

## Ionized Serum Calcium and Serum Total Magnesium, Predicts Outcome in Neonatal Hypoxic-Ischemic Encephalopathy

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**Abstract:** Perinatal asphyxia, more appropriately known as hypoxic ischemic encephalopathy (HIE), is characterized by clinical and laboratory evidence of acute or subacute brain injury due to asphyxia. The primary causes of this condition are systemic hypoxemia and/or reduced cerebral blood flow. Birth asphyxia causes twenty three percent of all neonatal deaths worldwide. Hypoxic-ischemic cerebral injury is a recognized cause of permanent long-term neurologic disability in children. Data about changes of serum ionized calcium and total serum magnesium concentrations described in asphyxiated infants were controversial. This study aimed to determine the serum levels of total serum magnesium (Mg<sup>2+</sup>) and ionized calcium (Ca<sup>2+</sup>) levels in newborn infants with hypoxic ischemic encephalopathy in the first two days of life and its relation to the severity of hypoxic-ischemic encephalopathy and the disease outcome. Our study was case-control study conducted on 25 term newborn infants in their first two days of life and born in Bab-Elsheria University Hospital and referred to the neonatology unit of pediatric department within the first 24 hours of life with the diagnosis of HIE. A control group of 25 matched (weight and gestational age) full-term newborns with no history of perinatal asphyxia. Exclusion criteria included newborns with neonatal sepsis confirmed by laboratory studies, congenital anomalies, intrauterine growth retarded newborns, and prematurity. All studied newborns were subjected to thorough history, full clinical examination, and clinical staging according to Sarnat and Sarnat's criteria into 3 groups: group I (mild HIE; 8), group II (moderate HIE; 8), group III (severe HIE; 9). Serum levels of total magnesium and ionized calcium were estimated via sampling during the 1<sup>st</sup> 48 hr of life (median age of sampling was 30 hr). Babies were managed according to unit's management protocol. From our study we concluded that significant correlation between severity of HIE and decreased serum levels of total magnesium and ionized calcium, significant relation between decreased serum levels of total magnesium and ionized calcium decreased survival rate.

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### 1. Introduction

Perinatal asphyxia is an insult to the fetus or newborn due to a lack of oxygen (hypoxia) and/or a lack of perfusion (ischemia) to various organs. Hypoxic-ischemic encephalopathy implies the interruption of supply of nutrients to the brain, mainly oxygen and glucose, sufficiently substantial to cause irreversible damage (*Martinez et al., 2013*).

Fifteen percent to twenty percent of affected newborns will die in the postnatal period and an additional twenty five percent will develop severe and permanent neuropsychological sequelae, including mental retardation, visual motor or visual perceptive dysfunction, increased hyperactivity, cerebral palsy and epilepsy (*Wu, 2012*).

There are multiple, biochemical mechanisms and pathways that contribute to the progression of the brain damage that subsequently follows a hypoxic-ischemic insult. These events include energy failure, membrane depolarization, an increase of neurotransmitter release, an increase of intracellular calcium, the production of oxygen-free radicals, and the release of inflammatory mediators (*Verklan,*

*2009*). N-Methyl-D-aspartic (NMDA) acid channel is normally closed by magnesium ions in a voltage dependent manner. Hypoxia decreases the blocking effect of magnesium ion the channel thus causes a rapid influx of Ca<sup>2+</sup> into the cell which has been reported to be the major cause of cell death (*Calvert, 2005*).

### Aim of The Work

The aim of this study is to assess and correlate the serum levels of total serum magnesium and ionized calcium in full term newborns with hypoxic ischemic encephalopathy in the first two days of life and compare that levels to levels in healthy controls.

### 2. Subjects and Methods

#### Study design:

Prospective study including twenty five full term newborn infants born in Bab El-Sharyia University Hospital and referred to the neonatology unit of pediatric department with the diagnosis of HIE during the period of October 2013 - September 2014. A control group of 25 matched healthy full-term newborns with no history of perinatal asphyxia.

The following criteria were used for the diagnosis of HIE, with one or more of the following: (1) 5 minutes Apgar score < 6, severe metabolic acidosis (PH  $\leq$ 7), and/or delayed first breath beyond 5 minutes after birth; (2) mechanical ventilation at birth; (3) evidence of encephalopathy and (4) evidence of multisystem involvement (i.e. encephalopathy and at least one other system) (*American Academy of Pediatrics, 1994*).

Infants were excluded from the study if they had any of the following:

1. Premature < 37 week
2. Infant with Apgar score > 6 at 5 minute
3. Infant with early neonatal sepsis confirmed by positive sepsis screen was excluded later on from the study.
4. Congenital malformations.
5. Intracranial hemorrhage

#### Methods:

All studied newborns were subjected to thorough history, full clinical examination with stress on neurologic evidence of HIE including reflexes, tone, posture, movement, seizures and cranial nerve function.

Patients group were classified according to **Sarnat and Sarnat's (1976)** criteria into 3 groups:

**Group I (Mild HIE):** This group included 8 full term newborn infant.

**Group II (Moderate HIE):** This group included 8 full term newborn infants.

**Group III (Severe HIE):** This group included 9 full term newborn infants.

Estimation of serum levels of total magnesium and ionized calcium, and other investigations as required. Short term outcome was estimated at time of discharge as alive or death.

#### Sample Collection:

Blood samples were obtained by veinpuncture of a peripheral vein under strict aseptic techniques. Samples obtained from patients and controls were allowed to clot in plastic test tubes for 30 minutes at room temperature. Next, the tubes were centrifuged for 5 minutes at room temperature.

#### Principle of ionized calcium assay:

Ionized calcium in the sample was determined by potentiometric ion selective electrode. It was done on AVL-908 (Roche diagnostic). It was based on the measurement of an electrical potential difference between 2 electrodes (half-cell) in an electrochemical cell (*Baker et al., 2000*).

#### Principle of magnesium:

The magnesium ions in the sample is determined by xylidyl blue colorimetric method producing a coloured complex its intensity is directly proportional to the magnesium ions concentration present in the sample. Glycoetherdiamine- N,N,N'-tetracetic acid (GEDTA) performs as a chelating agent (*Bohuon, 1962*).

### 3. Results

The results of this work were statistically analyzed and illustrated in the following tables:

**Table (1): Gender, birth, weight and mode of delivery of controls and patients with different grades of HIE. \***

		Control Group		Patients group		Chi-square test	
		No.	%	No.	%	X <sup>2</sup>	P value
Sex	Female	13	52.0%	13	52.00%	0.000	1.000
	Male	12	48.0%	12	48.00%		
Mode of delivery	C.S	11	44.0%	10	40.00%	0.082	0.774
	Vaginal	14	56.0%	15	60.00%		
Gestational age	Mean $\pm$ SD	39.36 $\pm$ 1.25		39.52 $\pm$ 1.12		0.475	0.637
	Range	37-41		38-41			
Birth weight	Mean $\pm$ SD	3.09 $\pm$ 0.37		3.12 $\pm$ 0.39		0.277	0.783
	Range	2.63-3.85		2.66 – 3.9			

This table shows gender, birth weight, mode of delivery and gestational age of control group and patients with different grades of HIE. Chi-square test test was used where no significant difference is evident.

**Table (2): Comparison between the two studied groups regarding ionized Ca<sup>2+</sup> and serum total Mg<sup>2+</sup>.**

	Control group		Patients group		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
Ionized Ca <sup>2+</sup> (mmol/L)	1.27	0.08	1.05	0.15	6.630	0.000
Serum Mg <sup>2+</sup> (mg/dL)	2.02	0.13	1.36	0.42	7.370	0.000

This table shows highly significant differences between patients and controls regarding mean ionized Ca<sup>2+</sup> and mean serum total mg<sup>2+</sup>.

**Table (3): Comparison between patients with mild HIE and control groups regarding ionized Ca<sup>2+</sup> and serum total Mg<sup>2+</sup>**

	Control		Mild HIE		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
Ionized Ca <sup>2+</sup> (mmol/L)	1.27	0.08	1.14	0.07	4.199	0.000
Serum Mg <sup>2+</sup> (mg/dL)	2.02	0.13	1.58	0.21	6.986	0.000

There is a highly significant lower mean ionized Ca<sup>2+</sup> and mean serum total Mg<sup>2+</sup> in patients with mild HIE compared to controls.

**Table (4): Comparison between patients with moderate HIE and control groups regarding ionized Ca<sup>2+</sup> and serum total Mg<sup>2+</sup>.**

	Control		Moderate HIE		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
Ionized ca (mmol/L)	1.27	0.08	1.10	0.07	5.572	0.000
Serum Mg (mg/dL)	2.02	0.13	1.38	0.18	10.745	0.000

There is a highly significant lower mean ionized Ca<sup>2+</sup> and mean serum total Mg<sup>2+</sup> in patients with moderate HIE compared to controls.

**Table (5): Comparison between patients with severe HIE and control groups regarding ionized Ca<sup>2+</sup> and serum total Mg<sup>2+</sup>.**

	Control		Severe HIE		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
Ionized Ca <sup>2+</sup> (mmol/L)	1.27	0.08	0.92	0.17	8.489	0.000
Serum Mg <sup>2+</sup> (mg/dL)	2.02	0.13	1.16	0.61	6.736	0.000

There is a highly significant lower mean ionized Ca<sup>2+</sup> and mean serum total Mg<sup>2+</sup> in patients with sever HIE compared to controls.

**Table (6): Serum ionized Ca<sup>2+</sup> and total serum Mg<sup>2+</sup> levels in patients with favorable and unfavorable outcome.**

	Unfavorable		Favorable		Independent t-test	
	Mean	±SD	Mean	±SD	t	p-value
Ionized ca (mmol/L)	1.00	0.17	1.23	0.10	5.948	0.000
Serum Mg (mg/dL)	1.26	0.51	1.86	0.30	3.793	0.000

Patients with unfavorable outcome showed highly significant lower serum ionized Ca<sup>2+</sup> and total serum Mg<sup>2+</sup> levels compared to those with favorable outcome.

#### 4. Discussion

This study was aimed to determine serum ionized Ca and total serum Mg levels in term neonates with hypoxic-ischemic encephalopathy (HIE) and correlating the results with the disease severity and outcome. This was done through evaluation of 25 term newborns diagnosed as HIE based on the inclusion criteria and 25 age and sex matched healthy full term newborns as control group recruited from NICU. Our results revealed that gestational age, birth weight, sex and mode of delivery were not significantly different in studied newborns.

In our study we found that neonates with HIE had significantly lower serum ionized Ca level (1.05±0.15mmol/L) as compared to control group (1.27±0.08mmol/L). Serum ionized ca was lower in mild, moderate and severe cases than control group.

This comes with agreement to *Petersen et al. (1981)*, *Jajoo et al (1995)* *Mehta et al. (1998)*, they described hypocalcemia in asphyxiated infants with more severe degrees of asphyxia. Also, *Yoneda et al. (2004)* showed that adjusted (Ca<sup>2+</sup>) shortly after birth was significantly lower in infants with HIE and that decrease in serum ionized Ca might be the result of hypoxic-ischemic reperfusion injury in the whole body. On the other hand, *Jajoo et al. (1995)* and *Ives et al., (2000)* found that good portion of asphyxiated infants had initially normal (Ca<sup>2+</sup>) level in cord blood sample at birth however decreased (Ca<sup>2+</sup>) level occurs on the second day of life. The difference in the above results shown by both authors and our results can be explained by the fact that we did not obtain our samples from cord blood but from serum samples in the first two days of life.

In our study we found that neonates with HIE had significantly lower mean total serum Mg<sup>2+</sup> level (1.36±0.42m/dl) as compared to mean total serum Mg<sup>2+</sup> level of control group (2.02±0.13mg/dl). The data about changes in total serum Mg<sup>2+</sup> level in asphyxiated term babies described by different authors were controversial (*Ives et al., 2000*).

Mean serum total magnesium was lower in mild, moderate and severe cases than control group.

This comes with agreement to *Geven et al. (1993)*, *Jajoo et al. (1995)* described hypomagnesemia after birth asphyxia. Our data about lower Mg<sup>2+</sup> concentration in severe HIE infants support the suggestion of a possible neuroprotective effect of Mg SO<sub>4</sub> in asphyxiated infants (*Grether et al., 1998*; *Ives et al., 2000*; *Berger and Garnier, 2001*). After asphyxia, alterations in intra-and-extracellular Mg concentrations affecting cell function through its interaction with Ca<sup>2+</sup> by binding competitively to its same sites and by altering its distribution due to changes in Ca<sup>2+</sup> influx across cell membrane (*Levine and Coburn, 1994*).

The decreased concentration of total Mg<sup>2+</sup> may be involved in the development of HIE, as Mg<sup>2+</sup> takes part in the biochemical cascade after hypoxic-ischemic insult. Asphyxia can disturb the neurone's ability to maintain its membrane potential and reduce Mg<sup>2+</sup> blockade of the NMDA receptor resulting in increased Ca<sup>2+</sup> entry which is the key mechanism in delayed death in asphyxiated infants (*Delivoria-Papadopoulos and Mishra, 1998*, *Mehta et al. (1998)*).

On the other hand, (*Engel and Elin, 1970*; *Bachman et al., 1976*; *Jukarinen, 1979* and *Ives et al., 2000*). Described hypermagnesemia after birth, the cause of such hypermagnesemia may be attributed to acidosis which depolarizes the cell membrane leading to some loss of intracellular Mg<sup>2+</sup>. (*Cadell and Reed, 1989*) suggested that total Mg<sup>2+</sup> determination during acidosis may give a falsely elevated value and may mask a true total Mg deficiency which can be diagnosed only after homeostasis is reestablished. Hypermagnesemia may be a type of protective mechanism, through which the body try to increase extracellular Mg<sup>2+</sup> which blocks NMDA receptors preventing Ca<sup>2+</sup> entry to brain cells and thus preventing its injury (*Hoffman et al., 1994*).

From our study we found that neonates with HIE who showed unfavorable outcome (neurological abnormality or death) had significantly lower serum ionized Ca<sup>2+</sup> level compared to those with favorable outcome (clinically normal).

*Yoneda et al. (2004)*, also found that a significant low serum ionized calcium level occurs in infants with HIE who had abnormal outcome (death or cerebral palsy development) compared to those with normal outcome (full neurological recovery), suggesting greater intracellular shift of Ca<sup>2+</sup> into multiple organs, particularly the brain. Different results suggest that serum ionized calcium may be useful for predicting the outcome of infants with HIE (*Ilves et al., 2000*).

Also we found in our results that neonates with HIE who showed unfavorable outcome (neurological abnormality or death) had significantly lower total serum Mg level compared to those with favorable outcome (namely, clinically normal).

*Jajoo et al. (1995)* also found that hypomagnesemia was a frequent finding in seizures resulting from asphyxia brain damage, highlighting the potential role of Mg as a predictor to the outcome of HIE. Also our results comes in agreement to *Ilves et al. (2000)* who supported the link between hypomagnesemia and neuronal injury in severe HIE and hence poor outcome.

In conclusion we have determined that low serum ionized Ca<sup>2+</sup> and total serum Mg<sup>2+</sup> levels in neonates with HIE. Significant correlation between low serum ionized Ca<sup>2+</sup> and total serum Mg<sup>2+</sup> levels to HIE grade and the disease outcome. Therefore, both serum ionized Ca<sup>2+</sup> and total serum Mg<sup>2+</sup> have combined diagnostic and prognostic values in neonates with HIE.

## References

1. American Academy of Pediatrics/American College of Obstetricians and Gynecologists: Relation between perinatal factors and neurological outcome. In: Guidelines for Perinatal Care. 3rd ed. Elk Grove Village, Ill 1994; 221-34.
2. Bachman KD, Feenders O, and Dominick H Chr: Die klinische Bedeutung des Magnesiums in der Neugeborenenperiode. Geburtsh und Frauenheilkunde 1976; 36: 308-13.
3. Baker E, Pertsch E, and Buhlmann P.: Selectivity of potentiometric ion sensors, Anal Chem.; 72: 1127-1133, 2000.
4. Berger R and Garnier Y: Perinatal brain injury. J Perinat Med 2001; 29 (1): 85-6.
5. Bohuon: Magnesium assay with calmagite or erichrome black T reagents. Anal. Chim, Acta, 7: 811-817, 1962.
6. Cadell JL and Reed GF. Unreliability of plasma magnesium value in asphyxiated neonates. Magnesium 1998; 8: 11-6.
7. Calvert J. W.: Pathophysiology of an hypoxic-ischemic insult during the perinatal period: Neurol Res 2005; 27: 246-260.
8. Delivoria-Papadopoulos M and Mishra OP: Mechanisms of cerebral injury in perinatal asphyxia and strategies for prevention. J Pediatr 1998 132: 30S-4S.
9. Engel RR and Elin RJ.: Hypermagnesemia from birth asphyxia. J Pediatr 1970; 77: 631-7.
10. Geven WB, Monnens LAH, Willems JL: Magnesium metabolism in childhood. Miner Electrolyte Metab 1993; 19: 308-13.
11. Grether JK, Hoogstrate J, Selvin S *et al.*: Magnesium sulphate tocolysis and risk of neonatal death. Am J Obstet Gynecol 1998; 178: 1-6.
12. Hoffman DJ, Marro PJ, McGowan JE, Mishra OP, Delevoria- Papadopoulos M *et al.* Protective effect of Mg sulfate infusion on NMDA receptor binding characteristics during cerebral cortical hypoxia in the newborn piglet. Brain Res 1994; 664:144-9.
13. Ilves P, Kiisk M, Soopold T, *et al.*: Serum total magnesium and ionized calcium concentrations in asphyxiated term newborn infants with hypoxic-ischemic encephalopathy. Acta Paediatr 2000 ;89: 680-5.
14. Jajoo D, Jajoo A, Shankar R, *et al.*: Effects of birth asphyxia in serum calcium levels in neonates. Indian J Pediatr 1995, 62(4): 455-9.
15. Jukarinen J: Plasma magnesium concentrations during the first five days of live. Acta Paediatr Scand 1979; S222: 5-55. for reduction of birth asphyxia induced brain damage.
16. Levine BS and Coburn JW: Magnesium, the mimic/antagonist of calcium. N Eng J Med 1994; 310:1253-5.
17. Martinez-Biarge M, Diez-Sebastian J, Wusthoff CJ, *et al.*: Antepartum and intrapartum factors preceding neonatal hypoxic-ischemic encephalopathy. Pediatrics 2013; 132:e952.
18. Mehta KC, Kalkwarf HJ, Mimouni F, *et al.*: Randomized trial of magnesium administration to prevent hypocalcemia in infants of diabetic mothers. J Perinatol 1998; 18(5): 352-6.
19. Petersen S, Christensen NC, Fogh-Andersen N.: Effect on serum calcium of 1 -hydroxy-vitamin D3 supplementation in infants of low birth weight, infants with perinatal asphyxia, and infants of diabetic mothers. Acta Paediatr Scand 1981; 70: 897-901.
20. Sarnat HB and Sarnat M: Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976; 33:696-705.
21. Verklan MT: The Chilling Details: Hypoxic-Ischemic Encephalopathy. J Perinat Neonatal Nurs 2009; 23 (1): 59-68.
22. Wu Y: Brain injury in newborn babies: we can't afford to get it wrong. Ann Neurol 2012; 72:151.
23. Yoneda S, Ibara S, Kobayashi K, Kato E, Maruyama Y, Maruyama H, Sumida Y, Sunami R, Sakai M, Ikenoue T and Saito S *et al.*: Low adjusted serum ionized calcium concentration shortly after birth predicts poor outcome in neonatal hypoxic-ischemic encephalopathy. J Obstet Gynecol Res Vol. 31, no. 2004; 6: 57-64.