Auditory brainstem responses in term newborns with hyperbilirubinemia

Potencial auditivo evocado de tronco encefálico em recém-nascidos de termo com hiperbilirrubinemia

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ABSTRACT

Objective: To evaluate auditory brainstem responses in term newborns with hyperbilirubinemia. Methods: Seventy-one newborns, 35 with aggravated physiological neonatal jaundice (group I), 24 with ABO blood incompatibility (group II) and 12 not suffering from jaundice or any other disease were submitted to auditory brainstem responses. Statistical analysis of variance was performed to evaluate waveform reproducibility, absolute and interwave latencies, and Pearson coefficient was used to evaluate the association between the level of serum bilirubin and abnormalities in the auditory brainstem responses. Results: According to the criteria assumed in the present paper waveform alterations were more frequently found in group II than in the control group (p = 0.023). No significant differences were observed between groups I and II (p = 0.083) or between control group and group I (p = 0.166). Wave I latency at 80 dBHL for good reproducibility responses and III-V interwave latency at 40 dBHL for poor reproducibility responses of newborns with hyperbilirubinemia showed significant findings in relation to the control group (0.008 and 0.004 respectively). There was positive, weak (9%) association between serum indirect bilirubin levels and auditory brainstem responses only when the two groups were analyzed together. Conclusions: Neonatal hyperbilirubinemia changed the conduction of auditory stimulus in term newborns with jaundice caused by ABO blood incompatibility. There was poor positive association between plasma levels of bilirubin and abnormalities in auditory brainstem responses.

Keywords: Infant, newborn; Jaundice; Hyperbilirubinemia; Evoked potentials, auditory; Hearing tests; Kernicterus

INTRODUCTION

One of the most appropriate methodologies to perform early detection of auditory problems in newborns is brainstem evoked response audiometry or auditory
brainstem responses (ABR). This test reflects the activity of several neuroelectric events along the auditory pathway, from the auditory nerve to the brainstem, which may be captured by surface electrodes placed on the skull(1). It is quite an accurate electrophysiological exam, non-invasive, and may be collected during natural sleep in the neonatal unit, with the newborn in his own crib(2).

ABR is a test that registers the electric responses obtained during the first 10 milliseconds after the presentation of an auditory stimulus, when we may observe a series of five to seven waves or peaks. Waves are generated by one or more structures (generators) along the auditory pathway(3). The classification of these generators has been described as follows: wave I, distal portion of the auditory nerve; wave II, proximal portion of the auditory nerve; wave III, cochlear nucleus; wave IV, superior olivary cochlear complex; wave V, lateral lemniscus, and wave VI, inferior colliculus(4).

Absolute latency is understood as the time interval, measured in milliseconds, between the stimulus onset and the peak of the response. Wave I latency represents the time spent for the peripheral conduction. Waves III and V latencies, and interwave latencies I-III, III-V and I-V, represent all time measurements of central conduction. The latency between wave peaks I and V (interwave latency I-V) refers to the time spent for brain conduction(5). Latencies increase as sound intensity decreases(2). In addition to this analysis, wave amplitude may diminish or even disappear in response to an insult.

The morphological changes in response reproducibility, making them poorly defined, unstable or difficult to read, may also demonstrate some degree of impairment by hyperbilirubinemia(6).

The Joint Committee on Infant Hearing (JCIH), in its report in the year 2000, showed a list of five risk factors that places the newborn in a high risk group for hearing loss(7); however, hyperbilirubinemia was not included as an independent factor. The 1994 report had specifically mentioned that neonatal hyperbilirubinemia at levels that indicate exchange transfusion was considered a risk factor(8).

Several studies have evaluated the relationship between the levels of serum bilirubin and ABR. Most of them identify some type of abnormality, such as: increased central conduction time; absence of waves; peripheral changes; transient changes; auditory behavior, visual and cry changes(9-10). These abnormalities may disappear after exchange transfusion(11-17) and, according to some authors, they do not depend on high levels of total serum bilirubin(18-21) but may be related to free bilirubin concentrations(22-23).

As shown above, the issue is still quite controversial, urging for new studies that may shed some light on the subject.

**OBJECTIVE**

The aim of the present study was to determine the conduction of the auditory stimulus in the auditory pathway by using ABR in term newborns with hyperbilirubinemia, with indication for phototherapy or exchange transfusion, in relation to normal anicteric newborns.

**METHODS**

Setting: Neonatal Unit of Hospital Israelita Albert Einstein.

Type of study: Cross sectional, prospective, retrospective study.

Sample.

Inclusion criteria: Term newborns according to gestational age calculated by Naegle rule(24) and gestational age estimated by Capurro’s method(25), with neonatal jaundice whose serum levels showed need for treatment, according to the criteria established by the University of Kentucky Medical Center, which was adopted by the Neonatal Unit(26) at the time of the study. Neonates had no respiratory or metabolic disorder, nor risk for hearing loss (except for hyperbilirubinemia). They were tested under ideal technical conditions, as follows: a-during the collection it was observed that morphology (waveform definition) changes were more frequent when the newborn was either awake or in light sleep. To make the method more trustworthy, only the responses of newborns that were sound asleep during the evaluation were included in the study; b-only the records obtained with balanced electrode impedances and values less or equal to 5 k ohms were included in the study.

Exclusion criteria: Newborns with family history of hearing impairment, gestational age < than 37 weeks, first minute Apgar score < 7, signs of respiratory or metabolic disorder, cranial-facial malformation or having received ototoxic medication. Newborns awake or in light sleep at the test, or with unbalanced electrode impedances of more than 5 k ohms were excluded from the study.

Out of four hundred and eighty newborns tested during the study period, 71 newborns were enrolled in the study, according to the inclusion criteria.

Procedures:

The tests were performed in the neonatal unit, after explaining the procedures to the parents and receiving their free consent. ABR were evoked with alternating clicks from an insert earphone at intensities of 80 dBHL and 40 dBHL, 20 clicks per second, band pass filtered (100 and 3000 Hz), 15 ms window, sample of 1,500 stimuli; two collections were made for each intensity. The test was performed at the highest peak of serum bilirubin levels.
ABR were performed using three surface electrodes placed on the ipsilateral mastoid (reference electrode) and contralateral mastoid (ground electrode) and on the newborn forehead (active electrode). The two ears were assessed randomly. The equipment used was Audit V (Nicolet).

The group of jaundiced newborns, according to etiology, was divided into two groups:

a) Group I (GI), comprising 35 newborns with jaundice designated as “agravated physiological jaundice”, with no maternal-fetal blood incompatibility and no hemolysis according to routine blood tests in use at the neonatal unit at the time of the study (hemoglobin plasma levels, erythrocyte creatine, reticulocyte count).

b) Group II (GII), comprising 24 newborns with ABO blood group incompatibility, identified by immune-hematological tests and with some degree of hemolysis, according to the criteria adopted in the neonatal unit at the time of the study (presence of A or B antibodies in serum or eluate; Coombs test (if positive, only confirmed the presence of incompatibility but, if negative, did not rule it out).

The control group comprised 12 normal term newborns, without clinical signs of jaundice, maternal-fetal blood incompatibility or any other abnormality, plus no risk factor for hearing loss.

Main measurements:

The recorded ABR were analyzed by the researcher who did not know about the clinical conditions and laboratorial tests of the newborns. The analysis considered the absolute latency of waves I, III and V and its interwave latencies I-III, III-V and I-V as well as waveform reproducibility.

ABR latency analysis was performed for the three waves (I, III and V) and interwave latencies (I-III, III-V and I-V) at 80 and 40 dB HL. The latencies that followed the normal range criteria for the same age group proposed by Gorga were considered normal.

ABR waveforms were categorized depending on response types, based on reproducibility and presence of waves I, III and V (figure 1). In term newborns, it is expected that ABR detect 12 replicable waves (waves I, III and V at 80 dB HL and waves I, III and V at 40 dB HL for each ear, as shown in figure 2).

The analysis was performed for the three waves (I, III and V), two ears and at two intensities (80 and 40 dBHL) totaling 12 waves for each newborn. The changes expected in waves I, III and V are absence of wave, poor definition or poor reproducibility.

These aspects have been considered to categorize the waveform types into good or poor reproducibility responses. Good reproducibility represented presence of all waves and poor reproducibility represented presence of any type of affection.
Statistical methods:
a) To verify whether there were differences in ABR latencies among the groups, for different intensities, an analysis of variance (ANOVA) was performed for repeated measures (univariate model) on the defined responses (good reproducibility) and on the undefined responses (poor reproducibility). The distribution of the good and poor reproducibility responses among the groups and whether there was any association between ABR changes and bilirubin levels were also studied. The significance level adopted was p < 0.05.
b) Pearson coefficient was calculated to study the presence of association between bilirubin and ABR between the two variants, in G I and G II, and considering the two groups altogether.
c) When the analysis of the variance was significant, Tukey test was used to check in which groups there were differences.

The study was approved by the Institutional Ethical Review Board.

RESULTS

Table 1 shows the average, range and standard deviation of the characteristics of the newborns in G I, G II and in the control group such as birth weight, gestational age, maximum level of indirect serum bilirubin (IB) and day of life when maximum indirect serum bilirubin occurred.

There were no significant differences in gestational age for the three groups and also no differences between indirect bilirubin levels in groups G I and G II. Regarding birth weight, G II showed higher average birth weight than the other two groups.

Waveform study

Table 2 shows the distribution of waveform types and good and poor reproducibility for the groups; no poor reproducibility responses were found in the control group.

Consideration poor reproducibility responses, waveform changes were more frequent in the group with ABO blood incompatibility (G II) than in the group of jaundice without blood incompatibility (G I) and they were absent in the control group, a difference that was statistically significant.

From the total of 852 studied waves, 55 alterations (6.4%) were found: 22 in G I and 33 in G II and none in the control group. The most frequent abnormalities were detected at 40 dBHL intensity in waves I (n = 16, 29%), III (n = 15, 27%) and V (n = 15, 27%) and, less frequently, at 80 dBHL intensity in wave III (n = 2, 3.6%) and wave V (n = 7, 13%).

Latencies and interwave latencies analysis

The study of latencies was analyzed according to waveform types and classified as good or poor reproducibility.

A) Good reproducibility

Tables 3 and 4 show the data referring to average latencies, interwave latencies and standard deviation for both ears in G I, G II and control, for intensities at 80 dBHL and 40 dBHL respectively, for good reproducibility responses.

When analyzing the latencies for good reproducibility responses, mean wave I latency at 80 dBHL was significantly different among the other groups (p = 0.008) (table 3). Results from Tukey test
showed differences between control and G II and between G I and G II. No significant differences were found between the control group and G I. Figure 3 shows individual and average values variability.

For absolute wave V at 40 dBHL, a tendency ($p = 0.054$) could be suggested to demonstrate the differences among G I, G II and the control group (table 4).

Regarding the values of interwave latencies and good reproducibility responses, there were no significant differences among the values of interwave latencies I-III, III-V and I-V for intensities at 80 and 40 dBHL for the different groups ($p > 0.05$).

## Table 3. Average latencies, interwave latencies and standard deviation (SD) in G I, G II and control for the intensity of 80 dBHL, for good reproducibility responses

<table>
<thead>
<tr>
<th>Wave</th>
<th>G I</th>
<th>G II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.77</td>
<td>1.70</td>
<td>1.81</td>
</tr>
<tr>
<td>II</td>
<td>4.59</td>
<td>4.57</td>
<td>4.63</td>
</tr>
<tr>
<td>V</td>
<td>6.92</td>
<td>6.84</td>
<td>6.94</td>
</tr>
<tr>
<td>III</td>
<td>2.82</td>
<td>2.67</td>
<td>2.83</td>
</tr>
<tr>
<td>III-V</td>
<td>2.33</td>
<td>2.26</td>
<td>2.31</td>
</tr>
<tr>
<td>I-V</td>
<td>5.15</td>
<td>5.14</td>
<td>5.13</td>
</tr>
</tbody>
</table>

*significant ($p<0.05$); NS = non significant.

## Table 4. Average latencies, interwave latencies and standard deviation (SD) in G I, G II and control group for intensity of 40 dBHL for good reproducibility responses

<table>
<thead>
<tr>
<th>Wave</th>
<th>G I</th>
<th>G II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.83</td>
<td>2.75</td>
<td>2.89</td>
</tr>
<tr>
<td>II</td>
<td>5.50</td>
<td>5.41</td>
<td>5.62</td>
</tr>
<tr>
<td>V</td>
<td>7.65</td>
<td>7.49</td>
<td>7.74</td>
</tr>
<tr>
<td>III</td>
<td>2.67</td>
<td>2.66</td>
<td>2.72</td>
</tr>
<tr>
<td>III-V</td>
<td>2.15</td>
<td>2.08</td>
<td>2.12</td>
</tr>
<tr>
<td>I-V</td>
<td>4.82</td>
<td>4.74</td>
<td>4.85</td>
</tr>
</tbody>
</table>

*tendency; NS = non significant.

B) Poor reproducibility

Tables 5 and 6 show data referring to average latencies, interwave latencies and standard deviation for both ears found in G I and G II for intensities at 80 dBHL and 40 dBHL, respectively, for poor reproducibility responses.

The analysis of latencies I, III and V and interwave latencies I-III, III-V and I-V values for poor reproducibility responses in G I and G II were compared to those in the control group and between themselves.

Values for wave I, III and V latencies for poor reproducibility responses did not show significant differences among the groups when compared to latency values found in the control group.

Regarding the values of interwave latencies I-III, III-V and I-V for intensities at 80 and 40 dBHL, significant differences were observed in interwave latency III-V at 40 dBHL among the groups ($p = 0.004$) (Table 6). Results of Tukey test showed differences between the control group and G I and between G I and G II (figure 4).

## Table 5. Average latencies, interwave latencies and standard deviation (SD) in G I, G II at 80 dBHL intensity for poor reproducibility responses

<table>
<thead>
<tr>
<th>Wave</th>
<th>G I</th>
<th>G II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1.70</td>
<td>1.70</td>
<td>1.89</td>
</tr>
<tr>
<td>II</td>
<td>4.71</td>
<td>4.62</td>
<td>5.52</td>
</tr>
<tr>
<td>V</td>
<td>7.18</td>
<td>6.96</td>
<td>7.74</td>
</tr>
<tr>
<td>III</td>
<td>3.02</td>
<td>2.91</td>
<td>7.74</td>
</tr>
<tr>
<td>III-V</td>
<td>2.47</td>
<td>2.34</td>
<td>7.74</td>
</tr>
<tr>
<td>I-V</td>
<td>5.49</td>
<td>5.25</td>
<td>7.74</td>
</tr>
</tbody>
</table>

NS = non significant.

## Table 6. Average latencies, interwave latencies and standard deviation (SD) in G I, G II at 40 dBHL intensity for poor reproducibility responses

<table>
<thead>
<tr>
<th>Wave</th>
<th>G I</th>
<th>G II</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.84</td>
<td>2.80</td>
<td>2.89</td>
</tr>
<tr>
<td>II</td>
<td>5.48</td>
<td>5.46</td>
<td>5.52</td>
</tr>
<tr>
<td>V</td>
<td>7.88</td>
<td>7.63</td>
<td>7.89</td>
</tr>
<tr>
<td>III</td>
<td>2.64</td>
<td>2.66</td>
<td>2.89</td>
</tr>
<tr>
<td>III-V</td>
<td>2.40</td>
<td>2.17</td>
<td>2.91</td>
</tr>
<tr>
<td>I-V</td>
<td>5.04</td>
<td>4.83</td>
<td>4.91</td>
</tr>
</tbody>
</table>

*significant ($p<0.05$); NS = non significant.
Association between bilirubin and ABR

In the analysis of absolute latencies (I, III, V) and interwave latencies (I-III, III-V, I-V) at the two analyzed sound intensities (80 and 40 dBHL) a positive association between indirect bilirubin levels and ABR was found. It was noticed only when the two groups (G I and G II) were analyzed together. This positive association was found with wave I at 80 dBHL (p = 0.026) and at 40 dBHL (p = 0.026) and wave III at 40 dBHL (0.035) and was considered poor association (9%) (figure 5 a, b, c).

DISCUSSION

Neonatal hyperbilirubinemia, although a common problem in newborns, remains as a constant worry for neonatologists because bilirubin may potentially cause brain damage.

Although the classic “Kernicterus” condition is rare and there is no reasoning for it to occur nowadays, high incidence of sensorineural hearing loss has been described in the absence of other clinical signs of bilirubinic encephalopathy, especially in premature newborns with hyperbilirubinemia and not always with high levels of serum bilirubin. The role of bilirubin as a direct cause of sensorineural hearing loss is still quite controversial and no safe limits or bilirubin levels have yet been established for this group of children. Nevertheless, it is known that the auditory system is highly sensitive to bilirubin toxicity and that auditory affections may occur in children with or without the classical signs of “Kernicterus”. The controversy is even greater when the subject is a term newborn, with hyperbilirubinemia with or without hemolysis.

It is also important to define criteria that would allow the clinician to monitor subjective ABR aspects such as waveform morphology. Thus, Amin et al. defined criteria to check ABR maturation, which was adapted to good or poor reproducibility responses in the present study.

There were reports of ABR alterations such as absence of waves III and IV-V, delayed latencies I and IV-V and increased brain conduction time (I-V) when total bilirubin levels were higher than 22.8 mg/dl; these affections disappeared after exchange transfusion, suggesting that this could be a transient effect. The authors in this study suggest exchange transfusion in cases of high hyperbilirubinemia (> 20 mg/dl), when it is not possible to monitor it by ABR, thus indicating that ABR has an important role in exchange transfusion indication. In the present study, the average bilirubin serum levels in the newborns in both groups were quite below the mentioned levels, however, we more frequently found ABR waveform changes in newborns with ABO incompatibility than in newborns in G I and in the control group.

Tan et al. performed serial ABR before, during and after exposing the infants to phototherapy and reported transient increase in latencies V, interwave I-V and III-V latencies in term newborns submitted to phototherapy with total bilirubin of 15.17 ± 0.31 mg/dl, when compared to anicteric newborns. In their study, the authors showed that the time of latencies was gradually decreasing during phototherapy and fell to normal values 24 hours after the end of the procedure.
In the present study, considering that the patients had similar gestational ages and days of life, wave I latency was significantly shorter in G II than in G I and the control group. Given that wave I latency represents peripheral conduction time, one could suppose that its shortening was due to changes in external and/or middle ear, therefore a peripheral change, and not due to a neurotoxic impact. Data from the above mentioned study\(^{(11)}\) also showed increase in interwave latencies III-V in the group of term newborns submitted to phototherapy. Similarly, in the present study, we found increase in interwave latency III-V among icteric (without ABO incompatibility) and anicteric infants and among icteric with and without ABO incompatibility. This increase could only be observed at 40 dBHL intensity, for poor reproducibility responses, what could be attributed to neurotoxicity or to the fact that less sound energy would be responsible for larger variability in responses. However, it is interesting to bear in mind that interwave latency III-V translates central conduction time\(^{(5)}\).

On the other hand, waves I, III and V latency values for poor reproducibility responses did not show significant differences among the groups when compared to latency values in the control group, but this could have happened because of the small number of analyzed cases with poor reproducibility responses.

Boo et al.\(^{(2)}\) observed that ABR alterations were not associated with high levels of bilirubin but rather with two other risk factors: the need of exchange transfusion and the early appearance of hyperbilirubinemia. Several other authors observed that ABR affections in term newborns without hemolysis with total bilirubin > 20 mg/dl were more frequently associated with free bilirubin serum levels equal or higher than 1 mg/dl than with total bilirubin levels, data that is also supported by experimental studies\(^{(14,23-24)}\).

In the present study, the groups with jaundice without blood incompatibility showed ABR waveform responses similar to that of the control group, and the unbound bilirubin level in G I showed levels indicating exchange transfusion in only one case. In the group with hemolytic jaundice due to ABO blood incompatibility, ABR behavior was different from that of the control group: there was a larger number of poor reproducibility responses in G II when compared to the control group, a difference that was statistically significant; although no differences between G I and G II occurred. We must point out that serum bilirubin levels were similar in G I and G II and there was only one case of exchange transfusion in GII. However, in the present study, free bilirubin was not evaluated in any case submitted to exchange transfusion.

These findings may lead us to suspect that the affections could have been caused not only by high serum bilirubin levels but by the influence of other factors such as anemia and duration of hyperbilirubinemia, as reported in the literature\(^{(30)}\). Nevertheless, the present findings do not enable us to validate this hypothesis.

When performing an overall analysis of the two icteric groups, a positive association between indirect bilirubin levels and alterations in ABR for waves I (80 and 40 dBHL) and III (40 dBHL) was observed. Although this association did seem weak, these findings should call the attention of healthcare professionals dealing with icteric newborns given that neonatal jaundice, even in term newborns without hemolysis, could represent a serious risk of late damage.

The present study did not focus on the follow-up of the newborns to check whether the affections would be definitive or transient. Thus, we suggest that further research in the area should be carried out.

**CONCLUSIONS**

1. ABR waveforms of jaundiced newborns due to ABO blood incompatibility showed significant alterations in morphology (poor reproducibility) when compared to anicteric newborns or jaundiced newborns without hemolysis.

2. Wave I latency at 80 dBHL and III-V interwave latency at 40 dBHL of newborns with hyperbilirubinemia showed significant abnormalities in relation to the control group.

3. There was positive but weak association between indirect bilirubin levels and ABR.

**REFERENCES**


