Early Human Development 91 (2015) 535–540

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

# Serum calcium concentrations and incidence of hypocalcemia in infants with moderate or severe hypoxic-ischemic encephalopathy: Effect of therapeutic hypothermia

Chatchay Prempunpong [a], Ionut Efanov [b], Guilherme Sant'Anna [b,*]

[a] Ramathibodi Hospital, Mahidol University, Bangkok, Thailand
[b] McGill University Health Center, Montreal Children's Hospital, Montreal, Canada

ARTICLE INFO

*Article history:*
Received 23 February 2015
Received in revised form 20 April 2015
Accepted 22 June 2015

*Keywords:*
Hypoxic-ischemic encephalopathy
Therapeutic hypothermia
Hypocalcemia
Calcium

ABSTRACT

*Background:* Hypocalcemia is a common morbidity in asphyxiated infants. Therapeutic hypothermia (TH), the standard of care for infants with moderate and severe hypoxic-ischemic encephalopathy (HIE), promotes neuroprotection by several mechanisms including a decrease in intracellular calcium ($Ca^{2+}$) influx which may improve serum $Ca^{2+}$ levels and homeostasis.
*Aims:* To evaluate the impact of TH on $Ca^{2+}$ homeostasis.
*Study design:* Historical, retrospective cohort analysis.
*Subjects:* Infants with moderate or severe HIE admitted to the hospital with ≤ 24 hours of age, gestational age ≥ 36 weeks, and birth weight ≥1800 g, before (pre-TH) and after (post-TH) TH was implemented.
*Outcome measures:* Minimum and maximum serum levels of ionized $Ca^{2+}$ ($iCa^{2+}$) and magnesium (Mg), $Ca^{2+}$ and Mg intakes, and incidence of hypo/hypercalcemia during the first week of life.
*Results:* A total of 67 infants were included: 29 pre-TH and 38 post-TH. Minimum $iCa^{2+}$levels were significantly lower in the pre-TH group; some infants required $Ca^{2+}$ boluses infusions. In the post-TH group, a significantly lower intake of $Ca^{2+}$ was necessary to maintain normal $Ca^{2+}$ levels and no infant required boluses. The incidence of hypocalcemia was higher in the pre-TH group with a statistically significant difference on day 2 of life (18 vs 0%; $p = 0.01$).
*Conclusions:* After the implementation of TH, $iCa^{2+}$ levels were within normal ranges despite lower $Ca^{2+}$ intakes. A lower incidence of hypocalcemia was observed during cooling. Our findings support the hypothesis that TH improves $Ca^{2+}$ homeostasis in HIE infants.



## 1. Introduction

Hypocalcemia is a common morbidity in infants with perinatal asphyxia [1–4]. A rapid influx of calcium ($Ca^{2+}$) into the cell, secondary to ATP-dependent $Na^{+}$–$K^{+}$ pump failure and membrane depolarization, has been reported as the major cause of either cell necrosis or apoptosis during the reperfusion phase following a hypoxic-ischemic insult [5]. *In vitro* studies have shown a simultaneous decrease in extracellular $Ca^{2+}$ during both types of cell death in multiple organs such as brain, heart, kidneys, and liver [6–10]. Therefore, this widespread influx of $Ca^{2+}$ into the cells of multiple damaged organs might lower the serum $Ca^{2+}$ concentration [2].

Based on the aforementioned pathways of cell dysfunction and death, several approaches toward neuroprotection have been investigated. In neonates, the use of therapeutic hypothermia (TH) became the standard of care for infants with moderate or severe hypoxic-ischemic encephalopathy (HIE) due to its beneficial effect of increasing survival, free of disability [11]. Several mechanisms by which TH promotes neuroprotection have been postulated and include a decrease in the accumulation of extracellular glutamate leading to decreased intracellular $Ca^{2+}$ influx [12]. In neonates, previous trials on the use of TH have not reported any differences in the incidence of hypocalcemia between cooled infants and controls [13–16]. However, the details on the daily $Ca^{2+}$ intakes and serum levels of $Ca^{2+}$ were not provided, making it difficult to delineate any potential impact of TH on $Ca^{2+}$ homeostasis. Therefore, we designed this retrospective cohort study including moderate and severe HIE infants admitted and treated at our unit before (pre-TH) and after (post-TH) the introduction of TH as standard of care. The primary objective was to investigate for differences in daily serum $Ca^{2+}$ levels and incidence of hypocalcemia during the first week of life, between these two groups. We hypothesized that a better control of serum $Ca^{2+}$ levels and lower rates of hypocalcemia would be observed during the post-TH period.

* Corresponding author at: Department of Pediatrics, Division of Neonatology, Montreal Children's Hospital, McGill University, 2300 Tupper Street, Room C912. Montreal, Québec, Canada, H3H 1P3. Tel.: +1 514 412 4452; fax: +1 514 412 4356.
*E-mail address:* guilherme.santanna@mcgill.ca (G. Sant'Anna).

http://dx.doi.org/10.1016/j.earlhumdev.2015.06.008
0378-3782/

## 2. Methods

### 2.1. Patients

Patient records were selected using the following admission diagnosis: perinatal asphyxia, HIE, neonatal encephalopathy, TH, or seizure. These medical records were reviewed after approval from the Research Ethics Board of McGill University Health Centre.

### 2.2. Inclusion and exclusion criteria

All neonates admitted to the hospital within the first 24 hours of age, gestational age (GA) ≥ 36 weeks, birth weight (BW) ≥ 1800 g, and evidence of physiological and neurological criteria of hypoxic-ischemic encephalopathy as used by the NICHD whole body hypothermia trial were included [2]. Infants with major congenital anomalies, incomplete TH (<72 hours), death, or initiation of palliative life support within this time frame were excluded.

### 2.3. Time frame of data collection

Since TH was implemented in our center on September 2008, we separated the patients into the following two periods: pre-TH (Apr 2006 to Aug 2008) and post-TH (Sep 2008 to Sep 2010). The pre-TH period was longer than the post-TH (extended another 4 months) due to the smaller number of infants in this group. We could not extend it further because the diagnosis identification codes of our institution changed in April 2006. The selected groups (pre- and post-TH) are the same used in a previous study investigating the effects of TH implementation on fluid balance and incidence of hyponatremia [17].

### 2.4. Data collection

Maternal, perinatal, and neonatal characteristics collected included maternal age, maternal complications during pregnancy and labor, abnormal fetal tracing, mode of delivery, GA, BW, gender, neonatal resuscitation, Apgar scores, blood gas from umbilical cord or within the 1st hour of life, modified Sarnat score, temperature on admission, neonatal clinical and/or electrographic seizures, ventilatory support, and use of anticonvulsants, diuretics, sedation, inotropes, or vasopressors.

### 2.5. Data analysis and definitions

Total $Ca^{2+}$ and magnesium (Mg) intakes were calculated and recorded daily, from birth (day 1) to 7 days of age, from the parenteral nutrition (PN), any intravenous fluid administered (including boluses), and enteral feedings. A 'day' was defined as the period starting and ending at midnight. The minimum and maximum serum ionized $Ca^{2+}$ ($iCa^{2+}$), considered the best indicator of physiologic blood calcium activity, and Mg levels were also recorded daily during the same period. Hypocalcemia and hypercalcemia were defined as serum $iCa^{2+}$ level < 1.00 and > 1.40 mmol/L, respectively.

### 2.6. Outcomes

The primary outcomes were differences in the serum $iCa^{2+}$ levels and incidence of hypocalcemia, during the first week of life, between the pre- and post-TH groups. Secondary outcomes were differences in the Mg levels, total $Ca^{2+}$, and Mg intakes and incidence of hypercalcemia between the two groups.

### 2.7. Statistical analysis

Data were expressed as mean ± (SD) or median [interquartiles] for continuous variables, and counts (%) for the categorical variables. For statistical analysis, chi-square test, or Fisher's exact test was used for categorical variables, and Student's *t*-test or non-parametric test was used for continuous variables. A $p$ value < 0.05 was considered statistically significant. Analysis was performed using Stata SE 10.0 (Stata, College Station, TX, USA).

## 3. Results

A total of 146 infants were identified and reviewed; 49 in pre-TH and 97 in post-TH group. Accordingly to our pre-specified criteria, 29 and 38 infants were enrolled in the pre-TH and post-TH groups, respectively (Fig. 1). Maternal, perinatal, and neonatal characteristics were similar between the groups (Table 1).

### 3.1. Hypocalcemia

Minimum $iCa^{2+}$ levels were significantly lower in the pre-TH group between day 1 and 3 of life with no difference after rewarming (Table 2). Five out of 29 infants in the pre-TH group required boluses of $Ca^{2+}$ during this period. In 3 of these infants, bolus was given only once at day of life 2 (2.5 mmol/kg, 3.7 mmol/kg, and 9.8 mmol/kg). Two patients required more than one bolus administration: one on days 1 and 2 (4.9 mmol/kg and 4.9 mmol/kg) and the other one on days 2, 3, and 4 (9.9 mmol/kg, 7.4 mmol/kg, and 2.5 mmol/kg).

In the post-TH group, minimum $iCa^{2+}$ levels were within the normal range throughout the study period (Fig. 2a) despite a significantly lower intake of $Ca^{2+}$ on day 2 and from days 4 to 6 of life (Table 3). Furthermore, no infant in the post-TH group received $Ca^{2+}$ boluses. The incidence of hypocalcemia was higher in the pre-TH group (from day 1 to 5), with a statistically significant difference on day 2 of life (18 vs 0%; $p = 0.01$). In the post-TH group, there were no cases of hypocalcemia during the cooling period (Fig. 3a). (See Table 3.)

### 3.2. Hypercalcemia

Maximum $iCa^{2+}$ levels were significantly higher on day 1–2 of life in the post-TH group, but all values were within the normal range. After the cooling period (day of life 4–7), the maximum $iCa^{2+}$ levels were also in the higher normal range in the post-TH group but similar between groups (Fig. 3b).

### 3.3. Magnesium

There were no significant differences on Mg levels between the groups during days 1–7 of life but total Mg intake was significantly higher in the pre-TH group between days 3 and 6 of life (Table 4).

## 4. Discussion

In moderate or severe HIE infants, a better $Ca^{2+}$ homeostasis was observed after the implementation of TH as the standard of care. This was evidenced by normal serum levels of $iCa^{2+}$ with a significant lower incidence of hypocalcemia in the post-TH group throughout the study period. Interestingly, both findings were achieved despite lower intakes of $Ca^{2+}$ supporting the hypothesis that TH improves $Ca^{2+}$ homeostasis in this population.

In normal term infants, the levels of serum $Ca^{2+}$ decline after birth and reaches a nadir around 24 hours of age. After a period of stabilization, these levels rise, reaching similar levels to those found in childhood by 1 week of age [4]. In our HIE infants during the pre-TH period, minimal $iCa^{2+}$ levels reached this nadir between 24 and 48 hours of life. This decline, however, was not observed in the cohort of infants treated with therapeutic hypothermia where minimum $iCa^{2+}$ levels slowly increased over time (Fig. 2, panel A).

Early hypocalcemia is also seen in preterm infants, infants of diabetic mothers (IDM), and infants with intrauterine growth restriction (IUGR). Since some infants with hypoxic-ischemic encephalopathy may also be