Background: Latent iron deficiency (LID) at birth is associated with prolonged latencies in auditory brainstem response (ABR), a surrogate for neural maturation. This study aimed to compare wave and inter-peak latencies of ABR at birth and at 4-6 months of age in infants ≥35 weeks of gestation with normal iron status (NIS) and LID.

Methods: Neonates born at ≥35 weeks were screened. Cord ferritin value ≤75 ng/mL and >75 ng/mL were classified as LID and NIS, respectively. ABR was performed within 48 h of birth. The absolute latencies of waves I, III, and V, and inter-peak latencies I-III, III-V, and I-V were computed. Infants were reassessed at 4–6 months of age for hemoglobin, serum ferritin levels, and ABR latencies.

Results: In total, 160 neonates were enrolled. The mean (SD) birth weight and gestational age of the study population were 2843 (384) g and 38.3 (1.1) weeks, respectively. Approximately 122 infants completed follow-up until 4–6 months of age: 37 in the LID group and 85 in the NIS group. Overall, the wave and interpeak latencies in both groups at birth were comparable. At 4–6 months, the absolute latencies of waves I, III, and V, and inter-peak latencies I-III, III-V, and I-V were decreased and were comparable in both groups. Among small-for-gestational-age neonates, inter-peak latencies in I-III and I-V at birth were significantly longer in the LID group than in the NIS group. Nine (24.3%) infants in the LID group and none in the NIS group were iron-deficient at 4–6 months of age.

Conclusion: There was no difference in wave or inter-peak latencies at birth and at 4–6 months of age in neonates aged ≥35 weeks with or without LID. However, infants with LID at birth have a significant risk of iron deficiency at 4–6 months of age.

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1. Introduction

Iron is a ubiquitous mineral that is found in trace quantities in humans. It accounts for approximately 0.0075% of human body composition. The total iron content of the term, appropriate for gestational age, is 75 mg/kg body weight, 55 mg/kg of which is complexed with hemoglobin. Iron deficiency is estimated to be the most common nutritional deficiency worldwide and is particularly common among infants and children. Term neonates are considered to have sufficient iron stores to last for 4–6 months. Preterm neonates, small for gestational age (SGA) neonates, and infants of diabetic mothers, however, are at high risk for iron deficiency during early infancy. Currently, direct measurement of brain iron in newborn infants is not feasible. Total body iron and iron storage estimates are based on measurements of serum markers such as hemoglobin (Hb), soluble transferrin receptor, hepcidin, free erythrocyte or zinc protoporphyrin concentrations, and ferritin concentrations. The serum ferritin concentration has been used as a standard measurement of iron stores in infancy in neonates with LID.2-4 Term neonates are considered to have sufficient iron stores to last for 4–6 months.5 Preterm neonates, small for gestational age (SGA) neonates, and infants of diabetic mothers, however, are at high risk for iron deficiency during early infancy.6-8 Currently, direct measurement of brain iron in newborn infants is not feasible. Total body iron and iron storage estimates are based on measurements of serum markers such as hemoglobin (Hb), soluble transferrin receptor, hepcidin, free erythrocyte or zinc protoporphyrin concentrations, and ferritin concentrations. The serum ferritin concentration has been used as a standard measurement of iron stores in the absence of inflammation.9

In addition to erythropoiesis, Iron (Fe) is an essential micronutrient that plays a significant role in various critical cellular functions. Fetal iron insufficiency affects multiple developmental processes during brain growth including myelination, dendritic growth, synaptic function, and energy metabolism.10 There is accumulating evidence that infants with iron deficiency have poor neurodevelopmental outcomes, which are tightly linked to alterations in myelination. The critical cells responsible for myelin formation are oligodendrocytes. These cells are particularly sensitive to iron deprivation, resulting in an altered composition and amounts of myelin in the white matter. Altered myelination manifests as delayed conduction in auditory and visual systems. Both sensory systems are critical for learning and social interaction. Simultaneously, during myelination, apart from the visual and auditory pathways, other intracerebral structures dependent on iron are also affected. Thus, impaired myelination may underlie other poor neurodevelopmental outcomes. This can lead to long-term cognitive or motor impairment and poor psychosocial adaptation.11-14 Auditory Brainstem Response (ABR) is a commonly used noninvasive neurophysiological tool for the assessment of auditory neural maturation, which is a surrogate marker of myelination and brain development.

Latent iron deficiency (LID) is a state in which hemoglobin is normal but iron stores are low. Recent data suggest 10–30% of neonates above 35 weeks of gestation may have LID defined as cord ferritin values less than 75 ng/mL.15-19 Few studies in preterm and term neonates have shown an association of LID at birth with neuronal maturation, indicated by prolonged wave and inter-peak latencies on ABR.15-18 There are limited data to demonstrate the progression of neuronal maturation from birth through infancy in neonates with LID.19 We conducted this prospective observational study to compare the effects of LID and normal iron status (NIS) on auditory neural maturation from birth to 4–6 months of age in infants ≥35 weeks of gestation.

2. Patients and methods

This prospective observational study was conducted at a tertiary care center between April 2017 and March 2018. The study was approved by the Institutional Research Review and Ethics Board. Written parental consent was obtained from all the subjects enrolled in the study. Infants born at ≥35 weeks’ gestation during the study period were evaluated for enrollment. Infants with craniofacial anomalies, chromosomal disorders, hemolytic disease (Coomb’s positive), multiple gestation, a history of maternal infections within 2 weeks of delivery, clinical chorioamnionitis, Apgar score <5 at 5 min, toxoplasmosis, other infections, rubella, cytomegalovirus infection, herpes simplex virus infections, clinical or culture-proven sepsis, history of admission to the neonatal intensive care unit, and refusal of consent were excluded. Demographic and clinical profiles, along with anthropometry (weight, length, and head circumference), were recorded using standard techniques.20 Cord blood was collected from all eligible infants in plain and ethylenediaminetetraacetic acid vials. Serum ferritin, hemoglobin, and hematocrit levels were also measured. Infants with anemia (cord Hb < 13.2 gm/dL)21 and those with hemolyzed blood samples were excluded from the study. Infants with serum ferritin levels ≤75 and > 75 ng/mL were categorized as having LID and NIS, respectively. Study subjects were further classified into appropriate and small for gestational age groups, according to Fenton’s growth reference.22

3. Outcome measures

3.1. ABR wave and inter-peak latencies

A bilateral monaural ABR test was performed at 24–48 h of life using a Bio-logic Navigator Evoked Response System (Bio-logic Systems, USA) by a trained audiologist who was blinded to the cord ferritin levels. It was performed in a quiet room using 80 dB nHL broadband click stimuli with insert earphones. The clicks were presented at a repetition rate of 29.9/second, and three runs of 2000 repetitions were recorded for each ear. ABR findings from the better ear (defined as the ear with shorter wave V latency) were used for the final analysis. The absolute latencies of waves I, III, and V, and inter-peak latencies (IPL) I–III, III–V, and I–V were computed.

3.2. Follow-up

At 4–6 months of age, ABR was repeated using the same technique described previously. Hemoglobin and serum ferritin levels were also estimated at the follow-up visits. At 4–6 months of age, serum ferritin levels >10 ng/mL were defined as iron-sufficient.23 Infants with a history of re-hospitalization for jaundice or other illnesses were excluded from the assessment.

3.3. Sample size

The sample size was computed using Stata/SE for WINDOWS version 11.0. Based on our previous study, the inter-peak
interval (I-V) was 5.1 (0.57) in the latent iron deficiency group and 4.7 (0.56) in the normal iron status group. To detect this difference with a power of 90%, alpha error of 0.05, and allocation of 1:3, we estimated that 128 infants (32 with LID and 96 with NIS) would be required. With an expected follow-up loss of 20%, 160 infants were planned to be enrolled in the study.

3.4. Statistical analysis

Data were prospectively recorded using a pre-designed Microsoft Access 2007 database. The results were analyzed using IBM SPSS Statistics 17.0. Quantitative data with normal distributions were compared using the Student’s t-test, and those with skewed distributions were analyzed using the Mann–Whitney U test. Categorical data were compared using the Chi-square or Fisher’s exact test, as applicable.

4. Results

During the study period, 1230 infants with a gestational age of >35 weeks were born. Of these, 1070 infants were excluded and 160 were enrolled. Approximately 20 infants were excluded later: 18 required readmissions for hyperbilirubinemia, and 2 were treated for neonatal sepsis. In total, 122 infants completed the follow-up at 4–6 months of age and were analyzed (Fig. 1). The maternal and neonatal characteristics of the study groups, which are LID and NIS, are shown in Table 1. The baseline characteristics were comparable in both groups. The mean birth weight and gestation in the study population were 2843 (384) g and 38.3 (1.1) weeks, respectively. The ABR waves and inter-peak latencies are shown in Table 2. Overall, the wave and inter-peak latencies in infants with NIS and LID at birth and at 4–6 months were comparable. They decreased significantly from birth to 4–6 months in both groups. Among SGA infants, I-III, and I-V, inter-peak latencies at birth in the LID group were significantly prolonged as compared to NIS group, 2.76 (0.3) and 5.01 (0.4) vs. 2.24 (0.4) and 4.54 (0.7), respectively. The latencies in these groups were comparable at 4–6 months.

The mean (SD) hemoglobin and median (IQR) serum ferritin levels at birth and at age 4–6 months in the NIS and LID groups are shown in Table 3. At birth, the incidence of LID in the overall, SGA and appropriate for gestational age (AGA) populations was 30.3%, 31.5%, and 25.9%, respectively. At 4–6 months of age, 9 (24.3%) infants in the LID group and none in the NIS group were iron-deficient.

![Figure 1](study_flow_chart.png)  
**Study flow chart.**
5. Discussion

We evaluated the iron status and latencies of ABR waveforms in infants ≥35 weeks of gestation, at birth, and at 4–6 months of age. The overall incidence of latent iron deficiency in our study was 30.3%, which is similar to the observations in previous studies (19.4%–26.6%).\textsuperscript{15–17} The primary objective of our study was to compare the ABR wave and inter-peak latencies at birth and 4–6 months of age in infants with LID and NIS. We did not find significant differences in wave and interpeak latencies between the NIS and LID groups at birth or at 4–6 months of age. Contrary to our findings, two previous studies observed significantly prolonged interpeak latencies in infants with latent iron deficiency compared to those with a normal iron status.\textsuperscript{16,17} Difference in population characteristics among these studies could be the reason for the varied observations. Amin et al. enrolled infants ≥35 weeks of gestation with at least one risk factor. Approximately half and 1/3 of the population have maternal diabetes and pregnancy-induced hypertension, respectively.\textsuperscript{16} In the study by Choudhary et al., 2/3 of enrolled infants had maternal diabetes or growth restriction.\textsuperscript{17} We observed a lower incidence of gestational diabetes and gestational hypertension in our study population. It is possible that risk factors, especially growth restriction, rather than LID alone, play an important role in neuronal maturation.\textsuperscript{24–26}

On further analysis of SGA infants, we observed significantly prolonged inter-peak latencies between I-III and I-V in the LID group as compared with the NIS group. The prolonged latencies in SGA infants with LID in our study are consistent with the findings of delayed ABR maturation.
birth. Similar patterns in wave and interpeak latencies from birth in infants aged 35 weeks, with or without LID. However, among SGA infants, those with LID had significantly prolonged I-III and I-V interpeak latencies. Infants with LID at birth have a significant risk of iron deficiency at 4–6 months of age. These findings suggest that iron deficiency in utero in the presence of growth restriction may contribute to delayed neuronal maturation at birth.

Declaration of competing interest

None to disclose.

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References


