factors for insufficient fetal iron allotment include

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Conflicts of Interest: None.

maternal anemia, gestational diabetes, premature birth, fetal undergrowth or overgrowth (small for gestation-SGA or large for gestation-LGA), and mothers from certain ethnic or lower socioeconomic groups.[1,3-7] Because obesity may also predispose to IDA and diabetes during pregnancy, as well as result in larger newborns at birth,[8,9] this maternal attribute should also be considered as an additional risk factor.

Recent findings indicate that an impaired fetal iron allotment and the progression to anemia during the first year of life may disrupt neurological and behavioral development.[1,2] A history of untreated IDA may cause problems in language comprehension, fine motor skills and/or self-regulation of attention and emotion among school-age children,[2] whereas early treatment has been shown to ameliorate deficits. Because of the increasing recognition that the detrimental effects of ID and IDA may be persistent, the American Academy of Pediatrics (AAP) Committee on Nutrition recently recommended utilizing the known maternal and prenatal risk factors to more aggressively screen for pre-anemic ID in addition to screening at younger ages.[1] Thus, the primary aim of our study was to evaluate the synergistic impact of perinatal risk factors for depleted fetal iron status at delivery, long before the emergence of anemia.

We hypothesized that multiple risk factors for ID in neonates would have a summative impact and produce poorer iron status at delivery. In addition, we hypothesized further that LGA newborns born to women with a history of obesity and diabetes during pregnancy would have a disrupted fetal iron supply from obesity-induced gestational ID and gestational diabetes-induced placental dysfunction, combined with higher fetal iron needs for a larger fetus, resulting in poorer iron profiles than those with at least three other risk factors.

Materials and Methods

This investigation of maternal and child risk factors for IDA was approved by the University of Wisconsin and Meriter Institutional Review Boards. English and Spanish speaking women, 18-40 years of age, delivering newborns ≥35 weeks of gestation at Meriter Hospital were eligible to participate. Consent was obtained from those with ≥1 medical or demographic risk factors for depleted newborn or infant iron status, including prenatally diagnosed maternal IDA, pre-gestational or gestational diabetes mellitus, SGA or LGA newborns, maternal ethnic minority (African-American, Latina or Asian), or low socioeconomic status, using the surrogate of self-pay or Medicaid. Specimens from a total of 309 newborns with 1 or more risk factors were analyzed. Although not a previously reported risk factor nor criterion for enrollment, we included maternal BMI >30 kg/m^2 as a risk factor in our analysis is because it predisposes to maternal IDA, gestational diabetes, and fetal overgrowth, all risk factors for depletion of newborn iron stores. To provide a reference comparison, we generated cord blood laboratory data from a recent representative population of 188 healthy newborns born at ≥35 weeks of gestation, delivered from mothers of all ethnic backgrounds at Meriter Hospital without other known risk factors for IDA.[6,10,11]

Umbilical blood collected at delivery, stored at 4° C, was assayed within 8 days. Complete blood cell counts were performed by pocH-100i (Sysmex, Mundelein, IL). After washing to remove pigments, cord zinc protoporphyrin/heme (ZnPP/H) was measured with Front-Face Hematofluorometry (Aviv Biomedical Co., Lakewood, NJ).[11] Reticulocyteenriched ZnPP/H (RE-ZnPP/H) was measured from the lightest 6.25% of cells to assess ZnPP/H in recently-made erythrocytes.[11] Serum ferritin (Bio-Quant, San Diego, CA) was assessed as an index of storage iron and serum transferrin (Immunology Consultants Lab, Newberg, OR) determined as a reflection of transport iron.

The 2 study groups of interest were demarcated as either high-risk (≥3 risk factors) or medium-risk (1-2 risk factors), and compared to the control neonates born to mothers without designated risk factors. Birth weight was *z*-scored for gestational age, and used to ascertain whether the neonate exceeded the criterion for LGA as >2 or SGA as <−2.[10,12] One-way and multiple ANOVA with Fisher *post hoc* testing were used to compare differences between the groups and the Pearson test was used to evaluate correlations between outcome measures. Chi square analysis was used for nominal demographic variables. The alpha value for statistical significance was set at $p<0.05$. Natural log conversions were applied to normalize the distribution of ZnPP/H, RE-ZnPP/H and ferritin values. The data portrayed in figures are the mean ± SEM.

Results

Compared to the reference values from 188 healthy newborns, the pooled data from all 309 newborn enrollees in the current IDA study were born with poorer iron indices, including higher mean cord ZnPP/H and RE-ZnPP/H ratios, and lower mean levels of serum ferritin and serum transferrin, ($p<0.01$ for all measures). Next, the number of subjects experiencing each numerical risk factor was determined in order to define medium-risk and high-risk groups. Number of risk factors (number of subjects) is listed as follows: 1 risk (n=76), 2 risks (n=114), 3 risks (n=88), 4 risks (n=21), 5 risks (n=9) and 6 risks (n=1). Table 1 shows the demographic characteristics and erythropoietic indices of the control, medium-risk (1-2 risks), and high-risk (3-6 risks) groups. The cord blood iron indices from pregnancies with either medium-risk or high-risk groups were each poorer than controls (Figure 1A-D), p<0.001. However, the high-risk neonates exhibited higher mean ZnPP/H and RE-ZnPP/H and lower mean serum ferritin than the medium-risk neonates ($p<0.001$ for all three indices).

Mean cord hemoglobin levels and mean cord erythrocyte (RBC) counts were higher in either the high- or medium-risk group, compared to controls (hemoglobin, $p<0.02$ and RBC, $p<0.001$, respectively), indicative of increased fetal erythropoiesis (Table 1). As evidence for iron-limited erythropoiesis in the two at-risk groups, the mean cell hemoglobin concentration (MCHC) was lower ($p<0.0001$) and RBC distribution widths (RDW) higher ($p<0.0001$) in cord blood from either the high- or medium-risk neonates as compared to the controls (Table 1). As additional evidence for iron-limited erythropoiesis, either cord blood ZnPP/H or RE-ZnPP/H ratios were positively correlated with the RDW ($r=0.37$, $p<0.0001$ and $r=0.37$, $p<0.0001$, respectively) and with absolute reticulocyte count ($r=0.15$, $p<0.015$ and $r=0.24$, $p<0.0001$, respectively).

A subset of 18 newborns in the ≥3 risk group met criteria to be designated as LGA with a maternal history including both obesity and diabetes (LOD). This LOD group was compared to 94 newborns with any other combination of ≥3 risks (≥3 other RF). The LOD neonates evinced higher mean ZnPP/H ($p<0.04$) and RE-ZnPP/H ($p<0.025$) and lower mean serum ferritin levels ($p<0.001$) than those with ≥ 3 other RF (Figure 2A-D). Mean plasma transferrin levels, mean hemoglobin and mean erythrocyte counts for these LOD neonates did not differ significantly from the other neonates in ≥3 other RF group ($p=0.15$, $p=0.7$, $p=0.4$, respectively). The biological factors impacting the LOD group are not independent. Diabetes ($F_{1,309}=9.94$, p<0.002) and newborn birth weight *z* score ($F_{1,309}=26.2$, p<0.0001) both have significant effect on delivery BMI, as well as a significant interactive effect ($F_{1,309}=5.87$, p<0.02) on delivery BMI.

Discussion

This study is the first to show that an accumulation of multiple risk factors for impaired maternal-to-fetal iron transfer may worsen neonatal iron status in an incremental manner.

We found that the LGA neonates born to women with comorbid obesity and diabetes had the worst iron profile, in keeping their high growth and metabolic demand for transplacentally transferred iron.

Newborns born to women with a history of maternal and prenatal risk had higher hemoglobin level, higher RBC counts, and lower MCHC, in addition to a strong relationship between reticulocytosis and inadequate erythrocyte iron (ZnPP/H). Consistent with our previous work assessing the offspring of diabetic mothers,[13] ZnPP/H in cord blood was directly correlated with the RDW values, supporting the notion of exaggerated erythropoiesis in the context of a limited iron supply. Whether due primarily to poor iron supply or to the increased iron demands, this compensatory response is of concern because when fetal stores are limited, iron may be directed away from fetal tissues, including the maturing brain and toward erythrocytes.[2] Neurodevelopment is known to be adversely impacted by both severity and timing of ID because iron-containing enzymes and hemoproteins are integral to the synthesis of myelin by the oligodendrocytes, can undermine the production of essential monoamine neurotransmitters, and affects structural proteins involved in synaptogenesis and dendritogenesis.[2] Studies show that fetal iron status at birth can track the same percentiles over the first year, perhaps a portend of the demand for iron for postnatal infant growth exceeding available stores and resources from nutritional sources.[14,15] Multiple studies have documented poorer cognitive, motor, and/or social functioning in children after the period of IDA in infancy.[2] Of additional concern is the recent animal research demonstrating that ID in early development appears to program a state of upregulated erythropoiesis, resulting in a higher set point for hemoglobin concentrations later in childhood.[16] This compensatory response may be maladaptive, as greater iron needs for erythropoiesis can partition iron away from the body, including the brain, worsening tissue depletion.[16] Additionally, typical screening at one year of age uses hemoglobin as the sole diagnostic barometer of ID, such that higher programming of hemoglobin could also obscure the detection of tissue iron depletion.

Based on their cord blood parameters, the LGA neonates born to obese mothers with diabetes appeared to evince the worst indications of an unfulfilled high demand for iron during the fetal period. Maternal BMI, diabetes and birth weight *z* score are three dependent interactive factors, such that further studies should examine the relative importance of each in a biological etiology. We previously found that the abnormal iron status in newborns after insulin-dependent diabetic pregnancy was due to poor supply from dysfunctional placental transport, compounded by greater demand due to accelerated growth and larger blood volumes.[13] Our findings complement the work of Petry, et al.,[17] who demonstrated that transferrin receptors on the placentae of diabetic mothers are dysfunctional and unable to rectify the iron shortfall in the fetal compartment. The current study extends these findings from frank diabetes to noninsulin-dependent gestational diabetes, a finding quite concerning given the prevalence of obesity among women of child-bearing aging and the known association between maternal obesity at conception, gestational diabetes and likelihood of birthing LGA newborns. There is an interactive effect between Larger babies at birth are also likely to continue growing at a faster rate across the first year of life, another documented risk factor for the emergence of pediatric IDA.[18] It would be important to examine whether more aggressive iron supplementation before or after birth would succeed in improving iron status of offspring when pregnancy is complicated by obesity and/or diabetes.

A limitation of the study is that it cannot infer direct biological causality, but generates hypotheses that can be tested in greater detail. Although our study was limited by the cross-sectional analysis conducted at the time of delivery rather than using a prospective evaluation beginning earlier in pregnancy, the strength of this design was random enrollment

of at-risk newborns representative of our hospital population. In addition, we employed multiple measures that have been shown sensitive to a history of ID in cord blood. At term, and even during the first months of life, diagnostic tests of ID are challenging to interpret and do not definitively predict the later emergence of IDA. However, we and others have shown that the underutilized ZnPP/H ratios, are reflective of erythrocyte iron at birth and are abnormal in those with impaired fetal iron status.[5,10,19] Examining ZnPP/H in the reticulocyte fraction, the most recently generated erythrocytes, improves the sensitivity of ZnPP/H to detect iron-limited erythropoiesis at an earlier stage.[11] Our simple nonautomated ZnPP/H and RE-ZnPP/H methodology [11] can be refined further and added as channels in automated clinical flow cytometry analyzers. Recent work also indicates that cord blood ferritin levels may provide a general measure of storage iron at birth,[20] especially when utilizing neonatal-specific normal ranges that are numerically much higher than normal ranges of ferritin in older children or adults. Serum transferrin levels were lower than the control group as well, but the reason for this observation was unclear. The transferrin assays were performed using the same assay kit. Perhaps the finding of lower transferrin is another maladaptation caused by the higher hemoglobin levels, as transferrin levels in iron-depleted, but anemic individuals generally rise in an attempt to improve iron transport.

AAP recommends universal screening at 1 year of age and adds that earlier screening may be warranted in the presence of risk factors for ID and IDA.[1] The AAP statement cited socio-demographic, perinatal and postnatal risk factors for infantile IDA, including many that were utilized in our study,[1] but not maternal obesity or large overgrown newborns. Our findings highlight the value of identifying specific risk factors that can direct clinicians toward the need for an earlier screening for pre-anemic ID and IDA. Identifying high-risk newborns may facilitate earlier treatment with iron, potentially preventing or shortening the period of iron depletion, thus preventing the negative long-term cognitive and behavioral deficits.

Acknowledgments

We also acknowledge the participating families, Meriter Hospital Birthing Center and Blood Bank, D. Krumpos RN, S. Shafranski RN, P. Green-Sotos RN, M. E. Chen MD, R. J. Baxter MD, V. Sridhar MBBS, M. E. Bacsik BS, M. L. Katcher M.D., Ph.D., A. P. Auger Ph.D., and staff of the Kling Laboratory Research Team.

Funding: NIH 1 ULRR026011 UW CTSA Program, NIH T32HD048302 Health Disparities Research Scholar (BAF), University of Wisconsin Shapiro Research Program (HMM), University of Wisconsin Cardiovascular Research Center (HMM), Meriter Foundation (PJK), Wisconsin Partnership Collaborative Health Sciences Program Grant (PJK, CLC, DQDP), Thrasher Research Fund (PJK), UW Graduate School Competition (PJK), Salary support (CLC) HD057064, HD38305, Gates Foundation OPP1046203).

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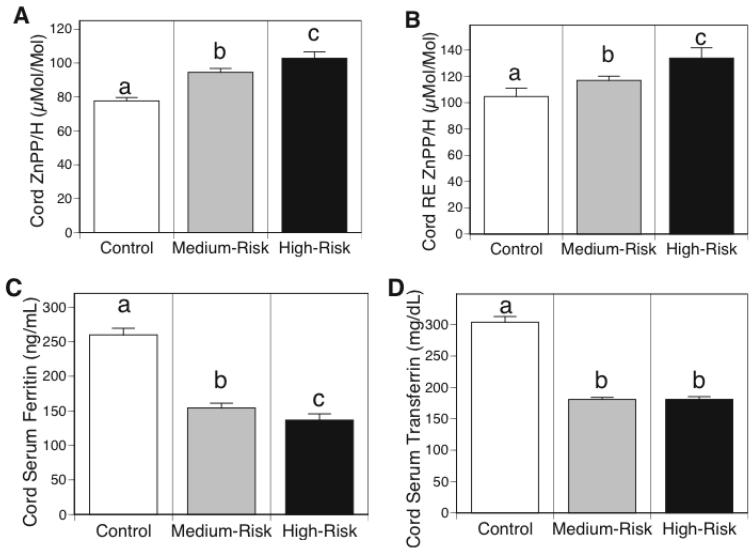

Figure 1.

Cord blood iron indices in control, medium-risk and high-risk newborns. The letters indicate the results of *post hoc* testing, with different letters connoting significant differences. **A**. Cord blood ZnPP/H was incrementally higher in neonates with more risks, $F_{2,406}=36.82$, $p<0.0001$. **B**. RE-ZnPP/H from the reticulocyte enriched fraction was also incrementally higher with increasing risk factors, $F_{2,321}=7.27$, $p<0.001$. **C**. Serum ferritin from cord blood was incrementally lower in neonates from mothers with more risks, $F_{2,380}=61.94$, $p<0.0001$. To convert serum ferritin to SI units (pmol/L), multiply by 2.247. **D**. Serum transferrin was lower than controls, $F_{2,384}=146.60$, $p<0.0001$, but values for the two at-risk groups did not differ. To convert serum transferrin to SI units (g/L), multiply by 0.01.

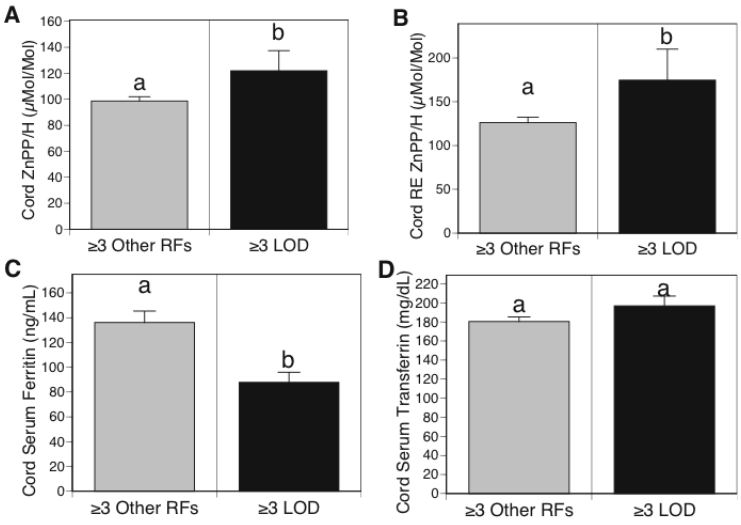

Figure 2.

Cord blood iron indices for the 18 LGA newborns of mothers with comorbid obesity and diabetes (LOD) to the 94 neonates born with ≥3 risk factors comparing who did not meet criteria for being LGA. Letters indicate the results of *post hoc* testing, with different letters connoting significant differences. **A**. ZnPP/H was higher in the LOD neonates, $F_{1,111}=4.32$ $p<0.04$. **B**. RE-ZnPP/H in the reticulocyte fraction, $F_{1,106}=6.15$ $p<0.02$. **C**. Serum ferritin from cord blood was significantly lower in the LOD neonates, $F_{1,108}=6.93$, $p<0.01$. **D**. Serum transferrin levels did not differ between LOD neonates and the ones with ≥3 other RFs, although collectively their serum transferrin levels were below those of the control neonates without prenatal risk factors.

Table I

Demographics and Erythropoietic Indices by Group

	Control	Low-Risk	High-Risk	*p* Control vs Low	*p* Control vs High
n	188	190	119		
Gestation (weeks)	39.20 ± 0.06	39.25 ± 0.11	39.33 ± 0.10	NS	NS
Birth Weight (kg)	3.640 ± 0.36	3.546 ± 0.044	3.627 ± 0.061	NS	NS
Female (%)	50%	47%	46%	NS	NS
AA/C/L/O (number)	45/102/38/3	8/170/11/9	28/57/24/10	$p<0.0001$	$p<0.05$
Hemoglobin (g/dL)	15.5 ± 0.2	16.4 ± 0.2	16.2 ± 0.3	$p<0.02$	$p<0.02$
RBC Count ($10^6/\mu L$)	4.17 ± 0.05	4.51 ± 0.06	4.53 ± 0.07	$p<0.0001$	$p<0.0005$
MCHC (g/dL)	34.1 ± 0.2	32.0 ± 0.1	32.4 ± 0.1	$p<0.0001$	$p<0.0001$
RDW (%)	16.89 ± 0.13	17.86 ± 0.90	18.15 ± 0.14	$p<0.0001$	$p<0.0001$

To convert Hemoglobin to SI units multiply by 10 (g/L) multiply by 0.153 and MCHC to SI units (g/L), multiply by 10.

A=African American, C=Caucasian, L=Latina, O=Other (Asian, Asian Indian).

Ethnicity Distribution Chi Square = 68.83, $p<0.0001$, comparisons above.

One-way ANOVA with Fisher *post hoc* testing shown above.

Hemoglobin $F_{2,405}=3.95$, $p<0.02$; RBC $F_{2,406}=11.39$, $p<0.0001$;

MCHC $F_{2,406}=67.40$, $p<0.0001$; RDW $F_{2,404}=29.71$, $p<0.0001$