Latent iron deficiency at birth influences auditory neural maturation in late preterm and term infants

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ABSTRACT

Background: In utero latent iron deficiency has been associated with abnormal neurodevelopmental outcomes during childhood. Its concomitant effect on auditory neural maturation has not been well studied in late preterm and term infants.

Objective: The objective was to determine whether in utero iron status is associated with auditory neural maturation in late preterm and term infants.

Design: This prospective cohort study was performed at Sir Ganga Ram Hospital, New Delhi, India. Infants with a gestational age ≥34 wk were eligible unless they met the exclusion criteria: craniofacial anomalies, chromosomal disorders, hemolytic disease, multiple gestation, third-trimester maternal infection, chorioamnionitis, toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex virus infections (TORCH), Apgar score <5 at 5 min, sepsis, cord blood not collected, or auditory evaluation unable to be performed. Sixty consecutive infants with risk factors for iron deficiency, such as small for gestational age and maternal diabetes, and 30 without risk factors for iron deficiency were enrolled. Absolute wave latencies and interpeak latencies, evaluated by auditory brainstem response within 48 h after birth, were measured and compared between infants with latent iron deficiency (serum ferritin ≤75 ng/mL) and infants with normal iron status (serum ferritin >75 ng/mL) at birth.

Results: Twenty-three infants had latent iron deficiency. Infants with latent iron deficiency had significantly prolonged wave V latencies (7.10 ± 0.68 compared with 6.60 ± 0.66), III–V interpeak latencies (2.37 ± 0.64 compared with 2.07 ± 0.33), and I–V interpeak latencies (5.10 ± 0.57 compared with 4.72 ± 0.56) compared with infants with normal iron status (P < 0.05). This difference remained significant on regression analyses after control for confounders. No difference was noted between latencies I and III and interpeak latencies I–III.

Conclusion: Latent iron deficiency is associated with abnormal auditory neural maturation in infants at ≥34 wk gestational age. This trial was registered at clinicaltrials.gov as NCT02503397.


Keywords: latent iron deficiency, infants, auditory neural maturation, in utero iron status, myelination

INTRODUCTION

Iron is an essential nutrient for brain development (1). During early human development, iron is required for multiple neurodevelopmental processes, including myelination, dendritic growth, synaptic function, monoamine metabolism, and energy metabolism (2–5). To meet the iron requirements of a developing brain, active transfer of iron occurs across the placenta during the last trimester of pregnancy; therefore, most term infants have iron-replete status at birth (6, 7). However, maternal iron deficiency during pregnancy, a global health problem, can negatively affect fetal iron status (7–9). In addition, prematurity, maternal diabetes mellitus, pre-eclampsia, maternal smoking, and intrauterine growth restriction during pregnancy have also been associated with decreased iron transfer to the fetus and often lead to in utero iron deficiency (7, 10–12). The cord serum ferritin (SF)6 concentration at birth provides a good measurement of fetal tissue iron storage concentrations and is therefore often used to evaluate in utero iron status (13, 14). A hierarchical loss of tissue iron occurs during progressive negative iron balance, and iron depletion occurs in the brain before it occurs in red blood cells (15). The negative iron status that occurs when brain iron may be depleted but red blood cells are not affected is defined as latent iron deficiency (LID). Emerging evidence suggests that in utero LID, defined as cord SF ≤75 ng/mL in late preterm and term infants, is associated with delayed neuronal maturation and abnormal neurodevelopmental outcomes during childhood (16–18).

The absolute latencies and interpeak latencies (IPLs) on auditory brainstem response (ABR) are often used as surrogate outcome measures for neural maturation in neonates (19–22). The ABR waveform comprises 3 major waves (I, III, and V), which can reliably be measured in infants with a gestational age (GA) of ≥34 wk (23). The absolute latencies for each of these ABR waves and the IPLs (I–III, III–V, and I–V) are influenced by the degree of myelination, neuronal development, synaptic function, and axonal growth in the auditory nervous system. The

1 The authors reported no funding received for this study.

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Abbreviations used: ABR, auditory brainstem response; GA, gestational age; IPL, interpeak latency; LID, latent iron deficiency; SF, serum ferritin; SGA, small for gestational age.

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absolute latencies and IPLs decrease as the auditory neural system matures with age in neonates (23, 24).

Although iron is essential for auditory neural maturation during the perinatal period, a paucity of data exists regarding the concomitant effect of in utero LID on auditory neural maturation in late preterm and term infants. The current prospective study was performed with an objective to determine the association between in utero LID and abnormal auditory neural maturation at birth in infants with a GA ≥34 wk.

METHODS

Study design

A prospective cohort study was conducted to compare auditory neural maturation (as assessed by absolute latencies and IPL) at birth between infants with a normal in utero iron status (SF >75 ng/mL, or 168.5 pmol/L) and infants with in utero LID (SF ≤75 ng/mL, or ≤168.5 pmol/L) at ≥34 wk GA. The study was conducted at Sir Ganga Ram Hospital, New Delhi, India, from July 2011 to March 2012. The study was approved by the Institutional Research Review and Ethics Board. Written parental consent was obtained for each subject enrolled in the study.

Study population

To study enough subjects with LID, as determined by the sample size calculation, we included two-thirds of the subject population with known risk factors for iron deficiency: infants of diabetic mothers and small-for-gestational age (SGA) infants. SGA status was defined as birth weight less than the 10th percentile for GA per the Fenton growth chart (25). The remaining one-third of the subject population was included from the infants without known risk factors for iron deficiency. Infants with craniofacial anomalies, chromosomal disorders, hemolytic disease (Coomb’s positive), multiple gestation, a history of third-trimester maternal infections, clinical chorioamnionitis, an Apgar score <5 at 5 min, toxoplasmosis, other infections, rubella, cytomegalovirus infection, and herpes simplex virus infections (TORCH), clinical or culture-proven sepsis, and history of admission to the Neonatal Intensive Care Unit were excluded. In addition, infants for whom cord blood was not collected for SF measurement and for whom an ABR test could not be performed because of unavailability of an audiologist were also excluded.

Exposure variable: iron status evaluated by cord SF

Blood samples were collected from eligible mothers on admission to the labor and delivery room and immediately centrifuged to separate and store the serum in a refrigerator at 4°C until delivery of the infant. Cord blood was collected from all eligible infants soon after birth and immediately centrifuged to separate and store the serum in a refrigerator at 4°C. Once an infant was enrolled in the study, both maternal and cord samples were sent to the institutional laboratory for SF measurement by using the chemiluminescence immunoassay method. Each infant was evaluated for anemia or polycythemia if clinically indicated at the discretion of the attending physician. For analyses, the study subjects were divided into 2 groups based on cord SF concentrations: 1) infants with a cord SF concentration ≤75 ng/mL (LID) and 2) infants with a cord SF concentration >75 ng/mL.

Outcome measure: auditory neural maturation

Bilateral monaural ABR tests were performed by an audiologist skilled in administering such tests to newborns. ABRs were recorded for each subject with a Bio-logic Navigator Evoked Response System (Bio-logic Systems) between 24 and 48 h after birth. ABR tests were performed by using an 80-dB nHL broadband click stimuli with insert earphones and while the subject was supine in a quiet room and had a normal skin temperature. The clicks were presented at a repetition rate of 29.9/s, and 3 runs of 2000 repetitions were recorded for each ear. The 2 most replicable runs for each ear were averaged and used for the analysis. The ABRs were analyzed by an audiologist with no knowledge of the iron status. We predetermined that the ABR findings from the better ear (defined as the ear with the shorter wave V latency) would be used for outcome measures. Absolute latencies of waves I, III, and V and IPLs (I–III, III–V, and I–V) were measured and considered outcome measures for auditory neural maturation at different levels of auditory pathway.

Sample size calculation

An approximate sample size was determined for the number of infants to be studied based on the reported findings of absolute latencies in infants ≥34 wk GA. We assumed a ratio of 1:3 for infants with LID and infants with normal iron status in our subject population. Considering a reported SD of 0.4 for absolute latencies in infants ≥34 GA infants, 20 infants with LID and 60 infants with normal iron status (ratio of 1:3) would allow detection of an actual difference of 0.3 ms (equal to 0.75 SD) for absolute latencies with an α level of 0.05 and a power of 0.80 (26). We assumed an incidence of LID of ~30% in infants ≥34 wk GA with known risk factors for iron deficiency. Therefore, we estimated that a total of 90 infants—60 infants with known risk factors for LID and 30 infants without known risk factors for LID—would be adequate for the proposed study.

Statistical analyses

Statistical analyses were performed by using Stata version 10 (Stata Corporation). The 2 groups of infants (LID compared with normal iron status) were compared by using 2-sample t tests for continuous variables with normal distribution or with the Mann-Whitney U test for skewed distribution. Categorical variables were measured by using the chi-square or Fisher’s exact test as applicable. All tests were 2 sided, and P < 0.05 was considered statistically significant. Variables identified to be associated with iron status or auditory neural maturation on bivariate analysis (P < 0.1) were considered potential confounders and were included in model building. Regression analysis was performed by using the backward selection method. Covariates that did not make a significant contribution to the model (P < 0.1) were removed from the model. The final regression model was evaluated for goodness of fit.

RESULTS

A total of 657 infants with a GA ≥34 wk were admitted to the newborn nursery during the study period. Of these infants, 156 met the exclusion criteria. Of the eligible infants, 98 consecutive infants admitted to the newborn nursery with risk factors for
LID, such as SGA and maternal diabetes, were approached. Of these 98 mother-infant dyads, 60 mothers consented. Similarly, 48 consecutive mother-infant dyads without known risk factors for LID were approached; 30 mothers consented. Overall, 90 infants were studied, 40 (44%) of whom were late preterm infants. The mean maternal and cord SF concentrations were 37.7 \pm 21 and 134 \pm 83.3 \text{ng/mL}, respectively. A significant positive correlation was found between maternal SF and cord SF concentrations ($r = 0.42$, $P < 0.001$). None of these infants had anemia. Of 90 infants, 23 (25.5%) had in utero LID and 67 had normal iron status in utero. The overall incidence of LID among infants with risk factors was 31.6%, whereas the incidence of LID among those without risk factors was 13.3%. The incidences of LID among SGA infants and infants of diabetic mothers were 26.7% and 36.7%, respectively.

The demographic and clinical characteristics of infants at delivery, as a function of iron status, are shown in Table 1. No significant differences were found between the 2 groups in GA, birth weight, sex, mode of delivery, and Apgar score at 5 min. In addition, no significant differences in pre-eclampsia, maternal diabetes, and the proportion of SGA infants were found between the 2 groups. A significant difference in maternal SF concentrations was found between the 2 groups; the infants with LID had lower maternal SF concentrations than did the infants with normal iron status.

On evaluation of possible confounders for the association with auditory neural maturation, we found mode of delivery, sex, and SGA to be associated with wave V latencies and IPLs III–V and I–V ($P < 0.001$). Therefore, in addition to maternal SF, these variables were included as confounding variables in the regression model.

**Absolute wave latencies**

No significant differences in absolute latencies I and III were found between the 2 groups (Table 2). However, wave V latencies were significantly prolonged in infants with LID compared with infants with normal iron status. The association between LID and wave V latencies was significant in both male and female infants when evaluated independently. On regression analyses, SGA and sex did not contribute to individual models and were removed by using the backward selection method. The association between LID and wave V latencies remained significant (coefficient = 0.71; 95% CI: 0.38, 1.04; $P = 0.001$) even after maternal SF and mode of delivery were controlled for.

**Interpeak latencies**

No significant difference in IPL I–III was found between the 2 groups; however, IPLs III–V and I–V were significantly prolonged in infants with LID compared with infants with a normal iron status (Table 2). The association between LID and IPL I–V was significant in both male and female infants, when evaluated independently. However, the association between LID and IPL III–V was significant only in female infants. Therefore, sex was used as an effect modifier in the regression model for the evaluation of the association between LID and IPL III–V. On regression analyses, SGA, mode of delivery, and sex did not contribute to individual models and were removed from the models by using backward selection method. After maternal SF was controlled for, infants with LID had a significantly prolonged IPL III–V (coefficient = 0.36; 95% CI: 0.14, 0.57; $P = 0.001$) and I–V (coefficient = 0.47; 95% CI: 0.18, 0.75; $P = 0.002$) but not IPL I–III compared with infants with a normal iron status.

**DISCUSSION**

Iron deficiency is one of the most common nutritional deficiencies in the world (27). More importantly, iron deficiency is common among women during pregnancy and has the potential to adversely influence fetal brain development (6–9, 27). Furthermore, conditions such as maternal diabetes and intrauterine growth restriction, which may be associated with in utero LID, are also common (28, 29). Although the critical role of iron in brain development has been known, little data exist regarding

**Table 1**

<table>
<thead>
<tr>
<th>Latent iron deficiency ($n = 23$)</th>
<th>Normal iron status ($n = 67$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, wk</td>
<td>37.1 ± 2$^2$</td>
<td>37.4 ± 1.8</td>
</tr>
<tr>
<td>Late preterm, %</td>
<td>47.8</td>
<td>46</td>
</tr>
<tr>
<td>Maternal serum ferritin, ng/mL</td>
<td>25 ± 17</td>
<td>41 ± 20</td>
</tr>
<tr>
<td>Maternal pre-eclampsia, %</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Infant of diabetic mother, %</td>
<td>39</td>
<td>28</td>
</tr>
<tr>
<td>Cesarean delivery, %</td>
<td>65</td>
<td>55</td>
</tr>
<tr>
<td>Apgar score at 5 min</td>
<td>8.8 ± 0.49</td>
<td>8.8 ± 0.38</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td>2502 ± 635</td>
<td>2496 ± 605</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>70</td>
<td>51</td>
</tr>
<tr>
<td>Small for gestational age, %</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Cord serum ferritin, ng/mL</td>
<td>47 ± 23</td>
<td>163 ± 75</td>
</tr>
</tbody>
</table>

$^1$Iron deficiency defined as serum ferritin $\leq 75$ ng/mL.

$^2$Mean ± SD (all such values).

$^3$t test.

$^4$Defined as $34\%$ to $36\%$ wk.

$^5$Chi-square test.

$^6$Mann-Whitney U test.
Absolute latency, ms during the later part of pregnancy is more likely to have a greater influence in term infants (23, 24). Therefore, in utero LID occurring during fetal life in relation to auditory neural maturation. The concomitant effect of in utero iron status on human brain development in late preterm and term infants at risk of LID. Our findings suggest that in utero LID is associated with abnormal auditory neural maturation, as evaluated by ABR, soon after birth in late preterm and term infants.

Our finding of an association between in utero LID and abnormal auditory neural maturation in late preterm and term infants agrees with a previously reported finding of similar association in very premature infants (17). However, compared with the study in very premature infants in whom it is difficult to measure IPL because of an immature ABR waveform, we were able to measure IPL in late preterm and term infants. Our findings of prolonged IPL III–V and I–V, which reflect myelination in the auditory pathway, agree with the findings of a recent study that showed that in utero LID is associated with abnormal myelination in the auditory pathway in late preterm and term infants (18). The difference in demographic and clinical characteristics of the study population, including racial differences, may explain the shorter IPL in our subjects compared with the IPLs reported by the earlier study (18). However, compared with a previous study, we performed a more comprehensive evaluation of auditory maturation, and our study population was different from that in the previous study. The subject population was characterized by a high prevalence of maternal iron deficiency anemia during pregnancy compared with a normal maternal iron status during pregnancy in the previous study. Furthermore, we found a strong positive correlation between maternal and cord SF, which suggests that maternal LID was one of the risk factors for LID in infants. These findings suggest a possible role of early screening and appropriate maternal intervention to improve fetal iron status and related neurodevelopment in a population with a high prevalence of maternal iron deficiency. Our findings of significantly prolonged wave V latencies and IPL III–V and I–V in infants with LID suggest that in utero iron status in infants ≥34 wk GA has a greater influence on the auditory pathway at the brainstem level. Although not evaluated, it may be related to the timing of iron deficiency during fetal life in relation to auditory neural maturation. The auditory neural maturation process in the caudal-rostral direction with maturation at the auditory nerve level preceding maturation at the brainstem level and is almost complete at birth in term infants (23, 24). Therefore, in utero LID occurring during the later part of pregnancy is more likely to have a greater influence on the auditory pathway at the brainstem level. It is not known whether these abnormal ABR changes secondary to in utero LID will improve spontaneously over time or are irreversible. The available literature from a longitudinal study in older infants suggests that correction of postnatal iron deficiency anemia with iron supplementation may not reverse the ABR changes (30, 31). This is not surprising because brain iron stores are depleted long before red blood cells are affected and iron deficiency anemia is established. Therefore, the preceding brain iron deficiency status during the critical period of brain development is more likely to result in irreversible ABR changes by the time iron deficiency anemia is diagnosed. Thus, iron supplementation when iron deficiency anemia is established may not improve ABR changes. It remains to be determined whether iron supplementation during the perinatal period may prevent ABR changes associated with LID.

One strength of our study was the objective assessment of auditory neural maturation with the use of ABR. Second, iron status was evaluated based on SF. A low SF concentration always indicates poor iron status or iron deficiency. Third, we used an SF concentration of 75 ng/mL as a cutoff to define LID because it has been used by most neonatal outcome studies and is consistently shown to be associated with abnormal neurodevelopment outcomes in term and premature infants (16–18). To avoid misclassification bias, we also excluded infants with sepsis, which may be associated with falsely elevated SF. In addition, neonatal clinical conditions, which may affect ABR findings, were excluded to limit confounding effects on the outcome. One limitation of our study was that we did not evaluate middle ear disease, which may influence the absolute latency of wave I in the corresponding ear. However, as previously done, we used the ABR findings from the better ear for the analyses to decrease the influence of any possible unilateral peripheral auditory dysfunction (17, 18). Moreover, the robust association observed between LID and abnormal wave V absolute latency is unlikely to become insignificant, even if a significant increase in peripheral auditory dysfunction was noted among infants with LID compared with infants with a normal iron status. More importantly, IPLs III–V and I–V are unlikely to be influenced by peripheral auditory dysfunction. Second, newer iron assays—such as serum soluble transferrin receptor and reticulocyte hemoglobin content—may have been useful to evaluate iron status in mother-infant dyads; however, these iron assays have not been standardized for use in neonates and are not readily available.

In summary, our findings suggest that LID is common among at-risk late preterm and term infants and that LID is associated with abnormal auditory neural maturation. The question remains whether at-risk late preterm and term infants should be routinely evaluated for iron status soon after birth. This may be critical for populations in which maternal iron deficiency during pregnancy is very common. Because late preterm infants have double the iron need of term infants, iron supplementation is essential for late preterm infants (32). However, iron supplementation is not currently recommended for term infants during the first few months after birth. Future well-designed studies are urgently required to answer these critical questions.

We thank Hongyue Wang for providing guidance and help with the statistical analyses and Mark Orlando for providing technical support and guidance for the audiological evaluation.

The authors’ responsibilities were as follows—SS and SBA: designed the research; VC: conducted the research; LMS: helped with the ferritin estimations; AA: performed the ABR on all subjects; SS and AS: analyzed the data.

### TABLE 2

<table>
<thead>
<tr>
<th>Latent iron deficiency</th>
<th>Normal iron status</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute latency, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wave I</td>
<td>2.00 ± 0.47</td>
<td>1.88 ± 0.40</td>
</tr>
<tr>
<td>Wave III</td>
<td>4.72 ± 0.62</td>
<td>4.52 ± 0.51</td>
</tr>
<tr>
<td>Wave V</td>
<td>7.10 ± 0.68</td>
<td>6.60 ± 0.66</td>
</tr>
<tr>
<td>Interpeak latency, ms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I–III</td>
<td>2.72 ± 0.69</td>
<td>2.64 ± 0.39</td>
</tr>
<tr>
<td>III–V</td>
<td>2.37 ± 0.64</td>
<td>2.07 ± 0.33</td>
</tr>
<tr>
<td>I–V</td>
<td>5.10 ± 0.57</td>
<td>4.72 ± 0.56</td>
</tr>
</tbody>
</table>

^1All values are means ± SDs.
^2Mann-Whitney U test.
^3t test.
and performed the statistical analyses; SS, VC, and SBA: wrote and revised the manuscript; and all authors: approved the final content of the manuscript. None of the authors declared a conflict of interest.

REFERENCES