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Carboxyhaemoglobin levels in infants with hypoxic ischaemic encephalopathy

Short title: COHb in HIE

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ABSTRACT

Objectives: Hypoxic ischaemic encephalopathy (HIE) is associated with oxidative stress. A potential marker of oxidative damage is carboxyhaemoglobin (COHb) which is the product of the reaction between carbon monoxide and hemoglobin and is routinely assessed on blood gas analysis. Our objective was to test the hypothesis that higher COHb levels would be associated with worse outcomes in infants treated for HIE.

Methods: A retrospective, observational study was performed of all infants who received whole body hypothermia for HIE at the tertiary neonatal intensive care unit at King's College Hospital NHS Foundation Trust, London, UK between January 2018 and August 2021. For each participating infant, the highest COHb level per day was recorded for days one, three and five after birth.

Results: During the study period, 67 infants with a median (IQR) gestational age of 40 (38-41) weeks underwent therapeutic hypothermia for HIE. The median (IQR) COHb level on day three was higher in infants without electroencephalographic seizures [1.4 (1.1-1.4) %] compared with infants with seizures [1.1 (0.9-1.3) %, $p=0.024$]. The median (IQR) COHb on day five was higher in infants without MRI brain abnormalities [1.4 (1.2-1.7) %] compared with infants with MRI abnormalities [1.2 (1.0-1.4) %, $p=0.032$]. The COHb level was not significantly different between the nine infants who died compared to the infants who survived.

Conclusions: COHb levels were higher in infants with HIE without seizures and in those with normal MRI brain examinations. We suggest that carbon monoxide has a potential protective role in HIE.

Key words: oxidation, neonates, seizures, MRI brain

INTRODUCTION

Hypoxic Ischaemic Encephalopathy (HIE) is a serious birth complication resulting from impaired cerebral blood flow and oxygen delivery in term infants [1]. Treatment response and outcome prediction are measured using a combination of the results of clinical examination, amplitude integrated electroencephalography (aEEG) and magnetic resonance imaging (MRI) of the brain [2]. The severity of the clinical picture, however, can be subjective depending on the assessor and the interpretation of aEEG can be affected by hypothermia treatment or anti-epileptic medication, while some infants are too unstable for transport to an MRI scanner [3]. As a result, the early predictive abilities of those modalities can be hindered.

The pathophysiology of HIE involves oxidative stress which is most marked during the secondary phase of injury occurring 6 to 15 hours after the initial injury, potentially as a result of reperfusion injury [4]. By-products of the oxidative processes may act as useful biomarkers in these infants, providing an additional non-invasive means of risk stratifying neonates with HIE [5]. A potential marker of oxidative stress in the neonatal population is carboxyhaemoglobin (COHb) [6] which is the product of the reaction between carbon monoxide and hemoglobin. COHb levels are available as part of routine blood gas analysis. Carboxyhaemoglobin levels have previously been shown to correlate with the length of invasive ventilation in paediatric intensive care [7] and the development of bronchopulmonary dysplasia and intraventricular haemorrhage in preterm infants [8]. We hypothesised that infants with severe HIE, that is those with abnormal MRI brain findings and/or who had clinical or electrical seizures, would have elevated COHb levels during the first days after birth compared to those with less severe HIE.

METHODS

Study design and subjects

A retrospective, observational study of all infants who received whole body hypothermia for HIE at the tertiary neonatal intensive care unit (NICU) at King's College Hospital (KCH) NHS Foundation Trust, London, UK between January 2018 and August 2021 was undertaken. Infants were identified via the BadgerNet Neonatal Electronic Patient Record (Clevermed, Edinburgh, UK). Infants with haemolytic jaundice and a positive direct antiglobulin test were excluded from the study as carbon monoxide is a by-product of heme degradation and is associated with increased COHb [9]. Infants were included if they had at least five arterial blood gases in the first three days after birth. The study was registered with the Clinical Governance Department of KCH. The Health Research Authority Toolkit of the National Health System, United Kingdom confirmed that the study would not need regulatory approval by a research ethics committee.

Information from the medical notes

The following information was collected from the medical notes: meconium stained amniotic fluid, emergency caesarean section, sex, gestational age (weeks), birth weight (kg), Apgar score at 1, 5 and 10 minutes, arterial cord blood pH, base deficit and lactate. Additionally, the highest blood aspartate aminotransferase (AST) and creatinine levels in the first three days, the duration of invasive ventilation (days), clinical or echocardiographic evidence of pulmonary hypertension [10], use of inotropic agents [11], electrical or clinical seizures [12], MRI abnormalities consistent with HIE [13] and survival to discharge from neonatal care were recorded. All infants

that received whole body hypothermia for HIE had a MRI brain before discharge from neonatal care, beyond the pseudo-normalisation window at the end of the first week of life [14].

Blood gas analysis

Arterial or capillary blood gas analysis was performed by the medical or nursing team as part of routine clinical care. Samples were analysed using the ABL90 FLEX PLUS analyser (Radiometer UK Ltd) and results were stored in the archived database on the blood gas analyser hard disk. All samples stored in the designated study period were extracted from the archived database for analysis of the COHb levels. COHb results were expressed as a fraction (%) of the total haemoglobin concentration. For each participating infant, the highest COHb level per day was recorded for days one, three and five after birth. This time period was selected as we have previously demonstrated that in the first month of life, the COHb levels were highest during the first three days and that during that period there was the greatest difference in levels between infants who developed oxidative diseases and those that did not [8]. Furthermore, the majority of infants did not have regular blood gas monitoring after day six.

Clinical management

Active whole body hypothermia was initiated within six hours from birth either at KCH or by the dedicated neonatal transport team attending the referring hospital and maintained for 72 hours. Assisted ventilation was initiated due to poor condition at

birth and maintained if there was an absence of adequate respiratory drive and the administration of sedative and anticonvulsive agents. The fraction of inspired oxygen was manually adjusted to achieve oxygen saturation levels measured by pulse oximetry (SpO₂) of 95–100% [15] and ventilation settings were adjusted to achieve a partial pressure of carbon dioxide of 4.5 kPa to 6.5 kPa [16].

Statistical analysis

Continuous data were tested for normality with the Kolmogorov-Smirnov test and found to be non-normally distributed and thus presented as median and interquartile range (IQR) and the Mann Whitney U test used to determine if differences were statistically significant. The strength of relationships between continuous variables was examined using Spearman's Rho non parametric bivariate correlation analysis. Statistical analysis was performed using SPSS software, version 27.0 (SPSS Inc, Chicago, Illinois, USA).

RESULTS

During the study period, 67 infants (44 male) underwent therapeutic hypothermia for HIE. Their median (IQR) gestational age was 40 (38-41) weeks and birth weight was 3.39 (2.08-3.66) kg (Table 1).

Seizures (electrographic or clinical) were reported in 33/66 (50%). MRI features consistent with HIE were present in 20/62 (30%). Nine infants (13%) died from HIE before discharge from neonatal care.

The median (IQR) COHb level on day three was higher in infants without EEG confirmed seizures compared to in infants with seizures ($p=0.024$, Table 2). The

median (IQR) COHb on day five was higher in infants without MRI abnormalities compared to in infants with MRI abnormalities ($p=0.032$, Table 2). The COHb levels were not significantly different between the nine infants who died compared to those who survived.

The COHb levels on any study day were not significantly different between infants with or without meconium stained amniotic fluid or pulmonary hypertension or who need inotrope support (Supplementary Table 1). The COHb levels on any of the study days did not significantly correlate with arterial cord blood pH, base deficit, lactate, AST or creatinine levels (Supplementary Table 2).

DISCUSSION

We have demonstrated that COHb levels were higher on day three in infants without seizures compared to infants with seizures and higher on day five in infants with normal MRI brain examinations compared to infants with abnormal MRI scans.

These findings do not support our hypothesis that infants with severe HIE would have higher COHb levels during the first days after birth compared to those with less severe HIE. It was interesting that on all three study days better outcomes (survival, no seizures, normal MRI examination) were associated with higher COHb levels compared to the unfavourable outcomes (death, seizures, abnormal MRI). We postulate that the consistency of these finding suggests that elevated COHb levels have a different mechanism in HIE than in our initial hypothesis.

Whilst carbon monoxide is known to be a toxic agent, recent evidence has suggested that when given at low doses it might have neuroprotective properties [17-19]. Elevated carboxyhaemoglobin levels may thus represent an increased

endogenous neuroprotective production of carbon monoxide in response to the hypoxic-ischemic insult. This effect might be mediated by the up-regulation of enzymes responsible for the heme catabolism which produce carbon monoxide as a by-product. Indeed, animal studies have highlighted the protective effect of carbon monoxide supplementation in ameliorating hepatobiliary dysfunction during catabolism of heme molecules in endotoxemic livers [20]. Furthermore, exogenous carbon monoxide has also been shown to have anti-inflammatory, antiapoptotic and cytoprotective effects in animal studies [18, 21]. In an animal study where mice were exposed to carbon monoxide at a concentration of either 200 or 250 ppm for a period of one hour after an hypoxic insult, it was demonstrated that 250 ppm of carbon monoxide were effective in preserving cortical volumes following a mild hypoxic-ischemic injury [3]. In further animal studies, Liu et al also showed that carbon monoxide reperfusion relieved oxidative injury and inhibited apoptosis and autophagy [22]. Our results then are interesting in pointing towards carbon monoxide not as an index of oxidative stress, but as a potential neuroprotective pathway that could be further explored as highlighted in the aforementioned animal studies. It is interesting to note that there were no significant correlations between cord gas variables (pH, BE, lactate), AST, platelets and creatinine levels and COHb levels on any of the study days. Those observations also highlight that COHb may be of limited clinical utility as a marker of oxidative stress in HIE.

This is the first human study pointing to a possible protective role of carbon monoxide in hypoxic ischemic injury. A limitation to our study is the lack of control samples, however, COHb levels would not be measured serially in infants without any pathology. In our cohort we also report a relatively low mortality rate so we are underpowered to report significant differences according to this outcome. The

mortality rate, however, is not dissimilar to those in previously published national reports [23].

CONCLUSION

In conclusion higher carboxyhaemoglobin levels were associated with normal MRI brain findings and the absence of electrical seizures in infants with HIE. Those findings are consistent with a possible cytoprotective effect of carbon monoxide. Trials are warranted to investigate the potential therapeutic effects of carbon monoxide in the context of HIE.

ACKNOWLEDGEMENTS

Ethic statement: The study was registered with the Clinical Governance Department of KCH. The Health Research Authority Toolkit of the National Health System, United Kingdom confirmed that the study would not need regulatory approval by a research ethics committee.

Informed consent: Not applicable

Author contributions: AJ: data collection, analysis and writing the first draft of the manuscript. SZ: data collection, revision of the manuscript. RB: data analysis and interpretation, revision of the manuscript. AG: conceptualisation, supervision, critical revision. TD: conceptualisation, supervision, analysis, critical revision.

Competing interests: None to declare.

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Data sharing statement: Data will be made available on reasonable request.

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Table 1: Demographics and clinical characteristics. Data presented as median (IQR).

Gestational Age (weeks)	40 (38-41)
Birthweight (kg)	3.39 (2.08-3.66)
Male	44/67 (65%)
Inborn	42/67 (62.7%)
Emergency LSCS	29/66 (43%)
Apgar at 1 min	2 (0-3)
Apgar at 5 min	4 (2-6)
Apgar at 10 min	6 (4-8)
pH cord gas	7.0 (6.9-7.2)
BE cord gas	-11.9 (-16.7 - -7.1)
Lactate cord gas (mmol/L)	10 (6-14)
Meconium stained amniotic fluid	23 (34%)
Pulmonary hypertension	9 (13%)
Need for inotropic support	27/67 (40%)

LSCS = lower segment caesarean section; BE = base excess]

Table 2: COHB levels according to presence of seizures and MRI brain results. Data are presented as median (IQR).

	No seizures	Seizures	P value	Normal MRI	Abnormal MRI	P value
COHb Day 1	1.4 [1.1-1.6]	1.3 [1.0-1.5]	0.534	1.4 [1.1-1.5]	1.45 [1.1-1.6]	0.360
COHb Day 3	1.4 [1.1-1.4]	1.1 [0.9-1.3]	0.024	1.3 [1.0-1.4]	1.2 [1-1.5]	0.946
COHb Day 5	1.4 [1.2-1.7]	1.4 [1.1-1.5]	0.391	1.4 [1.2-1.7]	1.2 [1.0-1.5]	0.032

Supplementary Table 1: Comparisons of COHb in infants with and without meconium stained amniotic fluid, pulmonary hypertension and inotropic support. The p values are presented.

	COHb - Day 1	COHb - Day 3	COHb - Day 5
Meconium stained amniotic fluid	0.603	0.473	0.886
Pulmonary hypertension	0.625	0.230	0.081
Inotropic support	0.857	0.176	0.427

Supplementary Table 2: Correlations of COHb with biochemical and haematological parameters. The p values are presented

	pH cord	BE cord	Lactate cord	AST	Creatinine	Platelets
COHb - Day 1	0.220	0.243	0.059	0.267	0.821	0.795
COHb - Day 3	0.872	0.485	0.184	0.412	0.112	0.667
COHb - Day 5	0.537	0.871	0.640	0.622	0.091	0.149
