The Effects of Hyperbilirubinemia on Sleep-Spindle Characteristics in Infants

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Objective: The purpose of this study is to investigate the effect of hyperbilirubinemia on sleep-spindle characteristics. Rhythmic activities, such as sleep spindles, may be abolished by hyperbilirubinemia.

Study Design: Electroencephalogram records were taken from 15 infants with hyperbilirubinemia and 18 healthy infants at the 12th week after birth. Sleep spindles of the 2 groups were compared according to location, density, duration, amplitude, frequency, asynchrony, and asymmetry.

Results: In the study and control groups, the density of the spindles was found to be 76.9 ± 23.7 and 105.2 ± 33.9, respectively, in a 1-hour non-rapid eye movement sleep period. The mean durations of the sleep spindles in the study and control groups were found to be 4105 ± 802 milliseconds and 5162 ± 1075 milliseconds, respectively. There was not any difference between the groups according to the amplitude and asymmetry. However, there was a significant difference between the 2 groups in the frequency of spindles. The mean frequency was found to be 12.5 ± 0.6 Hz in the study group and 13.2 ± 0.9 in the control group. The percentage of asynchronous spindles was higher in the study group than in the control group. There was a significant negative correlation between the bilirubin levels during the newborn period and density, duration, and frequency of spindles. However, there was a significantly positive correlation between the bilirubin levels and percentage of asynchronous spindles. There was a significant negative correlation between the duration of hyperbilirubinemia and spindle amplitude.

Conclusion: We suggest that studies on the critical maturation periods of sleep-spindle patterns might provide a sensitive tool for early diagnosis of neurophysiologic brain alterations during the first trimester of life in a population of at-risk children, such as jaundiced infants.

Key Words: Hyperbilirubinemia, electroencephalogram, sleep spindle, brain maturation, infant

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INTRODUCTION

HYPERBILIRUBINEMIA IS ONE OF THE MOST COMMON PROBLEMS DURING THE NEONATAL PERIOD. BILIRUBIN IS STORED, PARTICULARLY IN THE SUBTHALAMIC NUCLEUS, HIPPOCAMPUS, THALAMUS, GLOBUS PALLIIDUS, PUTAMEN, CRANIAL NERVE NUCLEI, AND CEREBRAL CORTEX IN THE BRAIN.1-4 Yellow staining of the brain has also been observed in infants without clinical symptoms of kernicterus and in whom only moderately elevated serum bilirubin values have been noted.5 Bilirubin inhibits oxidative phosphorylation and respiration in the mitochondria of the brain.6 Rhythm of the electroencephalogram (EEG) appears to be dependent on interactions between the cortex and thalamus, both of which have certain structural and functional features that lend themselves to the production of rhythmic activity. Rhythmic activities, such as sleep spindles, may be abolished by lesions directly involving either thalamic or cortical structures.7

A sleep spindle has been defined as a relatively sinusoidal “11- to 15-Hz burst,” but mostly at 12 to 14 Hz, occurring during quiet sleep and recorded on the central regions of the scalp. Rudimentary sleep spindles may be observed in full-term newborns at birth. Clearly distinguishable discrete bursts appear only between 6 and 8 weeks after term during quiet sleep.7,8

In this study, the effect of hyperbilirubinemia on sleep-spindle characteristics, which is a parameter showing the brain maturation on the EEG, was investigated. The goal of this study was to investigate the details of the changes in sleep spindles in infants with hyperbilirubinemia at 3 months of age.

MATERIAL AND METHODS

Fifteen infants who had unconjugated hyperbilirubinemia in the newborn period and 18 healthy infants from Pamukkale University Hospital Neonatal Care Unit were prospectively evaluated for a study aiming to observe changing sleep-spindle maturation on the EEG. All of the babies were born at 38 to 42 weeks gestation and were appropriate for gestational age according to the Dubowitz score.9 All infants were developmentally normal in the 12th week of life, by either neurodevelopmental testing or parental report. All of the babies’ families have been informed about the study, and written consent was signed. The institutional review board approved the study.

Babies with unconjugated hyperbilirubinemia during the newborn period constituted the study group, and the bilirubin levels were above the phototherapy limit.10 All of the babies were monitored for bilirubin levels 4 times each day during this period. The babies whose bilirubin levels were under phototherapy limits were treated as the control group. All of the infants were breastfed. All of the babies in the study and control groups were asymptomatic. Infants whose mothers had diabetes mellitus, an illness, eclampsia, or preeclampsia or used drugs during pregnancy and infants whose Apgar scores were below 7 were excluded. No infant suffered a major medical illness or required ventilatory assistance during the neonatal period. The babies did not have respiratory and cardiac diseases or congenital malformations.

Total and direct bilirubin concentrations were determined by using Modify Jendrassic Grof method in the Hitachi 902 automatic analyser (Abbott Laboratories, Abbott Park, III).

Each infant received an EEG examination in the first, second,
fourth, and 12th weeks. Sleep spindles were seen in only 2 subjects at the fourth week. We also did not see any sleep spindles at the first and second weeks. Because sleep spindles were observed in all patients in the 12th week, records in both groups were compared in terms of the spindle characteristics in the 12th-week records. Studies were performed in an environmentally controlled setting in which sound, light, humidity, and tactile stimulation were carefully monitored. All infants were studied during sleep in a prone position on an open bed. No infant received medication. The infants were admitted to the hospital before 10 AM for the EEG recording. After a routine pediatric examination, EEG was recorded at least 1 hour only in quiet sleep periods. Sleep spindles were evaluated during a total 1-hour period of quiet sleep in both groups. For each of the infants, we continued EEG recording until reaching a total of 1 hour of quiet sleep. We did not observe apnea, bradycardia, or oxygen desaturation during EEG recording in any of the infants. The EEG apparatus was produced by Medelec Profile Digital EEG Systems (Oxford Instruments, Old Woking, UK).

The digitized data from the EEG recordings were collected 21 cerebral electrodes were placed according to the international 10/20 system. Eight noncerebral channels and an event-marker proGram recorded viscerosomatic measures (2 channels of electrooculogram, 1 channel of submental electromyogram, 1 channel of respirogram, 1 channel of electrocardiogram, 1 channel of oxygen saturation, and 2 channels of skin and rectal temperatures). Records were performed with a high-frequency filter of 70 Hz, low-frequency filter of 0.16 Hz, and a sensitivity of 100 µV/mm. The signals were recorded on hard disk and transferred to CD-ROM for storage.

Sleep spindles were evaluated on the EEG records. The sleep spindle was as defined as a duration 0.5 seconds, amplitude 10 µV, and frequency 10 to 15 Hz. Sleep spindles were compared between the 2 groups according to the following criteria: location, density, duration, amplitude, frequency, asynchrony, and asymmetry. Spindles were measured at the loci of their maximum amplitude; however, a precise study of the location was not done. Density consisted of the number of spindles per hour; the spindles were counted on the location at which the amplitude of the spindles was maximal. The duration was determined, in seconds, for all spindles. Spindle amplitude was classified in 4 ranges: 10 to 25 µV, 25 to 50 µV, 50 to 75 µV, and 75 to 100 µV. Spindle frequency was classified in 3 ranges: 10 to 11.9 Hz, 12 to 13.9 Hz, and 14 to 15 Hz. Asynchrony was defined as an interval of more than 2 seconds between the middles of the 2 bursts on each hemisphere or an isolated burst (Figure 1). We considered a burst as asymmetrical when there was a voltage ratio greater than 2:1 between hemispheres.

The presence, location, density, asymmetry, and asynchrony of sleep spindles were evaluated manually by 2 observers. Manual evaluation was blinded. Interscorer agreement between the 2 scorers was checked and kappa values were found as 0.89, 0.84, 0.78, and 0.81, respectively, for the location, density, asymmetry, and asynchrony. However, the frequency, duration, and amplitude of manually detected sleep spindles were evaluated by computer. Statistical analyses were performed by Systat statistical software (version 9.0 for Windows; SPSS Inc, Chicago, Ill) The Student t test, χ2, Mann-Whitney U, Pearson correlation, and linear regression analysis were used for data analyses. Statistical significance was taken at P < .05. All data are presented as the mean ± SD.
RESULTS

There was no difference between the 2 groups regarding sex; Apgar score; conceptual age; birth weight, length, and head circumference; and age at 3 months of age (P > .05). The study group consisted of 15 babies (5 girls, 10 boys). There were 18 babies in the control group (7 girls, 11 boys). Apgar scores were 9 to 10 in the first minute and 10 in the tenth minute. Bilirubin levels in the study and control groups were 23.2 ± 5.4 mg/dL (16.3-33) and 7.9 ± 3.2 mg/dL (2-13.3), respectively. There was a significant difference between the bilirubin levels of the 2 groups (P < .001) (Table 1). In the study group, we detected Rh immunization (n = 1), ABO immunization (n = 3), urinary tract infection (n = 1), and G6PD deficiency (n = 3). Direct Coombs test was positive only in the case with Rh immunization. All of the infants in the study group required phototherapy, and 7 had exchange transfusions. There was no difference between the bilirubin levels of the 2 groups (P > .05). The study and control groups, respectively, had 3048 spindles (1154 spindles in the study group, and 1894 spindles in the control group). In the study and control groups, sleep-spindle duration was found to be 4105 ± 802 (2693-5686) milliseconds and 5162 ± 1075 (3512-7368) milliseconds, respectively. The duration of the sleep spindles was shorter in the control group than in the hyperbilirubinemia group (P < .05) (Table 2).

In both groups, there were no patients in whom sleep spindles did not develop. For all infants in the 2 groups, spindles were prominent in the frontocentral area (F3,C3, F4,C4).

In this study, 3048 spindles were analyzed (1154 spindles in the study group, and 1894 spindles in the control group). In the study and control groups, the mean number of the spindles was found to be 76.9 ± 23.7 and 105.2 ± 33.9, respectively. Spindle density in the jaundiced group was lower than in the control group (P < .05). In the study and control groups, sleep-spindle duration was found to be 4105 ± 802 (2693-5686) milliseconds and 5162 ± 1075 (3512-7368) milliseconds, respectively. The duration of the sleep spindles was shorter in the control group than in the hyperbilirubinemia group (P < .05).

The percentages of asynchronous spindles were 59% and 33% in the study and control groups, respectively (P < .001). There was also not any significant difference between the groups in terms of the amplitude (P > .05). However, there was a significant difference between the 2 groups in terms of spindle frequency (P < .05). Frequency was 12.5 ± 0.6 Hz (11.3-13.8) in the study group and 13.2 ± 0.9 Hz in the control group. Frequency was found to be 27% and 73%, respectively, between the 10 to 11.9 Hz and 12 to 13.9 Hz ranges in the study group. In the control group, frequencies between the 10 to 11.9 Hz, 12 to 13.9 Hz, and 14 to 15 Hz ranges were 11%, 61%, and 28%, respectively. Slow frequencies were higher in the hyperbilirubinemia group than in the control group (P < .05) (Table 2).

In this study, 3048 spindles were analyzed (1154 spindles in the study group, and 1894 spindles in the control group). In the study and control groups, the mean number of the spindles was found to be 76.9 ± 23.7 and 105.2 ± 33.9, respectively. Spindle density in the jaundiced group was lower than in the control group (P < .05). In the study and control groups, sleep-spindle duration was found to be 4105 ± 802 (2693-5686) milliseconds and 5162 ± 1075 (3512-7368) milliseconds, respectively. The duration of the sleep spindles was shorter in the control group than in the hyperbilirubinemia group (P < .05) (Table 2).

There was a significant negative correlation between the bilirubin levels during the newborn period and the density, duration, and frequency on the EEG records (density: P < .05, r = -0.38; duration: P = .001, r = -0.56; frequency: P < .05, r = -0.37) (Figures 2, 3, and 4). However, there was a significant positive correlation between the bilirubin levels and percentage of asynchronous spindles (P = .000, r = 0.64) (Figure 5). There was a significant negative correlation between the duration of hyperbilirubinemia

### Table 1—Data From Study and Control Groups

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Hyperbilirubinemia</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conceptual age, wk</td>
<td></td>
<td>40.1 ± 2.7</td>
<td>39.6 ± 0.8</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Birth length, cm</td>
<td></td>
<td>50.2 ± 2.3</td>
<td>50.3 ± 1.9</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Head circumference, cm</td>
<td></td>
<td>34.2 ± 1.1</td>
<td>34.5 ± 1</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Birth weight, g</td>
<td></td>
<td>3220 ± 638</td>
<td>3469 ± 486</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Third month weight, g</td>
<td></td>
<td>6307 ± 1295</td>
<td>6488 ± 1087</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Apgar scores</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First minute</td>
<td></td>
<td>9-10</td>
<td>9-10</td>
<td></td>
</tr>
<tr>
<td>Tenth minute</td>
<td></td>
<td>10</td>
<td>10</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Bilirubin level, mg/dL</td>
<td></td>
<td>23.2 ± 5.4</td>
<td>7.9 ± 3.2</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD.

### Table 2—Sleep Spindles in Study and Control Groups

<table>
<thead>
<tr>
<th>Sleep Spindle Measure</th>
<th>Hyperbilirubinemia</th>
<th>Group</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density</td>
<td>76.9 ± 23.7 (34-132)</td>
<td>105.2 ± 33.9 (50-167)</td>
<td>&lt; .05</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1154</td>
<td>1894</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration, ms</td>
<td>4105 ± 802</td>
<td>5162 ± 1075</td>
<td>&lt; .05</td>
<td></td>
</tr>
<tr>
<td>(2693-5686)</td>
<td>(3512-7368)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amplitude, mV</td>
<td>43.4 ± 19 (46-89)</td>
<td>53.3 ± 21.4 (23-87)</td>
<td>&gt; .05</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>33</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-25 %</td>
<td>40</td>
<td>22</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25-50 %</td>
<td>27</td>
<td>39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>50-75 %</td>
<td>—</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>75-100 %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>12.5 ± 0.6 (11.3-13.8)</td>
<td>13.2 ± 0.9 (11.8-15)</td>
<td>&lt; .05</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>27</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-11.9 , %</td>
<td>73</td>
<td>61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12-13.9 , %</td>
<td>—</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage of asynchronous spindles, %</td>
<td>59 ± 11.9 (35-82)</td>
<td>33 ± 7.8 (19-46)</td>
<td>.000</td>
<td></td>
</tr>
<tr>
<td>Percentage of asymmetry, %</td>
<td>3.1 ± 1.3 (1-6)</td>
<td>2.6 ± 1 (1-5)</td>
<td>&gt; .05</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD (range).
and amplitude ($P < .01, r = -0.53$) (Figure 6).

**DISCUSSION**

Jaundice is one of the major problems of newborns in Turkey.

Reversible depressive effects of bilirubin on neuronal functions have been shown with modern neurophysiologic diagnostic techniques such as brainstem, visual, and somatosensory evoked potentials.

Only 1 study is available in the literature about the effect of hyperbilirubinemia on EEG maturation. In our previous study, we investigated the electrophysiologic effects of bilirubin on the rhythmic oscillations with the long-term postnatal age in the hyperbilirubinemia group. We have found that, in records of the hyperbilirubinemia group in the first week, the theta, alpha, and beta frequencies and the amplitude levels are lower; however, the delta frequency is higher than in the control group, and these changes have been found to significantly correlate with bilirubin levels. The delta frequency is decreased, and the theta frequency is increased with the age. The changes between the groups disappear in the third month despite the differences at all regions of the brain. We have shown with these data that hyperbilirubinemia without clinical symptoms causes electrophysiologic changes of cerebrocortical activity in the brain. We thought that hyperbilirubinemia caused transient delay in brain maturation.

Bilirubin especially affects thalamus and cerebral cortex. Sleep spindles represent 1 of the few EEG patterns with a known anatomic generator, the reticular nucleus of the thalamus. Therefore, rhythmic activities, such as sleep spindles, may disappear as the results of lesions involving either thalamic or cortical structures. Sleep spindles have been defined as a relatively sinusoidal “11-15 Hz burst,” but mostly at 12 to 14 Hz, occurring during quiet sleep and appearing normally around the age of 8 weeks postterm.

In our study, sleep spindles were observed in all of the babies at 12 weeks postterm. Spindle patterns developed during quiet sleep in the first 3 months of infancy, possibly reflecting developmental changes in thalamocortical structures and myelinization and growth of dendrites. Spindle development during the first year of life might thus serve as an indicator of normal and abnormal central nervous system function and also, for some authors, as an electrophysiologic measurement of brain matura-
Abnormalities of sleep-spindle development have been described in several disorders, such as phenylketonuria, hypothyroidism, and trisomy 21. Absence of or a decrease in sleep spindles has been observed in mentally retarded children. However, the effects of hyperbilirubinemia on sleep-spindle maturation have not yet been described.

Many studies have been done on the development of sleep spindles in the first year of life in healthy infants. Previously indicated that, at 12 weeks, spindles were “fully developed.” Louis et al. called the third month the “turning point in the maturation process” and found that the longest sleep spindles appear at 1.5 and 3 months. In our study, we also examined sleep spindles at the 12th week of age.

Tanguay has reported that mean spindle frequency is 13.6 Hz in the healthy infants at the fourth month of life. Hughes and have also found that mean spindle duration is 5 seconds at the 10th week. Our results in the healthy infants are similar to the results of Tanguay and Hughes. Ellingson has reported that only half of sleep spindles are synchronous and symmetrical at the third month. However, Louis and et al. have found that the percentages of asynchrony and asymmetry were 25% and 4%, respectively, at the third month. Similar to these findings, we found that the percentages of asynchrony and asymmetry were 33% and 2.6%, respectively, in the healthy infants.

We detected that densities and durations of spindles were lower and frequency was slower in the hyperbilirubinemia group. The percentage of asynchronous spindles was also higher in the hyperbilirubinemia group. These findings show that hyperbilirubinemia affects the maturation of the thalamocortical region. This study supports our previous results, which show that hyperbilirubinemia affects the rhythm of the EEG. Although infants with hyperbilirubinemia are clinically well, their progression of brain maturation should be followed.

In conclusion, studies on sleep-spindle patterns during the critical maturation period—the first trimester of life—might provide a sensitive tool for the early diagnosis of neurophysiologic brain alterations in a population of at-risk children, such as jaundiced infants. We suggest that the sleep-spindle differences may be a surrogate neurophysiologic marker of the effects of bilirubin on the brain and that further research is needed to determine the respective predictive value of the peak serum bilirubin, associated changes in neurophysiologic measures, and long-term neurodevelopmental outcome.

REFERENCES

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