IS HYPONATREMIA IN NEONATES WITH HYPOXIC ISCHAEMIC ENCEPHALOPATHY WHO UNDERWENT THERAPEUTIC HYPOTHERMIA ASSOCIATED WITH POOR NEURO-DEVELOPMENTAL OUTCOME AT 12 MONTHS OF AGE?

by
Marang Molotsi

Thesis presented in fulfilment of the requirements for the degree of Master of Medicine in Paediatrics and Child Health in the Faculty of Medicine and Health Sciences at Stellenbosch University

Supervisor: Dr Gugu Kali
Co-supervisor: Prof. Johan Smith

December 2016

Declaration

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

December 2016

ABSTRACT

Introduction

Perinatal asphyxia occurs at an incidence of 1-2% per live births in developed countries, and much higher in developing countries. The main consequence of significant perinatal asphyxia is neonatal encephalopathy which, if severe enough, may result in death or long term neurological disability. Therapeutic hypothermia (TH) has emerged as a promising therapy in reducing its mortality and morbidity. Electrolyte abnormalities, including hyponatremia, are a common occurrence in neonates with HIE. Possible causes of hyponatremia are renal impairment secondary to acute kidney injury and the syndrome of inappropriate anti-diuretic hormone (SIADH). Therapeutic hypothermia itself has an impact on fluid and electrolyte balance. Serum sodium concentration is closely linked to serum osmolality and neuroprotective strategies following brain injuries usually include maintaining serum sodium within normal limits to minimize further damage. Studies done on adults have shown adverse effects of dysnatremia on neurologic outcome following brain injuries. These studies however looked at disease entities more common in the adult population. There are very few studies assessing dysnatremia in the neonatal population, hence the interest in carrying out this analysis.

Methods

This retrospective descriptive study is a sub-analysis of a previous prospective study for infants who met therapeutic hypothermia criteria after sustaining hypoxic ischemic encephalopathy. The infants were treated in the neonatal intensive care unit of the Department of Paediatrics and Child Health at the Tygerberg Children's Hospital

between 2008 and 2011. According to the cooling protocol, each infant had serum sodium levels measured on three consecutive days during cooling. After discharge the neurodevelopmental functional status was assessed during follow-up visits. The primary aim of the study was to determine if there is an association between hyponatraemia in neonates with HIE who underwent therapeutic hypothermia and neurodevelopmental outcome at 12 months of age, as assessed by Bayley Screening tool (BSID - III)

Results

The patient records search yielded 100 patients from 2008 to 2011. After excluding those who did not meet the inclusion criteria, fifty patients were studied. Twenty-one (21/50; 42%) patients had hyponatremic episodes. The remainder (29/50; 58%) were normonatremic. None were hypernatremic. Thirty-five (35/50; 70%) were assessed as normal at twelve-month follow up, nine (9/50; 18%) as mildly abnormal and 6 (6/50; 12%) as abnormal. There was no association between neurodevelopmental functional status at 12 months and frequency of hyponatremic episodes in the first week of life ($p = 0.444$). There was a significant association between number of hyponatremic episodes and HIE grade. We found no association between HIE grade and neurodevelopmental functional status at 12 months.

Conclusion

There was no significant association between hyponatremia after hypoxic-ischemic encephalopathy and neurodevelopmental outcome at 12 months. There was a positive correlation between HIE grade and frequency of hyponatremic episodes.

ACKNOWLEDGEMENTS

The author acknowledges the mentorships of Dr Gugu Kali and Professor Johan Smith for their commitment to the supervision of my MMED (Paediatrics) thesis.

The head of Department Professor Kruger for her commitment in ensuring that we commit adequate time to doing research work.

To Dr Netta Van Zyl, who performed the neurodevelopmental assessments on all the cooled infants. The guidance and assistance given by the research committee of University of Stellenbosch also goes a long way in shaping our understanding of the research process and structure

My appreciation also to those who have directly and indirectly contributed to this work.

TABLE OF CONTENTS

1. LIST OF ABBREVIATIONS

HIE	Hypoxic ischemic encephalopathy
NE	Neonatal encephalopathy NE
TH	Therapeutic hypothermia
HI	Hypoxic-ischemic
BSID	Bayley Scales of infant development

2. LIST OF TABLES AND FIGURES

3. INTRODUCTION

Hypoxic ischemic encephalopathy (HIE) refers to a form of neonatal encephalopathy (NE) where the underlying mechanism of brain injury is hypoxia and ischemia. NE in turn, as defined by the American Academy of Pediatrics (AAP) and the American College of Obstetrics and Gynecology (ACOG), is a clinically defined syndrome of disturbed neurological function in the earliest days of life in an infant born at or beyond 35 weeks of gestation, manifested by a subnormal level of consciousness or seizures, and often accompanied by difficulty with initiating and maintaining respiration and depression of tone and reflexes.

The definition of HIE has undergone modification over recent years. Previously, terms such as birth asphyxia and perinatal asphyxia were broadly applied to all neonates who presented with signs and symptoms of neurologic dysfunction, regardless of the etiology. With time, it became clear that a wide range of pathologies, both maternal and fetal in origin, occurring in the prenatal, perinatal or postnatal period, could manifest as NE. The term HIE is now reserved for those neonates who meet strict criteria, showing evidence of hypoxia and ischemia as the underlying pathology.

Prior to the advent of therapeutic hypothermia (TH), the management of HIE was limited to supportive care only. It was responsible for a large proportion of neonatal mortality as well as childhood neurologic morbidities, including cerebral palsy, epilepsy, visual and hearing problems. Studies performed on animals elucidated the underlying pathophysiologic mechanisms involved in hypoxic-ischemic (HI) injury and allowed

more targeted therapeutic strategies to be developed.

4. LITERATURE REVIEW

4.1 PATHOPHYSIOLOGY OF HYPOXIC-ISCHAEMIC INJURY

At the cellular level, HI injury is believed to result in a cascade of events that is initiated soon after the initial insult and continues for more than 48 hours afterwards. In the absence of oxygen, there is a shift to anaerobic metabolism, which is an inefficient ATP-depleting pathway. Once ATP is depleted, cellular functions that utilize ATP begin to fail, including sodium and potassium trans-cellular ion pumps. This results in loss of ionic gradients and widespread depolarisation. Pump failure results in the intracellular accumulation of sodium ions, chloride ions and water causing cytotoxic oedema. These events that occur at the time of the initial insult are referred to as primary energy failure[1-3].

Despite the re-establishment of perfusion, the pathologic cascade of events will continue for more than 48 hours, characterized by ongoing cellular injury and cell death from apoptosis. This secondary process (secondary energy failure) is mediated, amongst other substances, by the excitatory neurotransmitter glutamate (excitotoxicity) as well as calcium ions, which act as secondary messengers initiating a cascade of intracellular events that culminate in neuronal apoptosis. Depletion of ATP and failure of membrane pumps during the initial phase results in abnormal accumulation of glutamate at the synapses, and also causes an abnormal accumulation of calcium ions

in the cytosol. Under glutamate stimulation there is further depolarisation of membranes, further disruption of ionic gradients and a vicious cycle is perpetuated. Reperfusion also results in the production of an excess of reactive oxygen species that cause further cellular injury and inflammation[1-3].

4.2 CLINICAL MANIFESTATIONS OF HIE

The clinical manifestations depend on the severity of the HI injury. Infants with mild HI injury may have transient behavioral abnormalities such as irritability or drowsiness. The neurologic exam may reveal mildly increased tone with brisk reflexes. Infants with moderate HI injury may show a depressed level of consciousness with significant hypotonia, diminished or absent deep tendon reflexes and abnormal or absent primitive reflexes. There may also be seizures and abnormalities in respiration. A typical presentation in infants with severe HI injury would include stupor or coma, generalized hypotonia with depressed deep tendon reflexes, absent primitive reflexes. There are often abnormalities in breathing requiring mechanical ventilation. Seizures and autonomic dysfunction, manifesting as abnormalities in heart rate and blood pressure, also occur commonly[4].

In addition to the neurologic manifestations, infants with HIE often have multi-organ involvement. They may have myocardial dysfunction causing hypotension; pulmonary hypertension necessitating mechanical ventilation; renal failure leading to significant water and electrolyte imbalances; and gastrointestinal involvement which may manifest as elevated liver enzymes, delayed gastric emptying and abnormal peristalsis[5-7].

4.3 STAGING OF HIE

In 1976, Sarnat and Sarnat formulated a grading system that could be used to classify neonates into mild, moderate or severe HIE based on clinical findings. Since then, a number of other grading and scoring systems have been introduced[8-10], but the Sarnat grading system, or modified versions of it, remains the most widely used.

	STAGE 1 (mild)	**STAGE 2 (moderate)**	**STAGE 3 (severe)**
LEVEL OF CONSCIOUSNESS	**HYPERALERT**	**LETHARGIC/OBTUNDED**	**STUPOROUS**
Neuromuscular Control			
Muscular Tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch	Overactive	Overactive	Decreased/absent
Segmental myoclonus	Present	Present	Absent
Complex Reflexes			
Suck	Weak	Weak/absent	Absent
Moro	Strong	Weak	Absent
Oculovestibular	Normal	Overactive	Weak/absent
Tonic neck	Slight	Strong	Absent
Autonomic Function			
Pupils	Mydriasis	Miosis	Variable
Heart Rate	Tachycardia	Bradycardia	Variable
Bronchial/salivary secretions	Sparse	Profuse	Variable
Gastrointestinal motility	Normal/decreased	Increased/diarrhea	Variable
Seizures	None	Common/focal or multifocal	Uncommon
EEG	Normal/decreased	Early low voltage continuous delta and theta, later periodic, seizures focal 1-1.5Hz spike wave	Early periodic pattern with isopotential phases, later isopotential
DURATION	<24h	2-14 days	Hours - weeks

TABLE 1: Sarnat staging of neonatal encephalopathy

In their original study, Sarnat and Sarnat also found there was an association between HIE severity and neurodevelopmental outcome. Infants who had mild HIE had better outcomes than those with moderate or severe HIE. This has since been confirmed in other studies, using different grading sytems[8-10].

4.4 THERAPEUTIC HYPOTHERMIA FOR NEUROPROTECTION

With the understanding that brain injury continues to evolve over hours following the initial insult, it was possible to devise therapeutic strategies that target the latent period between primary and secondary energy failure in an attempt to prevent or limit this ongoing injury process. The initial use of therapeutic hypothermia (TH) in modern medicine dates back as far as the 1800s[11]. Regarding neuroprotection, it was in 1953 that Bigelow and McBirnie, using canine models, published a study reporting its beneficial effect for the brain and the heart during cardiac surgery[12]. During the ensuing 30-40 years however, its popularity waned due to the discovery of its deleterious effects. Positive data obtained from animal models supporting its benefits later lead to a resurgence of interest in the field[11]. Following prospective studies in adult patients who had suffered anoxic brain injury, the American Heart Association published guidelines in 2002 recommending its use as a treatment modality for out-of-hospital comatose victims of cardiac arrest[12].

The neuroprotective effects of TH on the immature brain following hypoxic-ischemic injury were also demonstrated in several animal studies[13]. Randomized controlled trials in human neonates were conducted from the early 2000s and they repeatedly showed a

reduction in the rate of death or neurologic disability following HIE[14]. Therapeutic hypothermia for HIE has since become the standard of care in most developed countries[15].

The exact mechanisms of neuroprotection by TH continue to be investigated and are believed to be multifactorial[16]. They include, amongst others, suppression of apoptotic pathways, suppression of microglial activation and the resultant inflammatory cascades, as well as suppression of excitotoxicity[16]. Although the exact point at which neuronal injury becomes irreversible is not known, there is evidence that for TH to be beneficial, there is a limited window of opportunity[17]. The current practice is to initiate TH within 6 hours of delivery and continue for a total of 72 hours.

In addition to the effects of TH on the brain as described above, lowering the body's temperature to subnormal limits is also associated with some undesirable physiologic effects on most systems in the body. Reported effects include sinus bradycardia, reduction in cardiac output with some studies reporting hypotension and a need for inotropic support[18], impaired clotting and immune function, decreased renal blood flow and glomerular filtration rate, delayed gastric emptying, disturbances of electrolytes and glucose homeostasis[18,19]. When offered in centres with adequate experience and equipment, the risks and adverse effects associated with TH could be minimized in favour of benefit. Overshooting of target temperatures, both in the cooling and rewarming phases, is also associated with complications and has been associated with increased mortality and morbidity[18]. This may be a problem particularly for the low-resourced or inexperienced centres.

4.5 NEUROLOGIC OUTCOME FOLLOWING HIE

Studies done both prior to and following the introduction of TH have shown that certain clinical findings, biochemical markers, imaging and electrophysiological findings were predictive of neurologic outcomes. Over time, some factors were consistently found to be predictive across multiple studies, while others had inconsistent results. Moreover, the predictive value of some was altered with the introduction of TH. While they could be used in the pre-cooling era, they would not be accurate or would be less predictive when used on cooled patients[19,20].

The severity of HIE (HIE grade), as determined by scoring systems, has been found to be a strong predictive marker of outcome for both cooled and non-cooled infants. It has however been found that the predictive value is reduced during TH and that it may be more accurate if used after TH has been completed[19]. The presence of abnormalities on amplitude integrated electro-encephalogram (aEEG) and the presence of seizures have also been associated with poorer outcomes. Other clinical factors that were commonly used for prediction of outcome in the pre-cooling era include APGAR scores, the need for prolonged resuscitation, time to establishment of spontaneous respiration and severity of metabolic acidosis. These also formed the basis of inclusion criteria in early TH trials. Similar to HIE grade, the predictive value of these variables has been altered with the introduction of TH[20].

4.6 HYPONATREMIA

Hyponatremia is defined as a serum sodium concentration of less than 135mmol/L/[21]. It is the most common disorder of body fluid and electrolyte balance and occurs with a frequency that varies from 25% to 65% in very ill neonates[22]. Studies have shown that hyponatremia occurs with a higher incidence in neonates with HIE than in healthy newborns[23,24]. In one multi-center randomized trial investigating whether TH improved neurodevelopmental outcomes after HIE, Gluckman *et al.* found that hyponatremia occurred both in neonates who underwent TH (49/112 [44%]) and those who had HIE but did not undergo TH (46/118 [39%])[25]. One study in addition, found that the degree of hyponatremia was directly proportional to the severity of HIE[24].

In the first few days after birth, there is movement of water both from the interstitium as well as the intracellular compartment, into the intravascular compartment [26,27]. This stimulates the release of atrial natriuretic peptide which causes sodium and water diuresis and loss of body weight[26,27]. This means that in a healthy neonate, for the first 48-72 hours post-delivery, there is a net negative sodium and water balance. Under the influence of the renin-aldosterone-angiotensin system, sodium and water balance then become positive and remain so into adult life[28].

4.6.1 CAUSES OF HYPONATREMIA

The etiologies of hyponatremia can be broadly classified into primary water excess or primary sodium depletion[28,29]. Primary water excess can either be the result of excess intake of hypotonic fluid (dilutional hyponatremia), or impaired excretion of water. Primary sodium depletion is either the result of insufficient intake or excessive loss of sodium. A combination of the two mechanisms can also exist in the same patient[29].

The majority of the hyponatremia seen in children is thought to be hospital acquired and occurring in children who receive hypotonic intravenous fluids[30]. Hypotonic IV fluids in children have been used based mainly on a recommendation made by Holliday and Segar more than 50 years ago[31]. Studies done since then have however showed that hypotonic fluids produce hyponatremia in hospitalized children[30]. Neonates with HIE frequently receive intravenous fluid as part of their routine care. Although studies done were mostly on older infants and children, this could also be a relevant cause in neonates.

Maternal hyponatremia caused by intravenous administration of large volumes of hypotonic fluid during labor, or oral ingestion of free water, has been shown to cause neonatal hyponatremia[29,32]. This will be evidenced by hyponatremia in the newborn at, or a few hours after birth[33]. Synthetic oxytocin, used for induction of labor is thought to have anti-diuretic properties and when administered to the mother during labor (usually in dextrose solutions) can also lead to water intoxication and resultant maternal and neonatal hyponatremia[34,35]. Other medications used by the mother that may result in neonatal hyponatremia include diuretics, laxatives, some anti-depressants (eg selective

serotonin reuptake inhibitor exposure [SSRI]) and recreational drugs such as ecstasy[33,36].

Acute kidney injury (AKI) is a common occurrence in babies with HIE and those with severe asphyxia are more likely to have renal involvement than those less severely affected[37]. It is believed to be a consequence of an adaptive mechanism during hypoxia-ischemia – the diving reflex. This reflex shunts blood away from the skin and splanchnic area to the heart, brain and adrenals[38]. As the kidneys are very sensitive to a lack of oxygen, perinatal asphyxia is one of the most frequent causes of AKI in neonates[37].

The incidence of AKI in neonates with perinatal asphyxia is reported to be 30-56% and is thought to be an underestimate due to the lack of a consensus definition of AKI in neonates[37]. One study reported an incidence of approximately 76%[39]. Another study, that looked at the incidence of AKI in neonates with HIE who underwent TH, reported an incidence of 38%[40]. Both non-oliguric and oliguric renal failure occur; there is conflicting data from studies regarding which occurs more commonly[23,39]. AKI may contribute to the occurrence of hyponatremia either due to impaired water excretion or impaired tubular reabsorption of sodium[23,39].

The release of anti-diuretic hormone (ADH) is stimulated by a rise in osmolality, a fall in central blood pressure and hypovolemia. Changes in osmolality are sensed by osmoreceptors located in the supra-optic nucleus of the hypothalamus, while changes in blood pressure and volume status are sensed by baroreceptors located in the cardiac atria, aorta and carotid sinus. The two principal actions of ADH are to increase

reabsorption of water by the kidneys and vasoconstriction[41].

It has been suggested by some authors that the syndrome of inappropriate ADH secretion (SIADH) occurs commonly in newborns[42]. Elevated levels of ADH and hyponatremia do occur frequently in sick newborns[28,29]. It has however been found that there are other non-osmotic and non-baroreceptor stimuli that increase the secretion of ADH[43]. In addition, the defense of blood pressure overrides that of tonicity, meaning that ADH secretion will continue as long as there is hypovolemia or hypotension, regardless of plasma osmolality, serum sodium concentration and volume status[44]. The non-osmotic and non-baroreceptor stimuli that have been identified include nausea, vomiting, pain, stress, trauma and medications such as opioids and anesthetic agents [44-46]. These findings have several implications. Firstly, neonates are at risk of intravascular volume depletion and hypotension[47] but the recognition of an inadequate intravascular volume can be difficult in children[48]. It is not known to what extent unrecognized baroreceptor stimulation of ADH release contributes to the occurrence of water retention and hyponatremia. The proportion of neonates erroneously diagnosed with SIADH in the presence of these non-osmotic and non-baroreceptor stimuli to ADH secretion is also unknown. Poorly controlled pain in neonatal intensive care units may also play a role, as pain is known to cause an immediate increase in ADH concentration in urine[28]. Some authors therefore believe that true inappropriate ADH secretion is probably rare in the newborn[28]. The diagnostic criteria for SIADH include hyponatremia with plasma hypotonicity, inappropriately concentrated urine, continued sodium excretion, and the exclusion of renal or endocrine disease. Stimuli that produce non-osmotic release of ADH, such as medications, hypovolemia, hypotension, pain, stress,

and nausea, must also be absent[49].

The causes of sodium depletion include renal tubular dysfunction such as that seen with pyelonephritis, obstructive uropathies, damage caused by nephrotoxic agents and medications such as diuretics[29]. Endocrine disorders, specifically the salt-losing forms of congenital adrenal hyperplasia and hypoaldosteronism may also cause hyponatremia[29]. Gastrointestinal diseases causing vomiting and stoma losses are another potential cause of sodium depletion[29]. These pathologies are however not likely to be a cause of hyponatremia in the immediate neonatal period.

The implementation of TH may have an impact on the body's fluid and electrolyte status. This was suggested by Prempunpong *et al.* when they investigated the effect of the implementation of TH on fluid balance and incidence of hyponatremia in neonates with moderate or severe HIE. They found that neonates who underwent TH had increased fluid retention (as evidenced by weight gain) and lower serum sodium concentrations than controls. They hypothesized that the application of a cold stimulus to the skin results in local vasoconstriction, reduced blood flow and reduced transepidermal loss of water[50]. These neonates are frequently intubated and ventilated, which reduces respiratory water loss[50], in addition to the other risk factors of impaired water excretion, as mentioned previously. The authors acknowledged the limitation of not directly measuring the amount of trans-epidermal water loss for the cooled infants. Further studies are necessary.

No other cooling studies have investigated the impact of TH on fluid and electrolyte balance. Some reported on the incidence of hyponatremia as a secondary outcome and

no significant effect of TH was observed between cooled infants and controls[50]. Hypothermia is known to suppress antidiuretic hormone, and in experimental animal models cooling was associated with a decrease in renal perfusion and glomerular filtration rate[51]. Meta-analysis of trials that reported the effect of hypothermia on urine output however showed no statistically significant difference in the occurence of oliguria between cooled and non-cooled infants[51,52].

4.6.2 THE EFFECTS OF HYPONATREMIA ON THE BRAIN

As the major cation in the extra-cellular compartment, sodium levels strongly influence serum osmolality. Hyponatremia results in a fall of serum osmolality. Without compensatory mechanisms, this leads to movement of water from the intravascular space into brain cells, down an osmotic gradient, resulting in brain edema[53,54]. There are however adaptive mechanisms in place that counteract brain swelling. The efficacy of these mechanisms in preventing brain swelling depend on how rapidly hyponatremia and resultant hypo-osmolality occurs. When hypo-osmolality develops at a rate that exceeds the brain's ability to regulate its volume, severe brain edema results, potentially leading to neurological dysfunction and sometimes death. Hypo-osmolality is considered to be acute when it develops over 24–48 h[54].

The initial adaptive response is movement of fluid from the interstitial space into the cerebrospinal fluid (CSF) and from there into the systemic circulation[55]. This is followed by the extrusion of intracellular inorganic ions (sodium, potassium and chloride), which will cause osmotic movement of water extracellularly. This is mediated by the sodium-potassium ATPase pump and is energy dependent[53]. Animal studies have shown that

this process is initiated within thirty minutes of induced hyponatremia and reaches its maximum by three hours[54].The second phase of the adaptive response involves the extrusion of intracellular organic solutes – mainly amino acids[53,54]. This phase takes longer to come into effect and is more useful for combatting chronic hyponatremia. The extrusion of these osmolytes is sustained for as long as hyponatremia persists[53].

There is evidence that age may influence the ability of the brain to adapt to hyponatremia and hypoosmolality in older children and adults[56,57]. The ratio of brain volume to skull vault size is higher in children due to the fact that the human skull does not attain full size until adulthood, although brain development is complete by six years of age[56,57]. In addition, brain water content is more than two-and-a-half times higher in the paediatric population[56]. The volume of CSF in adult brains is more than 10% greater than that in paediatric patients. This higher CSF volume allows for more space in which the brain can expand[56]. There is therefore less room for expansion of the paediatric brain in the skull than there is in adults. In addition to these physical factors, the paediatric brain has much less sodium-potassium-ATPase activity and higher intracellular concentration of sodium than adults, resulting in a steeper osmotic gradient during hyponatremia[56,57]. These studies focused on older children, whether neonates have these limitations in adapting to hyponatremia and hypo-osmolality is unclear but a possibility.

As mentioned earlier, during hypoxic-ischemic injury of the brain, there is failure of transcellular ion pumps, disruption of ionic gradients and cell death caused by cytotoxic edema as well as apoptosis. Since these ion pumps are important in the regulation of

brain cell volume, the adaptive response to hyponatremia, is severely blunted by hypoxia[58,59]. This results in worsening of brain edema and increases mortality[59]. Furthermore, it has been found that in patients with hyponatremic encephalopathy hypoxemia is a common finding and plays an important role in the eventual outcome. Two mechanisms have been proposed; respiratory depression secondary to brain edema and neurogenic pulmonary edema[60]. This suggests the possibility of a vicious cycle where the two pathologies have an additive adverse effect on the brain, greater than if either occurred alone, with a resultant increase in mortality.

4.6.3 NEUROLOGIC OUTCOME FOLLOWING HYPONATREMIA

Neuro-protective strategies usually include maintaining serum sodium levels within normal limits. Studies performed on adults, have shown adverse effects of dysnatremia on neurologic outcome [61-63] These studies however looked at disease entities more common in the adult population, namely strokes, sub-arachnoid haemorrhages and traumatic brain injuries. Very few studies have been done on the paediatric population.

Al-Zahraa et al[64]*.* carried out an analysis on 72 children, including 14 neonates, who were admitted for neurologic pathologies in a tertiary hospital. They were divided into 3 groups according to the serum sodium on admission – normal, mild deficit and moderate to severe deficit. The duration of stay of each patient in hospital was recorded and they were examined for the presence or absence of persistent neurologic deficits before their discharge and during follow-up visits. When analyzed according to the serum sodium grouping, the hospital stay was shortest for those with normal serum

sodium on admission. In terms of long term sequelae, the majority of patients (88.6%) with normal serum sodium on admission recovered completely. All those who had a mild deficit had neurodevelopmental sequelae, while all those with moderate to severe deficit were severely incapacitated, with 4 of them dying. The authors also found a significant inverse 'dose–response relationship' between the degree of hyponatremia and duration of hospital stay. They however emphasized that the group of patients that stayed longest contained a disproportionate number of conditions which tend to carry poor prognosis and hence longer stay. The serum sodium could have been an epiphenomenon reflecting severity of the children's underlying diagnosis[64].

Mcjunkin *et al.* studied a group of 127 children admitted with La Crosse encephalitis, a mosquito-borne disease, investigating the clinical manifestations and clinical course. They assessed factors associated with in-hospital clinical deterioration, which occurred in 13 (11%) of the children. They found that in the patients with in-hospital deterioration, the nadir serum sodium level during hospitalization was slightly lower than in those without deterioration[65].

Sporadic case reports of symptomatic dilutional hyponatremia secondary to poor feeding practices of infants, appeared in the 1980s.They were typically 3-6 months of age presenting with an acute neurological syndrome characterized by generalized tonic-clonic seizures, some lasting for several hours, apnoea or respiratory failure requiring ventilation in nearly half of the affected infants. Plasma sodium was usually below 120mmol/L and readily correctable. The neurological disturbances resolved promptly and these infants had good outcomes[66].

In 1989, Vanapruks et al published case reports on 2 previously healthy neonates who had been fed dilute formula and excessive water and developed severe hyponatremia with seizures. The serum sodium was 117mmol/L in one and 116mmol/L in the other. One was given 5% hypertonic saline, while the other was only fluid-restricted. The serum sodium normalized for both and no further seizures occurred[67]. Bruce et al also published 2 case reports in 1997 on two infants, also presenting with convulsions following water intoxication at home. Following saline infusions, these infants recovered completely with no sequelae[68].

The few studies reporting on hyponatremia in neonates with perinatal asphyxia mainly looked at the incidence of hyponatremia and did not analyze its impact on neurologic outcome[24,25]. Gupta et al carried out a prospective case controlled study to determine the incidence of renal failure in asphyxiated neonates and to correlate severity and type of renal failure with Apgar score and HIE grading of the neonates. It was observed that babies with asphyxia had significantly higher incidence of hyponatremia than healthy controls. In addition to oliguria and abnormal renal sonographic scan, hyponatermia was noted to be an ominous signs predicting mortality in this study[23].

The data appears to be conflicting, with some studies showing no impact while others suggest an adverse effect on neurologic outcome, hence the interest in carrying out this analysis.

5. AIMS /OBJECTIVES OF THE STUDY

Primary objectives:

1. To determine if there is an association between hyponatraemia in neonates with HIE who underwent therapeutic hypothermia and neurodevelopmental outcome at 12 months of age.
2. To determine the association of hyponatraemia with severity of HIE in the neonatal period.

Secondary objective

1. To determine if there is an association between HIE grade and neurodevelopmental outcome

6. METHODS

This is a retrospective descriptive study conducted at the Tygerberg Children's Hospital, Department of Paediatrics & Child Health, Stellenbosch University. The data was initially prospectively collected on infants who underwent therapeutic hypothermia for HIE from 2008 to 2011[69].

6.1 INCLUSION CRITERIA

- Infants who met the criteria for Therapeutic hypothermia (as outlined below)
- Infants who had serum sodium levels done on three consecutive days during their therapeutic hypothermia treatment.
- Infants who were followed up after discharge at 12 months of age.

EXCLUSION CRITERIA

- Infants without follow-up assessment at 12 months, including those who died.

The selection criteria that were used for therapeutic hypothermia:

A. Infants ≥ 36 completed weeks gestation with at least one of the following:

    - Apgar score ≤ 7 at 10 minutes.
    - Continued need for resuscitation, including endotracheal or mask ventilation, at 10minutes after birth.
    - Acidosis within 60 minutes of birth (defined as any occurrence of umbilical cord, arterial or capillary pH < 7.00.
    - Base deficit ≥ 16mmol in umbilical cord or any blood sample (arterial, venous or capillary) within 60 minutes of birth.

B. Infants who met criteria (A) were assessed for whether they met the neurological abnormality entry criteria for therapeutic hypothermia:

    - Altered state of consciousness (reduced or absent response to stimulation)
    - Abnormal primitive reflexes
    - Abnormal tone (focal or general hypotonia, or flaccidity)

For selection for cooling, modified Sarnat (moderate/severe) or an HIE Thompson score of 10 was used as a cut off for selection for cooling.

Exclusion criteria for therapeutic hypothermia were:

- Infants likely to require surgery during the first 3 days after birth.
- Co-existent major congenital abnormalities such as those suggestive of chromosomal anomalies or other syndromes that included brain malformations.
- Outborn infants (from primary or secondary level hospitals within Tygerberg Hospital's drainage area), that could not reach the treatment center within 6-8 hours of delivery.

6.2. TYGERBERG CHILDREN'S HOSPITAL THERAPEUTIC HYPOTHERMIA PROTOCOL

Cooling was started as soon as possible after resuscitation was completed, ideally within 6 hours of birth. Rectal temperatures were monitored with rectal probes and cerebral function monitoring/amplitude integrated electroencephalogram (CFM/ aEEG) commenced at the beginning of cooling (where available). The Tecotherm cooling system guidelines were followed. The initial mattress temperature was set to 28ºC and adjusted according to response. A comprehensive cooling booklet was maintained for each neonate showing cooling starting time, target stop date (72 hours from start), initial temperature and serial rectal temperatures aiming for 33-34 ºC (whole body cooling). For sedation the following drugs were used:

Phenobarbitone 10-20mg/kg (could be repeated up to 40mg/kg),

Lorazepam (Ativan) 0.05mg-0.1mg/kg/dose,

Morphine 50mcg/kg slow IV bolus over 10 minutes, then 50mcg/kg bolus 6hourly or infusion at 8mcg/kg/hour.

The doses would be adjusted according to response.

Rewarming:

Cooling was stopped 72 hours from the time of initiation. The rate of rewarming was not to exceed 0.5ºC / hour.

6.2.1 FLUID MANAGEMENT DURING COOLING

Neonates were kept nil by mouth during the cooling period. The total fluid intake was restricted to 50ml/kg/day on admission to ICU and only potassium-free solutions were used (10% Neonatalyte®). Depending on the renal function and urine output, fluid could further be restricted. For urine output less than 1ml/kg/hr, furosemide (1-2mg/kg intravenously) would be considered as needed.

Electrolytes were monitored and corrected as needed. The fluid restriction was released if the serum sodium, renal function and urine output were normal. For hyponatremia (serum sodium less than 135mmol/L), a 5% hypertonic saline solution was used for correction intravenously, at a rate that did not exceed 0.5mmol/hr.

6.3 METHOD OF DATA COLLECTION

6.3.1 SERUM SODIUM

Each patient received a booklet at the start of cooling, where daily records of serum electrolytes and other parameters were kept for the duration of the cooling and re-warming period. For the purpose of this analysis, data were obtained from these cooling booklets as well as from daily clinical notes in patients' files and from the hospital's laboratory system (for incomplete data).

6.3.2 NEURODEVELOPMENTAL ASSESSMENT

After completion of cooling, patients were discharged from the ICU to neonatal wards and eventually discharged home. They were followed up by a neurodevelopmental specialist. Neurodevelopmental assessment was done at 12 months of age, using the Bayley-III Screening Test. The data obtained from these follow-up visits was stored in a database. For the purpose of this analysis, data obtained from these visits will be used and augmented with data from patient records.

6.3.2.1 BAYLEY-III SCREENING TEST

The Bayley-III Screening Test is used to assess the cognitive, language, and motor functioning of infants and young children between 1 month and 42 months of age.

The test items are a subset of the cognitive, language, and motor items of the Bayley

Scales of Infant and Toddler Development, 3rd edition. The screening tool is used to determine if further, more comprehensive evaluation is needed. The child's performance is scored on the basis of a risk category classification: competent, emerging, or at risk.

CLASSIFICATION	DEFINITION
Competent	Child is considered at low risk for developmental delay and in most cases does not need further evaluation.
Emerging	Child has some risk for developmental delay, but further evaluation is made on the basis of other information collected. Can monitor development or refer for further evaluation.
At risk	Child is most likely in need of further evaluation to determine need for early intervention.

TABLE 2: Definitions of BSID-III Screening Classification[70]

In this study the terms competent, emerging and at risk were used to refer to the above performance categories.

6.4 STATISTICAL ANALYSIS

Each infant had serum sodium levels measured in the laboratory on three consecutive days (day 1 – day 3) during TH. Hyponatremia was defined as a serum sodium less than 135mmol/L and hypernatremia was defined as a serum sodium more than 145mmol/L. Since the majority of patients' sodium levels were between 130 and 134 mmol/L and too few infants had hyponatremia levels below 130 mmol/L, we determined the frequency or number of hyponatremic episodes on the three consecutive days of cooling. This approach increased the data points to 63 (3 x 21), giving us some statistical power. Out of the three serum sodium levels recorded per patient, the number

of times each neonate was hyponatremic or hypernatremic was noted.

This study seeks to determine if there is any association between the number of times a neonate was hyponatremic (0-3) and the neurodevelopmental outcomes at 12 months of age (competent, emerging and at risk) for the patients who survived. We also ascertained the relationship between the grade of HIE and hyponatremia. The three variables (neurodevelopment outcome, degree of HIE, number of hyponatremic episodes) represents categorical data hence the association between them was analysed using non-parametric methods - Kendall's tau-b and Spearman ranked order (rho) correlation coefficients. The associations between the following variables were measured:

- Number of hyponatremic episodes and neurodevelopmental outcome
- Number of hyponatremic episodes and HIE grade
- HIE grade and neurodevelopmental outocome

The calculations were done using IBM *SPSS Statistics 22* software.

Sample size determination

A sample size of 48 achieves 80% power to detect a difference of 0.30000 between the null hypothesis correlation of 0.00000 and the alternative hypothesis correlation of 0.30000 using a two-sided hypothesis test with a significance level of 0.05000

7. RESULTS

The patient records yielded 100 patients who were potential candidates for the study from 2008 to 2011. Of these patients:

- twenty-eight (28) patients were never seen post discharge.
- seventeen (17) patients died before 12 months
- Fifty-five (55) patients had post discharge follow-up:
    - Fifty patients were followed up at 12 months
    - Five patients were seen before 12 months:

50 patients were included in this study. These are patients who were seen at 12 months follow-up.

7.1. DESCRIPTIVE STATISTICS (PERINATAL PERIOD)

Out of 50 patients analyzed, 21/50 (42%) had hyponatremic episodes while the rest (29/50 [58%]) were normonatremic. Of the hyponatremic infants, 10/21 (47.62%) were male while the rest were female (52.38%). The mean birth weight of hyponatremic infants was 3032g and the average pH within the first hour of life was 7.08 (cord sample or neonatal-derived). Six of the hyponatremic infants (28.57%) had seizures detected by aEEG during the 3days of TH. Of the normonatremic infants, the majority were male (24/29) with only 5 females. The average birth weight of these infants was 3222g and the average pH was 7.02. Fifteen of these infants (51.72%) had seizures (on aEEG) in

the first few days of life. This is summarized in table 3. The distribution of HIE grades among hyponatremic and normonatremic infants is also shown. Hyponatremic infants constituted the majority (6/7;85.71%) of those with severe HIE as compared to normonatremic infants. The differences of the other variables between hyponatremic and normonatremic infants were not statistically significant.

	HYPONATREMIC (n = 21)	**NORMONATREMIC** n=29	**TOTAL N=50**
MALES	10 (47.62%)	24 (82.76%)	**34**
FEMALES	11 (52.38%)	5 (17.24%)	**16**
MEAN BIRTH WEIGHT (g)	3032.4	3223.0	
Standard Deviation	778.51	519.63	
MEAN PH	7.08	7.02	
Standard Deviation	0.097	0.178	
SEIZURES	6 (28.57%)	14 (48.28%)	**20**
HIE GRADE 1	8	14	**22**
2	6	12	**18**
3	6	1	**7**
Undocumented	1	2	**3**

TABLE 3: PATIENT CHARACTERISTICS

7.1.1 HIE grades

Forty-seven (47/50) of the patients had their HIE grades documented. The frequency of different grades is shown on the bar chart below

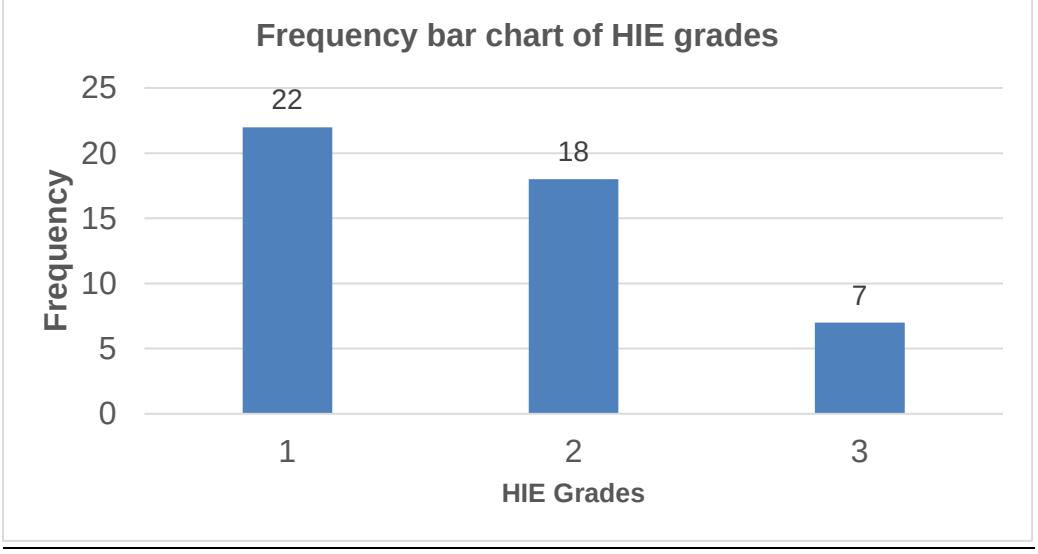

Figure 1 Frequency bar chart of HIE grades

7.1.2 SERUM SODIUM LEVELS

The patients had serial serum sodium levels measured on the first three consecutive days of life while undergoing therapeutic hypothermia. Of the fifty analysed patients, 21/50 (42%) had hyponatremia and the rest were normonatremic 29/50 (58%).

Of the hyponatremic patients;

- 14/21 had sodium between 130 - 134 mmol/L.
- 7/21 had sodium levels between 124 and 129.

The distribution of serum sodium levels for each of the three consecutive days is shown on the boxplot (figure 2) and the dispersion was measured using the standard deviation from the mean serum sodium level.

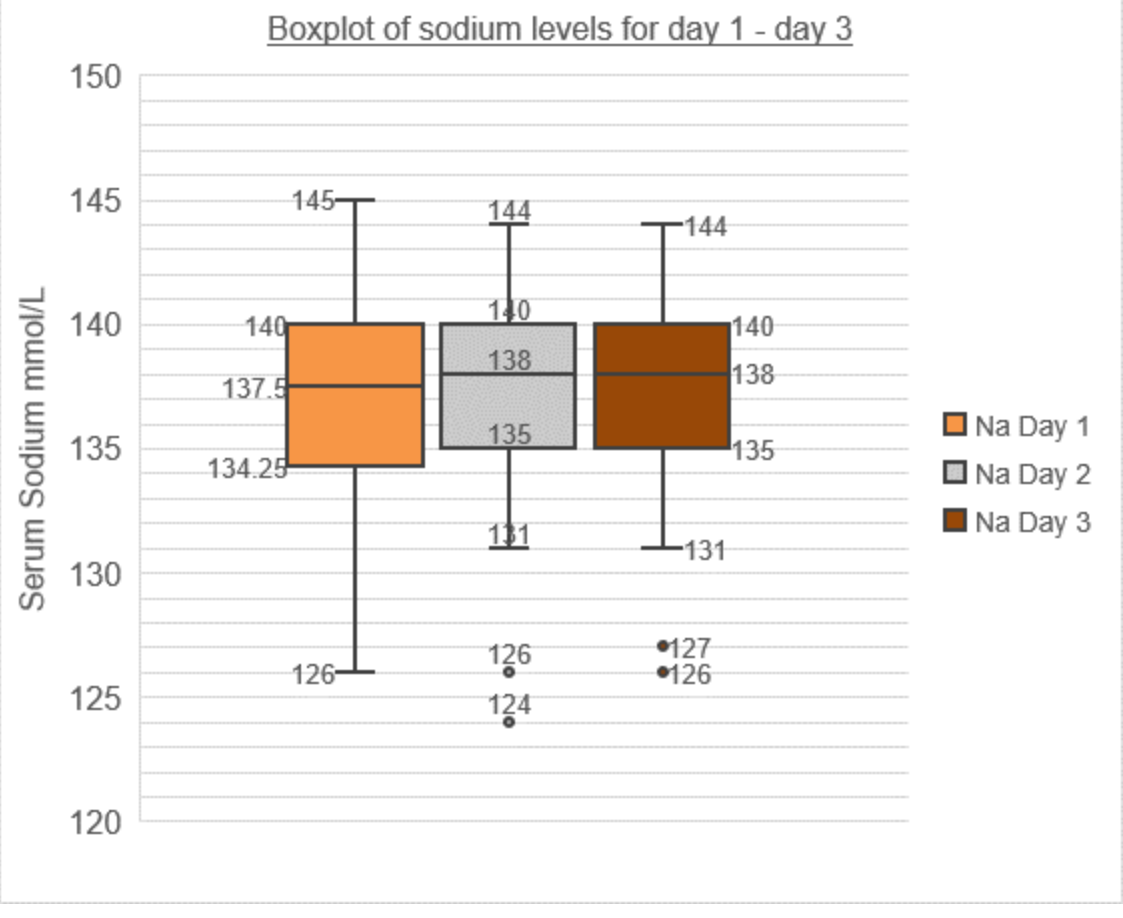

Figure 2: Boxplot of Sodium levels for days 1 to 3.

Hyponatremic infants tended to have a relatively high proportion with severe HIE grades compared to normonatremic infants. Of all the infants with HIE grade 1 (n = 22), 14 of them (63.64%) were normonatremic, while 6/7 (85.71%) of those with HIE grade 3 were hyponatremic. Twenty-nine percent (6/21) of hyponatremic infants had HIE grade 3 compared to 3% (1/29) in the normonatremic infants. A hyponatremic infant was 8 times

more likely to have an HIE grade of 3 than a normonatremic infant (RR = 8.29) as shown in table 4 and figure 3 below.

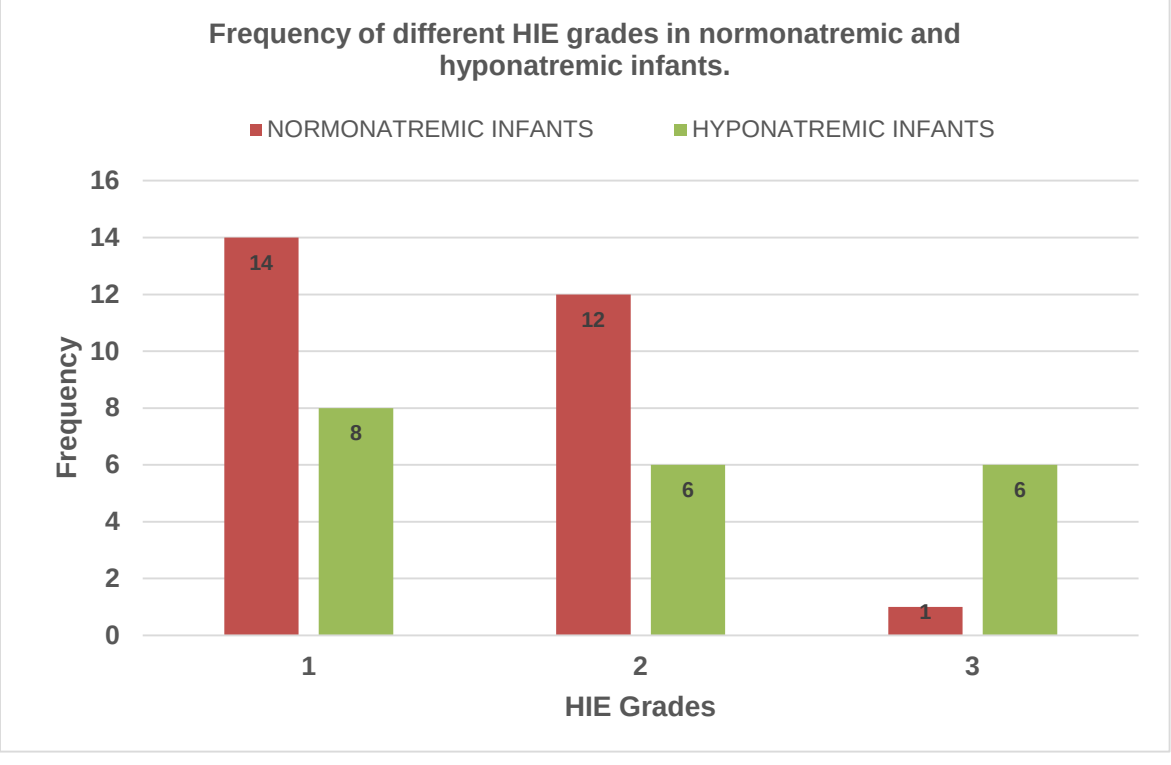

Figure 3: Frequency of different HIE grades in normonatremic and hyponatremic infants.

HIE GRADE	NORMONATREMIC INFANTS	HYPONATREMIC INFANTS	Rel. Risk
1	14	8	0.79
2	12	6	0.69
3	1	6	8.29
Undocumented	2	1	

Table 4: Frequency of HIE Grades in hyponatremic and normonatremic infants with relative risk

The frequency of hyponatremic episodes reflects how many days out of the three during TH an infant had hyponatremia. The majority of the hyponatremic infants had hyponatremia on one day out of three. Only 3/21 had hyponatremia on all three days, as shown in figure 4.

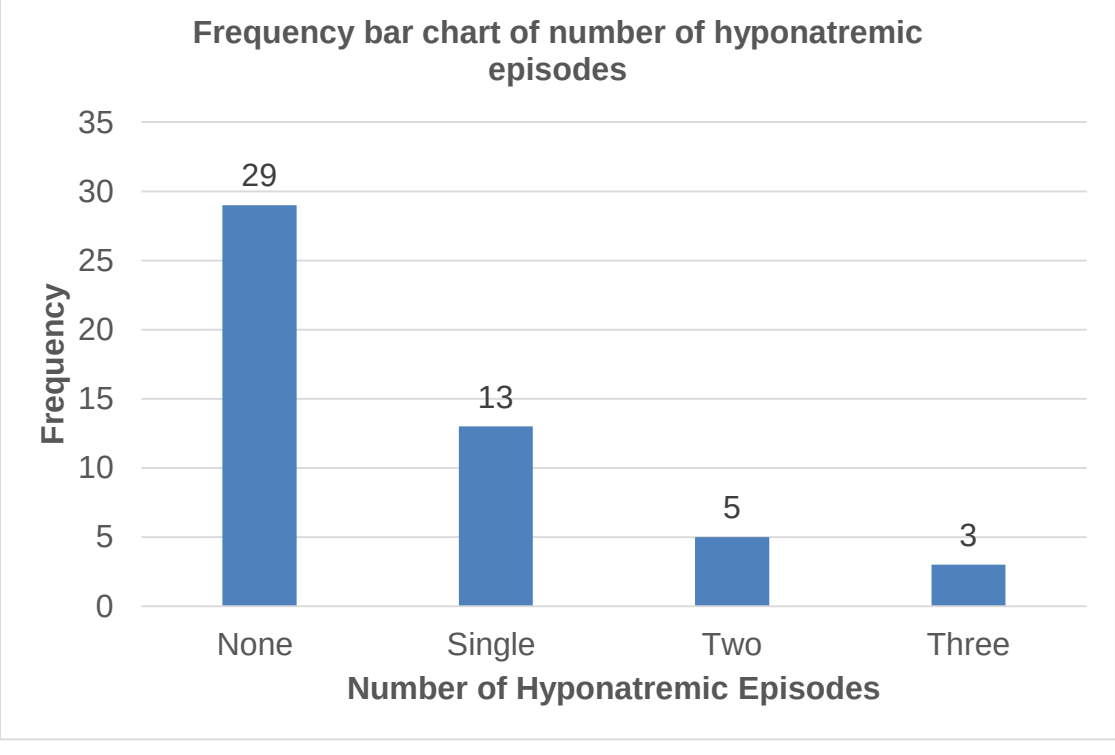

Figure 4: Frequency bar chart of number of hyponatremic episodes

7.1.3 NEURODEVELOPMENTAL OUTCOME

Neurodevelopmental assessment was done using the Bayley III Screening tool. The frequency bar chart below shows the distribution of neurodevelopmental functional levels as scored by the neurodevelopmental specialist at the 12-month follow-up. Thirty-

five (70%) were assessed as competent, nine (18%) as emerging and six (12%) as at risk (Fig 1.3).

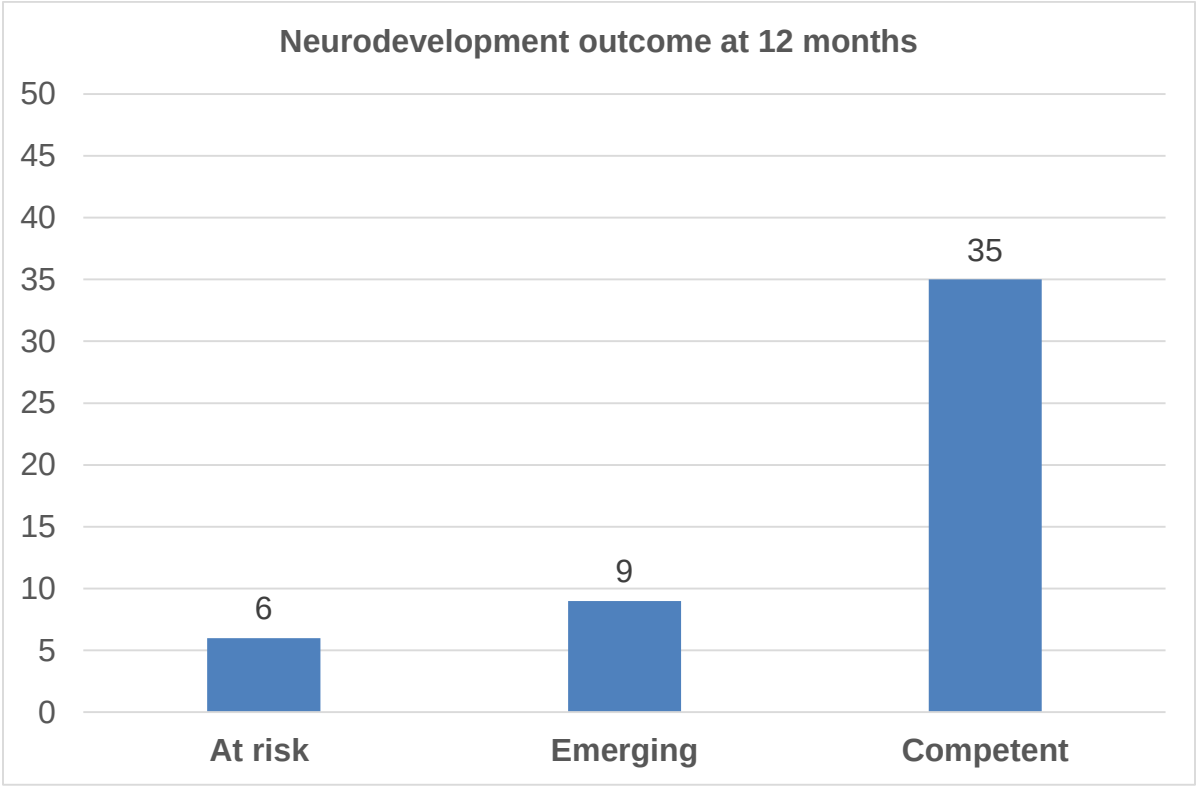

Figure 5 Frequency bar chart of neurodevelopmental functional status at 12 months

Of all the neonates who had been hyponatremic, 15/21 (71.43%) were assessed as “Competent” at 12 months, while 3/21 (14.29%) were assessed as “Emerging” and 3/21 as “At risk”. Of those who had been normonatremic, 20/29 (68.97%) were assessed as “Competent”, 6/29 (20.69%) as “Emerging” and 3/29 (10.34%) “At risk”.The difference between proportions of outcome in hyponatremic and normonatremic infants is not statistically significant, as shown in figure 6.

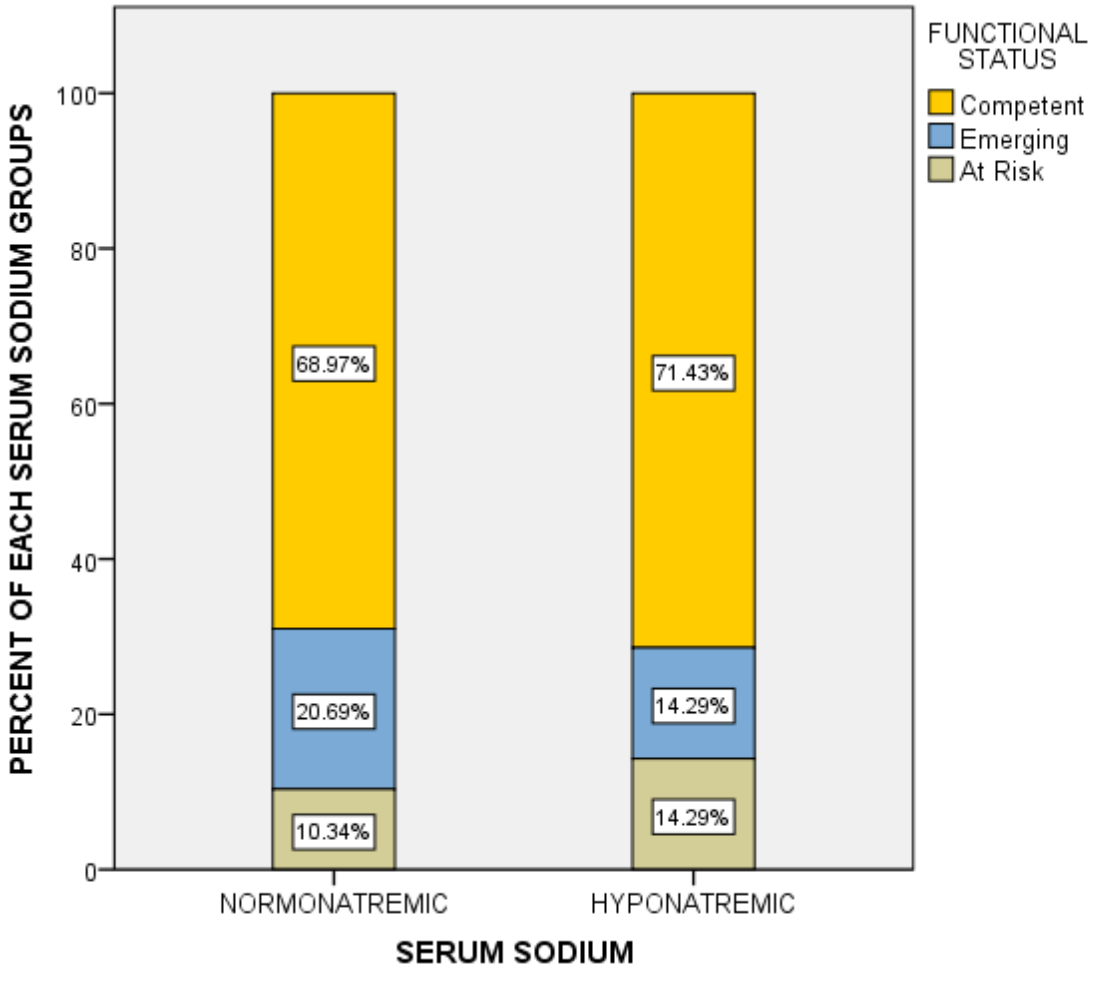

Figure 6 Neurodevelopmental outcome in Hyponatremic Vs Normonatremic Neonate

7.2. ANALYTIC STATISTICS

The research question is to investigate if there is any association between:

1. Neurodevelopmental outcome at 12 months of age and hyponatremia.
2. Hyponatremia and HIE grade

Two statistical tests (Kendall's tau b and Spearman's ranked order) were used to

assess for the presence of the above-mentioned relationships. The results were reproducible for both statistical tests (Table 3).

CORRELATED VARIABLES	TEST STATISTIC	CORRELATION COEFFICIENTS	SIGNIFICANCE
Number of hyponatremic episodes AND Neurodevelopmental functional status	Kendall's tau_b	0.018	0.444
	Spearman's rho	0.021	0.443
HIE grade AND Neurodevelopmental functional status at 12 months	Kendall's tau_b	0.123	0.184
	Spearman's rho	0.13	0.193
Number of hyponatremic episodes and HIE grade	Kendall's tau_b	0.224	0.024
	Spearman's rho	0.25	0.025

Table 5: Cross-tabulation for functional status vs number of hyponatremic episodes

There was no association between neurodevelopmental functional status at 12 months and number of hyponatremic episodes in the first 3 days of life (p = 0.444). There was significant positive correlation between number of hyponatremic episodes and HIE grade with both Kendall's tau_b and Spearman's rho test (p = 0.024 and p 0.025 respectively). We found no association between HIE grade and neurodevelopmental outcome (p = 0.184)

8. DISCUSSION

The primary objective of the study was to assess for an association between hyponatremia in neonates with HIE who underwent cooling, and neurodevelopmental outcome at 12 months of age. The secondary objective was to determine the association of hyponatraemia with severity of HIE in the neonatal period. Our study showed no association between hyponatremia and outcome at 12 months. Studies done previously showed conflicting results, with some showing complete recovery following hyponatremic encephalopathy[66-68], while others showed an increased risk of mortality and morbidity[56,64,65]. The reports of good outcomes were however of patients with no pre-existing comorbidities. We found a positive correlation between HIE grade and number (frequency) of hyponatremic episodes; neonates with severe HIE had a higher frequency of hyponatremia. This is in keeping with previous studies that showed that the degree of hyponatremia was directly proportional to the severity of HIE[24].

Hypertonic saline was administered to hyponatremic patients in this study. This may have had an impact on the results, especially since the majority (14/21) of them had mild hyponatremia (serum sodium 130 – 134mmol/L). Moreover, the serum sodium levels were taken at 24 hour intervals. Significant deviations might have been noted if levels were checked more frequently. The implementation of TH has been shown to modify the predictive value of variables that have previously been shown to be useful in predicting neurodevelopmental outcome after HIE[20]. This could be another reason why there was no association found between hyponatremia and outcome in this study.

Variables that had been shown to affect neurodevelopmental outcome following perinatal asphyxia pre-cooling include APGAR scores, pH within 1 hour of birth, base deficit, HIE grade, birth weight, presence/absence of seizures and aEEG abnormalities[19]. In this study, a significant difference between hyponatremic and normonatremic infants was noted in the distribution of HIE grades - 85.7% of those with grade 3 being hyponatremic as compared to 57.1% of those with grade 1. There was also a significant difference in the occurrence of seizures, the majority of infants who had seizures (15/21;71.4%) were normonatremic and only 6/21 (28.6%) had hyponatremia. Mean birth weight was lower in the hyponatremic group. A larger sample size would have allowed for multi-variate analysis in order to assess for potential confounders.

We also looked for an association between HIE grade and neurodevelopmental outcome at 12 months of age. No association was found between these two variables although previous studies have shown HIE grade to be predictive of outcome[19]. This again could be due to the fact that these patients underwent therapeutic hypothermia, altering the predictive value of HIE grade. Selection bias was also a possibility as only those with complete data present were studied.

A strength of the study is that it analyzed a uniform sample with sodium measurements on three consecutive days, regular neurological assessment and outcome data beyond discharge.

Future research perspectives:

A statistically powered study allowing us to do more subpopulation analysis would yield more information. This may potentially need a multi-center conducted study. This would allow stratification according to hyponatremia severity as well as multivariate analysis.

9. CONCLUSION

There was no association between neurodevelopmental outcome at 12 months and number of hyponatraemic episodes in the first week of life in neonates with HIE who underwent therapeutic hypothermia. There was significant association between number of hyponatremic episodes and HIE grade. We found no association between neurodevelopmental functional status at 12 months and HIE grade.

10. REFERENCES

1. James A, Patel V. Hypoxic ischaemic encephalopathy. *Paediatr Child Health*. 2014; 24(9):385-389.
2. Lai MC, Yang SN. Perinatal Hypoxic-Ischemic Encephalopathy. *J Biomed Biotechnol.* 2011; 11(3): 609813-609819.
3. Allen KA, Brandon DH. Hypoxic Ischemic Encephalopathy: Pathophysiology and Experimental Treatments. *Newborn Infant Nurs Rev.* 2011;11(3): 125-133.
4. Zanelli, SA, Kaufman DA, Stanley DP*. Hypoxic-Ischemic Encephalopathy Clinical Presentation: History, Physical, Causes*. Available from http://emedicine.medscape.com/article/973501. Accessed 09th November 2016.
5. Antonucci R, Porcella A, Pilloni MD. Perinatal asphyxia in the term newborn. *J Pediatr Neonat Individual Med*. 2014;3(2):e030269 doi: 10.7363/030269
6. Martín-Ancel A, García-Alix A, Gayá F, Cabañas F, Burgueros M, et al. Multiple organ involvement in perinatal asphyxia. J Pediatr 1995;127(5):786–93.
7. Shah P, Riphagen S, Beyene J, Perlman M. Multiorgan dysfunction in infants with post-asphyxial hypoxic-ischaemic encephalopathy. *Arch Dis Child Fetal Neonatal Ed*. 2004. 89(2): F152-F155.
8. Portman RJ, Carter BS, Gaylord MS, Murphy MG, Thieme RE, Merenstein GB.. Predicting neonatal morbidity after perinatal asphyxia: a scoring system. *Am J Obstet Gynecol.* 1990;162(1): 174-181.
9. Lipper EG, Voorhies TM, Ross G, Vannucci RC, Auld PAM. Early predictors of one-year outcome for infants asphyxiated at birth. *Dev Med Child Neurol*. 1986; 28(3): 303-309.

10. Bao XL, Yu RJ, Li ZS**.** 20 Item neonatal behavioural neurological assessment used in predicting prognosis of asphyxiated newborn. *Chin Med J*. 1993; 106(3): 211-215.
11. Karnatovskaia LV, Wartenberg KE, Freeman WD. Therapeutic Hypothermia for Neuroprotection: History, Mechanisms, Risks, and Clinical Applications. *Neurohospitalist.* 2014; 4(3): 153–163.
12. Varon J, Acosta P. Therapeutic Hypothermia Past, Present, and Future. *Chest*. 2008; 133(5):1267–1274.
13. Gunn AJ, Gunn TR, de Haan HH, Williams CE, Gluckman PD. Dramatic neuronal rescue with prolonged selective head cooling after ischemia in fetal lambs. *J Clin Invest.* 1997;99(2):248-256.
14. Shankaran S. Outcomes of hypoxic-ischemic encephalopathy in neonates treated with hypothermia. *Clin Perinatol.* 2014;41(1):149-159.
15. Perlman JM, Wyllie J, Kattwinkel J, Atkins DL, Chameides L, Goldsmith JP, Guinsburg R, et al. Neonatal Resuscitation Chapter Collaborators. Part 11: neonatal resuscitation: 2010 International Consensus on Cardiopulmonary Resuscitation and Emergency Cardiovascular Care Science With Treatment Recommendations. Circulation. 2010;122(suppl 2): S516–S538.
16. Drury PP, Gunn ER, Bennet L, Gunn AJ. Mechanisms of Hypothermic Neuroprotection. *Clin Perinatol.* 2014; 41(1):161–175.
17. Silveiraa RC, Procianoy RS. Hypothermia therapy for newborns with hypoxic ischemic encephalopathy. *J. Pediatr. (Rio J.).* 2015; 91(6): S78-83.
18. Sarkar S, Barks JD. Systemic complications and hypothermia. *Semin Fetal Neonatal Med*. 2010;15(5):270-275.

19. Sabir H, Cowan FM. Prediction of outcome methods assessing short- and long-term outcome after therapeutic hypothermia. *Semin Fetal Neonatal Med*. 2015; 20(2): 115-121.
20. Gunn AJ, Wyatt JS, Whitelaw A, Barks J, Azzopardi D, Ballard R, et al. Therapeutic Hypothermia Changes the Prognostic Value of Clinical Evaluation of Neonatal Encephalopathy. *J Pediatr.* 2008;152(1):55-58.
21. Modi N. Fluid and electrolyte balance. In; Rennie, JM.(ed). *Rennie and Roberton's Textbook of Neonatology*. 5th ed. Edinburgh: Churchill Livingstone/Elsevier; 2012. p.337.
22. Marcialis MA, Dessi A, Pintus MC, Irmesi R, Fanos V. Neonatal hyponatremia: differential diagnosis and treatment. *J Matern Fetal Neonatal Med*. 2011; 24(S(1)): 75-79.
23. Gupta BD, Sharma, P, Bagla J, Parakh M, Soni JP. Renal Failure in Asphyxiated Neonates. *Indian Pediatr*. 2005;42(9):928-934.
24. Basu P, Som S, Das H, Choudhuri N. Electrolyte Status in Birth Asphyxia. Indian J Pediatr. 2010; 77 (3):259-262.
25. Gluckman PD, Wyatt JS, Azzopardi D, Ballard R, Edwards AD, Ferriero DM, et al. Selective head cooling with mild systemic hypothermia after neonatal encephalopathy: multicentre randomized trial. *Lancet*. 2005; 365(9460), 663 – 670.
26. Chawla D, Agarwal R, Deorari AK, Paul VK. Fluid and Electrolyte Management in Term and Preterm Neonates. *Indian J Pediatr.* 2008; 75(3): 255-259.
27. Posencheg MA, Evans JR. Acid-Base, Fluid, and Electrolyte Management. In; Gleason CA, Devaskar SU. (eds). *Avery's Diseases of the New Born*. 9th ed. Philadelphia: Saunders, an imprint of Elsevier Inc; 2012, p 368.

28. Modi N. Hyponatremia in the newborn. *Arch Dis Child Fetal Neonatal Ed*. 1998;78(2): F81–F84.

29. Modi N. Fluid and electrolyte balance. In; Rennie, JM.(ed). *Rennie and Roberton's Textbook of Neonatology*. 5th ed. Edinburgh: Churchill Livingstone/Elsevier; 2012. p339.

30. Moritz ML, Ayus JC. Prevention of hospital-acquired hyponatremia: do we have the answers? *Pediatrics.* 2011;128(5):980-983.

31. Holliday MA, Segar WE. The maintenance need for water in parenteral fluid therapy. *Pediatrics*. 1957;19(5):823-832.

32. West CR, Harding JE. Maternal water intoxication as a cause of neonatal seizures. *J. Paediatr. Child Health*. 2004; 40(12): 709–710.

33. Valerio E, Fantinato M, Giovannini IAB, Baraldi E, Chiandetti L. Severe asymptomatic maternal antepartum hyponatremia leading to neonatal seizures: Prevention is better than cure. *Matern Health Neonatol Perinatol.* 2015; 1(25):1-5.

34. Schwartz RH, Jones RWA. Transplacental hyponatraemia due to oxytocin. *Br Med J*. 1978; 1(6106):152-153.

35. Tarnow-Mordi WO, Shaw JCL, Liu D, Gardner DA, Flynn FV. Iatrogenic hyponatraemia of the newborn due to maternal fluid overload: a prospective study. *Br Med J (Clin Res Ed).* 1981; 283(6292):639-642.

36. Modi N. Fluid and electrolyte balance. In; Rennie, JM.(ed). *Rennie and Roberton's Textbook of Neonatology*. 5th ed. Edinburgh: Churchill Livingstone/Elsevier; 2012. p336.

37. Durkan AM, Alexander RT. Acute Kidney Injury Post Neonatal Asphyxia. *J Pediatr.* 2011; 158(Suppl. 2): e29-33.

38. Rennie JM, Huertas-Ceballos A, Boylan GB. Neurological problems in the newborn. In; Rennie, JM.(ed) *Rennie and Roberton's Textbook of Neonatology*. 5th ed. Edinburgh: Churchill Livingstone/Elsevier, 2012. p1136.
39. Agrawal S, Chaudhuri PK, Chaudhary AK, Kumar D. Acute kidney injury in asphyxiated neonates and its correlation to hypoxic ischemic encephalopathy staging. *Indian J Child Health.* 2016; 3(3): 254-257.
40. Selewski DT, Jordan BK, Askenazi DJ, Dechert RE, Sarkar S. Acute kidney injury in asphyxiated newborns treated with therapeutic hypothermia. *J Pediatr. 2013*;162(4):725-729.
41. Modi N. Fluid and electrolyte balance. In; Rennie, JM.(ed). *Rennie and Roberton's Textbook of Neonatology*. 5th ed. Edinburgh: Churchill Livingstone/Elsevier; 2012. p338.
42. Rees L, Brook CDG, Shaw JCL, Forsling ML. Hyponatraemia in the first week of life in preterm infants. I. Arginine vasopressin secretion. *Arch Dis Child*.1984;59(4):414-422.
43. Skippen P, Adderley R, Bennett M, Cogswell A, Froese N, Seear M, et al. Iatrogenic hyponatremia in hospitalized children: Can it be avoided? *Paediatr Child Health.* 2008; 13(6): 502-506.
44. Singhi S. Hyponatremia in hospitalized critically ill children: Current concepts. *Indian J Pediatr*. 2004;71(9): 803 – 807.
45. Easley D, Tillman E. Hospital-Acquired Hyponatremia in Pediatric Patients: A Review of the Literature. *J Pediatr Pharmacol Ther*. 2013;18(2):105–111.

46. Friedman JN. Risk of acute hyponatremia in hospitalized children and youth receiving maintenance intravenous fluids. *Paediatr Child Health.* 2013;18(2): 102-104.
47. Wardrop, C.A., Holland, B.M. The roles and vital importance of placental blood to the newborn infant*. J Perinat Med.*1995; 23(1-2):139–143.
48. Gerigk, M., Gnehm, H.E., Rascher, W.Arginine vasopressin and renin in acutely ill children: implication for fluid therapy. *Acta Paediatr.*1996; 85(5):550–553
49. Diringer MN, Zazulia AR. Hyponatremia in neurologic patients: consequences and approaches to treatment. *Neurologist*. 2006;12(3):117-126.
50. Prempunpong C, Efanov I, Sant'Anna G. The effect of the implementation of therapeutic hypothermia on fluid balance and incidence of hyponatremia in neonates with moderate or severe hypoxic–ischaemic encephalopathy. *Acta Paediatr.* 2013; 102(11): e507-513.
51. Sarkar S, Barks JD. Systemic complications and hypothermia. *Semin Fetal Neonatal Med*. 2010;15(5):270-275.
52. Jacobs SE, Berg M, Hunt R, Tarnow-Mordi WO, Inder TE, Davis PG. Cooling for newborns with hypoxic ischaemic encephalopathy. *Cochrane Database Syst Rev*. 2013; 31;1:CD003311.
53. Giuliani C, Peri A. Effects of Hyponatremia on the Brain. *J. Clin. Med.* 2014; 3(4): 1163-1177.
54. Verbalis JG. Brain volume regulation in response to changes in osmolality. *Neuroscience*. 2010;168(4):862-70.
55. Fisher SK., Heacock AM, Keep RF, Foster DJ. Receptor regulation of osmolyte homeostasis in neural cells. *J. Physiol*. 2010; 588(18), 3355–3364.

56. Arieff AI, Ayus JC, Fraser CL. Hyponatraemia and death or permanent brain damage in healthy children. *Br Med J. 1992*; 304(6836): 1218–1222.
57. Ayus JC, Achinger SG, Arieff A. Brain cell volume regulation in hyponatremia: role of sex, age, vasopressin, and hypoxia. *Am J Physiol Renal Physiol.*2008;295(3): F619-F624.
58. Ayus JC, Armstrong D, Arieff AI. Hyponatremia with hypoxia: Effects on brain adaptation, perfusion, and histology in rodents. *Kidney Int.* 2006;69(8):1319-1325.
59. Vexler ZS, Ayus JC, Roberts TP, Fraser CL, Kucharczyk J, Arieff AI. Hypoxic and ischemic hypoxia exacerbate brain injury associated with metabolic encephalopathy in laboratory animals. *J Clin Invest.*1994; 93(1): 256–264.
60. Ayus JC, Arieff AI. Pulmonary complications of hyponatremic encephalopathy. Noncardiogenic pulmonary edema and hypercapnic respiratory failure. *Chest*. 1995;107(2):517-521.
61. Spatenkova V, Bradac O, Skrabalek P. Outcome and frequency of sodium disturbances in neurocritically ill patients. *Acta Neurol Belg*. 2013;113(2):139-145.
62. Hasan D, Wijdicks EF, Vermeulen M. Hyponatremia is associated with cerebral ischemia in patients with aneurysmal subarachnoid hemorrhage. *Ann Neurol*. 1990;27(1):106-108.
63. Qureshi AI, Suri MFK, Sung GY, Straw RN, Yahia AM, Saad M, et al. Prognostic significance of hypernatremia and hyponatremia among patients with aneurysmal subarachnoid hemorrhage. *Neurosurgery*. 2002;50(4):755–756.
64. Al-Zahraa Omar F, Al Bunyan M. Severe hyponatremia as poor prognostic factor in childhood neurologic diseases. *J Neurol Sci.* 1997; 151 (2):213–216.

65. McJunkin JE, de los Reyes EC, Irazuzta JE, Caceres MJ, Khan RR, Minnich LL, et al. La Crosse encephalitis in children. *N Engl J Med.* 2001;344(11):801-807.

66. No Author listed. Excess water administration and hyponatraemic convulsions in infancy. *The Lancet*. 1992; 339(8786):153 – 155.

67. Vanapruks V, Prapaitrakul K. Water intoxication and hyponatraemic convulsions in neonates. *Arch Dis Child*. 1989;64(5):734-735.

68. Bruce RC, Kliegman RM. Hyponatremic seizures secondary to oral water intoxication in infancy: association with commercial bottled drinking water. *Pediatrics*. 1997;100(6): E4.

69. Kali GT, Martinez-Biarge M, Van Zyl J, Smith J, Rutherford M. Management of therapeutic hypothermia for neonatal hypoxic ischaemic encephalopathy in a tertiary centre in South Africa. *Arch Dis Child Fetal Neonatal Ed.* 2015;100(6): F519-523.

70. Jackson BJ, Needelman H, Roberts H, Willet S, McMorris C. Bayley Scales of Infant Development Screening Test-Gross Motor Subtest: Efficacy in Determining Need for Services. *Pediatr Phys Ther*. 2012;24(1):58–62.