Assessment of brain oxygenation in term and preterm neonates using near infrared spectroscopy

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ABSTRACT

**Purpose:** The aim of this study was to determine brain oxygenation in full-term and preterm neonates using near infrared spectroscopy.

**Material and methods:** A total of 88 full-term and preterm newborn infants without hypoxic-ischaemic disorders admitted to the NICU were examined using NIRS on the first day of life and on day 28 of life. Additional measurements were taken at the end of the first week of life in the premature neonates group. Measurements of oxyhaemoglobin (HbO2), deoxyhaemoglobin (Hb), total haemoglobin (HbT) concentration and tissue oxygen saturation (Ox) were performed in 5 brain regions. Right and left frontal areas, the occipital area and right and left temporal areas were measured.

**Results:** In full-term healthy neonates a marked decrease in HbO2, Hb and HbT values was observed on day 28 of life in all brain regions except the occipital area. In the neonatal period the greatest changes in brain oxygenation occurred in the right and left frontal regions of the brain. In preterm neonates constant values of HbO2 and Ox were observed in the first 28 days of life. In preterm newborn infants, as well as in full term newborn infants, similar Ox and HbO2 values were obtained on day 28 of life.

**Conclusions:** NIRS is a safe method and can be used to evaluate brain oxygenation in newborn infants. The results of these measurements are in accordance with changes in brain oxygenation in the first month of life, which are predicated on the basis of the neonate's physiology.

**Key words:** near infrared spectroscopy, central nervous system, hypoxic-ischaemic injury, neonates

Introduction

Intrauterine hypoxia still remains a leading perinatal problem. It concerns full-term as well as pre-term newborn infants. In both groups it is the main cause of cerebral palsy in children [1]. The numerous methods of evaluating the state of a fetus or neonate, widely used in obstetrics and neonatology, such as examination of acid-base balance, fetal cardiac activity, fetal and newborn infant pulsoxymetry and Doppler ultrasonography, still do not give full information about cerebral perfusion changes, and above all, about the level of oxygen in the brain [2]. Measuring cell metabolism is most important in evaluating the influence of hypoxic injures on brain cells and, at the same time, for prognosticating the future development of infants. This is done by assessing the biochemical markers of brain damage, evaluating the density of highly energetic elements of neurons and, above all, by assessing the level of oxygen [3].

New possibilities in this field have been created by Near Infrared Spectroscopy (NIRS), a method using easy penetration of light waves through the skin and skull into the neonate’s brain, which allows for non-invasive examination of the cerebral haemodynamic condition [4,5]. In order to
examine tissue properties, NIRS uses laser light from a near infrared range of 700-1000 nm. In this range of wavelengths there is an absorption ‘window’ allowing for deeper light penetration with relatively low power lasers, and thus for the performance of diagnostic measurements. A great advantage of this method is the fact that it allows for real-time monitoring and has no side-effects. The radiation doses are very small and they do not cause any skin or intra-tissue changes. The examinations are carried out without influencing tissue temperature. The near infrared spectroscopy method opens a wide range of diagnostic possibilities [6]. This technique allows even tiny local changes in tissue oxygenation to be clearly identified, which, from the medical point of view, is very important mainly in all ischaemic states and in tissue oxygenation deficits.

In our study, frequency-domain spectroscopy was used. In contrast to the very simple continuous wave method, in which only changes of light attenuation are measured, the frequency-domain technique allows for estimation of the path length of light in the tissue under investigation and, in consequence, for analysis of absolute concentrations of oxy- and deoxyhaemoglobin. In frequency-domain NIRS the phase shift between intensity modulated optical waves emitted into the tissue and reemitted on the surface after propagation in the tissue is estimated [7]. This phase shift is directly related to the mean time of light of photons and the optical path length of reemitted photons. These quantities can be assessed in the laboratory by time-resolved NIRS, which is based on emission of short picosecond light pulses and analysis of the broadening of the pulse during its journey between the points of emission and detection [8,9]. Thus, the frequency-domain technique is a method which allows for estimation of the path length of light but it does not involve complicated laboratory equipment and can be applied at the bedside in a clinical environment.

There is very little data regarding the use of NIRS in sick newborn infants [10-12]. Most of the research done in this area is based on monitoring oxygenated and deoxygenated haemoglobin concentration in response to a standard stimulus of brain regions [13]. Interesting studies have been carried out regarding auditory brainstem response based on the hearing screening test in neonates [14,15], cortical neuronal activity response to speech processing [16], evaluation changes in regional cerebral haemodynamics caused by pain [17], evaluation of the sensorimotor cortex of newborn infants during passive knee movement under sedated sleep. [18] and function of the visual cortex during photic stimulation under the condition of natural sleep [15,19]. Our research on full-term healthy and preterm neonates without intrauterine hypoxia indices provides referential values for both groups and may be a basis for further research performed on newborn infants with different clinical disorders.

Materials and methods

Subjects
Studies were carried out in the Neonatal Department of Warsaw Medical University in 2006-2007. Eighty-eight newborn infants were examined. In the first group 42 newborn infants were selected from normal full-term healthy neonates (gestational age 38-42 weeks, mean birth weight 3359 g, min. 2560, max. 4700 g) delivered either by spontaneous vertex delivery or by elective caesarean section. Eighteen were males, twenty-four were females. They had normal arterial umbilical cord blood gases at delivery, mean pH 7.34 (min. 7.25, max. 7.39) and mean base deficit BE of 2.27 (min.-6, max.+0.7). There were no pregnancy or birth complications.

The second study group consisted of 46 premature neonates of mean gestational age 31 weeks (min. 22, max. 36 weeks g.) and mean birth weight 1670 g (min. 430, max. 3130 g). Twenty-six were male, twenty were female. All premature infants required mechanical ventilation or supplemental oxygen delivery. Arterial umbilical cord blood gases with a normal range and mean pH of 7.34 (min. 7.17, max. 7.47) and mean base deficit BE of -2.51 (min. -11.20, max. +5.30).

Neonates with congenital malformations affecting brain circulation, severe brain damage, seizures, or the cardiovascular system were excluded from this study. Characteristics of both study groups are presented in Table 1. Concentration of haemoglobin was estimated in capillary blood samples obtained after delivery.

Instrumentation
Brain oxygenation was measured using frequency domain near infrared spectroscopy. The dual Channel OxiPlex TS; 06208 model (ISS Inc., Champaign, IL, USA) was used. According to this technique light penetrates brain tissue

<table>
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<th>Table 1. Characteristics of study groups – mean (min.-max.)</th>
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<tr>
<td><strong>Healthy term group</strong></td>
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<tr>
<td>Number of patients</td>
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<tr>
<td>Gestational age (wks)</td>
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<td>Birth weight (g)</td>
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<td>Haemoglobin (g%)</td>
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and is modulated at a frequency of 110 MHz. After passing through the tissue the amplitude and phase change depending on absorption and scattering of light and optical path length. Measurement of amplitude of reemitted light and phase shift at several source-detector separations allows for estimation of the absorption coefficient of the tissue [20-22]. Using spectral properties of oxy- and deoxyhaemoglobin, the absorption coefficients are measured at two wavelengths, and this allows for the concentration of chromophores to be derived. [23].

The instrument is equipped with two class 3b laser diodes operating at wavelengths of 690 nm and 830 nm. A standard optode holder provided by the manufacturer with four different source-detector separations was used. The power of laser light on the surface of the tissue is lower than 1mW, which makes the measurement safe and assures that the light does not cause any thermal stimulation of the tissue. The optode holder was made from flexible and soft rubber and consisted of four emitting fibres and one detecting fibre bundle. The distances between emitting and detecting fibres were 1.90 cm, 2.37 cm, 2.91 cm and 3.40 cm, as shown in Fig. 1. The optical probes were calibrated prior to measurements on the neonates using a phantom of known optical properties. Measurements were carried out at sampling frequency of 4 Hz. The measurement signals of oxy- and deoxyhaemoglobin concentration received were used to derive total haemoglobin concentration and tissue oxygen saturation measurements defined as a ratio of oxyhaemoglobin and total haemoglobin concentration.

**Examination Protocol**

Measurements of (HbO$_2$) and (Hb) concentrations were performed in 5 brain regions, as shown in Fig. 2. The optode was placed in the right and left frontal area at the site of the frontal eminence; in the area of the right and left temple directly above the auricle and in the occipital area in the midline. Measurements in term neonates were performed in a separate quiet room and in preterm neonates in their incubators. To assess reproducibility, data was acquired more than once during the same measurement for the majority of neonates. Measurement sites were selected to ensure areas with homogenous skin (e.g. sites with no hair) in order to provide for unimpaired measurement. It must be stressed that the optical fibres were arranged in a row in a flexible black rubber optode, which lay flush with the skin. For all locations HbT concentrations and Ox value were also calculated.

Right (A) and left (B) frontal areas, occipital area (E) and right (C) and left (D) temporal areas were measured (Fig.2). During each measurement the optical probe was put in contact with the head for 15 seconds at each of the 5 locations. In both groups the first measurement was taken as soon as possible after birth (in the first day of life). The second measurement was taken at the end of the first week of life in 25 out of 46 premature neonates (54% of total), and at the end of the neonatal period (day 28 of life) in 14 of 42 healthy newborn infants (34% of total). The third measurement was taken in 23 out of 46 premature neonates on day 28 of life (50% of total).

The study was approved by the Ethics Committee of Warsaw Medical University (approval number KB/67/2006). Mothers of all newborn infants included in the study were informed about the methods and the aim of the experiment and their consent was obtained before measurements were carried out.

**Data analysis**

For all measurement positions on the head the measured HbO$_2$, Hb, HbT and Ox signals were averaged in 15 s recording windows, and obtained values were taken for further data analysis.

The preliminary analysis involved, for interval-scaled variables, the calculations of the means, the standard deviations and other simple statistics; for nominal variables, contingency tables measures and the association statistics. To identify significant risk drivers for brain oxygenation the effects of different factors were tested by fitting Generalized Linear Mixed Models (GLMM). Significance level for extraction acceptable factors was set to p-value<0.05. The Student’s T test was used for normally distributed data. Statistical analysis was performed using the SAS/9.2 statistical package.

**Figure 1.** ISS Optiplex TS optode holder consisting of four emitting fibres (E) and one detecting fibre bundle(D) fixed in a flexible soft rubber.

**Figure 2.** Optical probe placement: A - right frontal area, B - left frontal area, C - right temporal area, D - left temporal area, E - occipital area.
**Results**

**Full-term healthy neonates**

The highest values of (HbO$_2$) and (Hb) concentration were noted in the occipital region and the lowest in the frontal regions (Fig. 3 and Fig. 4). HbO$_2$ concentration recorded after birth was significantly higher than that at the end of the neonatal period only in frontal regions (Fig. 2: A p < 0.02 and B p < 0.001). On the other hand, there was a significant decrease in Hb concentration in each region during the neonatal period.

In the healthy infants group (Fig. 5), the total haemoglobin concentration (HbT) was approximately 40 μM for both frontal regions in the first day of life. At the same time, higher values of HbT (approximately 50 μM) were noted in both temporal regions. HbT was highest in the occipital region, as expected (67 μM). Because concentrations of HbO$_2$ and Hb in brain tissue decreased, we also observed a significant decrease in mean HbT values during the neonatal period (between the first and second measurement) in all regions except occipital region E.

Summing up results observed in the neonatal period, a marked decrease in HbO$_2$, Hb and HbT values in all brain regions was noted. As presented in Fig. 6 only slight changes in Ox were observed in the neonatal period. The Ox changes in A, C, and E. regions in both measurements taken on day 1 and day 28 of life were not statistically significant (Ox1 38-51%, Ox2 33-56% NS). There was, however, a significant difference in Ox values in regions B and D immediately after birth and at the end of the neonatal period (Ox B1-B2 < 0.02, D1-D2 < 0.002)

**Premature neonates**

In the group of premature neonates, as in the full-term healthy group, a distinct decrease in HbO and Hb concentrations was observed, as shown in Fig. 7 and Fig. 8. However, no statistically significant differences were found between the values of HbO$_2$ obtained in the next 3 measurements. On the other hand, we observed a significant decrease in Hb during consecutive three measurements at each location.

Statistical differences in HbT concentration were noted between the measurements taken on the first day of life and taken on day 28 of life in all regions. In contrast, the differences between values obtained on day 1 and day 7...
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were not statistically significant. In premature neonates HbT values were lower than in full–term newborn infants (30-49 μM, p<0.05) in the first measurement and (21-30 μM, p<0.05) on day 28 of life (Fig. 9).

In the group of premature neonates, as in the full-term healthy group, no significant statistical differences were found between the values of Ox obtained in 3 consecutive measurements. In this study group, mean Ox values were 31 - 41% on the first day of life, 32 - 42% on day 7, and 31 - 43% on day 28 (Fig. 10). The highest mean value of oxygen saturation Ox was noted in occipital location E and the lowest values in both frontal areas A and B.

Comparison of results for full-term and premature neonates.

In a comparison of the two study groups, no significant statistical differences were found between Ox values obtained on the first day of life in region A, B and D, whereas some statistically significant differences were noted in the regions C and E ( p<0.05 for C; p=0.02 for E). In all locations some statistically significant differences were also noted in haemoglobin concentration: HbT, Hb and HbO₂ (p < 0.05). Both study groups were compared at the end of the neonatal period (on day 28 of life). No statistically significant differences were found in HbO₂ concentration and Ox values in locations A and B. In contrast, statistical differences in Hb, HbO₂ and HbT concentrations and Ox values were noted for all other locations: C, D and E.

Discussion

Monitoring of brain function in newborn infants should be carried out using a portable non-invasive instrument which does not interfere with other diagnostic and therapeutic equipment in NICUs. Near infrared spectroscopy is used to make observations at the bedside and allows for assessment of intracerebral concentration of the chromophores: oxyhemoglobin (HbO₂) and deoxyhaemoglobin (Hb) as well as total haemoglobin (HbT) and oxidised cytochrome. It provides information about cerebral metabolism by measuring oxygen consumption. This method uses non-ionising radiation of very low energy and therefore it is non-invasive. The technique is based on analysis of absorption of near infrared light by brain tissue at wavelengths of 600 to 1,000 nm [24,25]. Most of the research done in this area is based
on monitoring oxygenated and deoxygenated haemoglobin concentration in response to a standard stimulus of brain regions [13]. There are also studies on brain monitoring of sheep fetuses using NIRS after clamping the umbilical cord. An increase in tolerance to hypoxia in repeated episodes of umbilical cord clamping was shown. In human fetuses with no fetal distress constant fluctuation of brain oxygenation proportional to the intensification of contractions of the uterus, can be observed [26]. Stimulation of the CNS by appropriate stimuli leads to changes in the circulatory system eg. by an increase in cardiac output, as well as in the cellular metabolism of appropriate regions of the brain. An increase in cardiac output causes a rise in cerebral blood volume (CBV), which is reflected by HbT concentration values. Together with an increase in HbT a rise in both HbO2 and Hb concentration can most often be observed. A greater increase in average Hb concentration than in HbO2 concentration indicates a rise in oxygen consumption as a result brain tissue activity.

Research on quantitative monitoring of infant brain development by frequency-domain infrared spectroscopy (FD-NIRS) was carried out by Franceschini et al. [10] in 47 infants in the first year of life. The average gestational age of the neonates examined was 37.9 weeks. The results were divided into those obtained in the first 6 weeks of life and those obtained after the neonatal period. Our investigations were mainly focused on measuring brain oxygenation in the neonatal period, ie up to day 28 of life. Franceschini et al. observed a significant decrease in HbT and S02 in all regions measured. Their results are similar to ours. In our research we also showed differences between particular brain regions investigated Hinz et al. [27] described the use of the NIRS method in several neonates of 32-33 weeks gestation. The Roche-Larabe study, based on simultaneous NIRS and EEG recordings, provides evidence that spontaneous physiological neuronal activity in premature infants is coupled with a transient haemodynamic pattern, consisting of deoxygenation followed by strong oxygenation [28]. In our study, we investigated both healthy and premature neonates, in order to observe the changes in brain oxygenation. In full-term neonates immediately after birth we observed an average total hemoglobin concentration HbT of 40 µM for both frontal regions. These results were similar to the ones obtained by other investigators [10]. In our investigations, the values obtained in frontal regions were the lowest. In contrast, HbT concentration was highest in the occipital region (67µm), as expected. The biggest vein in the brain - Galen's vein, is located in this region. That is why we found the highest concentration of HbT and the fewest statistically non-significant changes in HbO2, Hb and HbT in this location during the neonatal period. In the remaining regions of the brain we observed a decrease in HbO2, Hb and HbT concentration during the neonatal period. The most evident decrease in HbO2 concentration was observed in frontal regions. This was caused by a decrease in HbT concentration but also reflected the decrease in regional CBV in this region. In literature investigations of the brain using SPECT and FDG-PET also confirmed low cortical blood flow, low synaptic activity and low cortical glucose uptake in the first 4-6 weeks of life [29,30]. In our study HbO2 decreased by 26% in the right frontal regions and by 58% in the left frontal region on day 28 of life, as compared with values obtained on the first day of life. These changes may have been caused by two factors. Firstly, regression of the ‘physiological brain sparing effects’, which is characterized by an increase in CBV in the fetus towards the end of pregnancy, and the extension of this effect in the first day of life. During regression of this effect CBV decreases and reaches a plateau after day 4 of life [2,10]. This is caused by a global increase in oxygen in the organism. The second factor, also described by Franceschini et al. [10], is connected with the physiological transition from fetal to adult life. A considerable decrease in haematocrit (40%) can be observed during the neonatal period. Our investigations showed that the decrease in HbT concentration was most evident in frontal regions. A decrease of 21% was obtained in the right frontal region and a decrease of 43% in the left frontal region on day 28 of life.

Analysis of brain tissue oxygen saturation did not show any significant changes in the occipital region and right hemisphere during the neonatal period. However, we observed significant fluctuations in Ox values in the left hemisphere during the neonatal period. In the frontal region, Ox value decreased by 27%, while in the left temporal region it increased by 11%. Differences in Ox value obtained for frontal and temporal lobes may be due to the insufficient number of patients and the insufficient number of measurements taken. This undoubtedly limits our investigation. Differences between the right and left hemispheres indicate a lateralisation in the activity of brain hemispheres. Other researchers also report that lateralization of brain oxygenation occurs in this period of life [15,16,31,32].

In preterm neonates we observed constant HbO2 concentration and constant Ox values in the first 28 days of life. This may indicate a constant CBF in spite of a considerable decrease in HbT concentration on day 28 of life. In the frontal and temporal regions HbT concentration ranged from 31%-39% on day 28. The greatest HbT decrease (47%), in preterm newborn infants, as compared with full-term neonates, was observed in occipital region. We observed that the statistically significant decrease in Hb concentration was considerably greater than the decrease in HbO2 concentration, which confirms low oxygen consumption. This effect reflects low metabolic tissue activity. Constant HbO2 concentration and constant Ox values were observed in neonates requiring mechanical ventilation oxygen therapy and circulatory support. These results suggest that this type of therapy is effective. On account of prematurity anaemia, we observed differences in HbO2, Hb and HbT concentrations in preterm and full term neonates immediately after birth. In contrast
oxygen saturation Ox values were similar in both groups. Average HbT concentration in preterm neonates was 22% lower than average HbT in full term neonates.

In preterm newborn infants, as well as in full term neonates, similar Ox values and HbO₂ concentration obtained on day 28 of life indicate similar metabolic brain activity and CBF. However, differences in HbT and Hb were noted between the two groups. The advantage of our study is that it concerns a group of neonates without hypoxic-ischaemic indicators immediately after birth. That is why a comparison between these two groups is possible.

A comparison between full-term newborn infants and preterm neonates in week 40 after conception would be useful in the future. In our study only a few preterm neonates reached this age on day 28 of life. It would be valuable for future investigators to assess changes in brain oxygenation in preterm neonates depending on gestational age.

CONCLUSIONS

The results of our study on preterm and full-term healthy neonates show a significant decrease in HbO₂, Hb and HbT values in all brain regions except the occipital region during the neonatal period. The greatest changes in brain oxygenation occurred in the right and left frontal regions of the brain.

In our opinion NIRS is a safe method and can be used not only to evaluate brain oxygenation in newborn infants with brain injury but also to monitor the effectiveness of oxygen therapy. However, further investigations in this area needed.

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