WHOLE BODY COOLING (WBC) FOR NEWBORN INFANTS WITH PERINATAL ASPHYXIA: A FEASIBILITY TRIAL

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CERTIFICATE

This is to certify that the dissertation entitled "**Whole Body Cooling (WBC) For Newborn Infants With Perinatal Asphyxia: A Feasibility Trial"** is a bonafide, original work done by **Dr. Koshy C. George**, during his academic term – **March 2007 to February 2009**, at the Christian Medical College, Vellore, in partial fulfillment of the rules and regulations for the award of M.D (Branch VII – Paediatrics) degree of The Tamil Nadu Dr. MGR Medical University, Chennai to be held in March 2009.

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This work is dedicated to my father who was unable to realize his dream of watching his son step into the medical field. I love you, Dad. **Koshy C. George**

TABLE OF CONTENTS

INTRODUCTION

Neonatal mortality accounts for two-thirds of the infant mortality rate in India and other developing countries.[1] It is estimated that over 1 million newborn infants die during the first four weeks of life.[2] The current neonatal mortality rate in India is 39 per 1000 live births.[1] Asphyxia was the single most important cause of still-births, accounting for 45.1% of all intrauterine deaths and the primary cause for neonatal mortality, accounting for 28.8% of all the deaths.[2] Morbidity associated with perinatal asphyxia includes cerebral palsy, learning disabilities, visual and hearing impairments, behavioural abnormalities and residual motor or cognitive disabilities. These have a considerable effect on the surviving babies, their families and on society.[3,4]

The etiology of perinatal Hypoxic Ischemic Encephalopathy (HIE) includes those conditions that can affect the cerebral blood flow in the fetus and newborn compromising the supply of oxygen to the brain. They may develop antepartum (20%), intrapartum (30%), intrapartum and antepartum (35%) or postpartum (10%).[3] HIE that develops in the setting of perinatal asphyxia is part of a multiorgan system disease.[5]

After an asphyxial event, there may be an opportunity to intervene to minimize brain damage. The first phase of brain damage, early cell death, results from the primary exhaustion of cellular energy stores. Early cell death can occur within minutes. Damage to the brain is limited at this stage by immediate resuscitation to restore oxygen supply and blood circulation. A secondary phase of neuronal injury occurs some times after the initial insult. There are several mechanisms involved in this process. Treatment during the post resuscitation phase aim to block these processes thereby limiting secondary cell damage and minimizing the extent of potential brain damage.

Neuroprotection was achieved in animal models of brain ischemia by reduction in brain temperature by 2°C to 5°C.[6-11] Randomized control trials done in the developed countries using expensive equipment was shown to reduce mortality and morbidity among newborn survivors of perinatal asphyxia.[12-17] The present trial was conducted to evaluate whether whole-body cooling could be achieved in a low-resource setting using simple available cooling materials.

AIM AND OBJECTIVES

Aim and objectives

To study the feasibility of whole body cooling for newborn infants with perinatal asphyxia in a low resource setting.

Primary objective

Achievement of target temperature within 1 hour of initiation of treatment and within 6 hours of birth and maintaining the target temperature for 72 hours.

Secondary objectives

Monitoring adverse events and possible complications that could occur secondary to whole body cooling

1. Cardiac arrhythmia
2. Persistent hypoxemia
3. Hypotension despite full inotropic support
4. Skin changes
5. Thrombocytopenia
6. Life threatening coagulopathy
7. Arterial thrombosis
8. Hepatic and renal failure
9. Electrolyte disturbances
10. Death

LITERATURE REVIEW

Encephalopathy following perinatal asphyxia is the most common neurologic disease during the neonatal period (hypoxic ischemic encephalopathy, HIE). It is associated with a high mortality and morbidity including cerebral palsy, mental retardation, visual and hearing impairment, behavioural abnormalities and seizures. The incidence of HIE is 1.0-1.5% in most centers and is usually related to the gestational age and birth weight.[18]

Definition of perinatal asphyxia

There is no gold standard definition of perinatal asphyxia that exists to date.

The WHO defines perinatal asphyxia as a "failure to initiate and sustain breathing at birth".[19]

NNPD defines birth asphyxia[2] as

Definition I

- Moderate birth asphyxia: Slow gasping breathing at 1-minute of age.
- Severe birth asphyxia: No breathing at 1-minute of age.

Definition II

- Birth asphyxia: Apgar score of <7 at 1-minute of age.
- Moderate birth asphyxia: Apgar score of 4-6 at 1-minute of age.
- Severe birth asphyxia: Apgar score of <3 at 1-minute of age.

For HRRC sites: cry after 5 minutes of age or no cry at all.

The American Academy of Pediatrics (AAP) and the American College of Obstetrics and Gynecology (ACOG) criteria states that all of the following must be present for diagnosis of asphyxia[20]

i. Profound metabolic or mixed academia (pH < 7.00) in cord arterial blood

ii. Persistence of Apgar scores 0-3 for >5 minutes

iii. Neonatal neurologic sequelae (seizures, coma, hypotonia)

iv. Multiple organ involvement (kidneys, lungs, liver, heart intestine)

Pathogenesis of hypoxic ischemic brain injury

Impaired cerebral blood flow is the predominant pathogenetic mechanism underlying the neuropathology attributed to intrapartum hypoxia-ischemia. This is most likely to occur as a consequence of interruption in placental blood flow and gas exchange causing severe fetal acidemia which is defined as a fetal umbilical arterial pH of ≤7.00.[21] Hypoxia ischemia is associated with two phases of pathologic events that culminate in brain injury (Fig 1.). These phases are the primary and secondary energy failure based on the characteristics of the cerebral energy states as described in experimental animals.

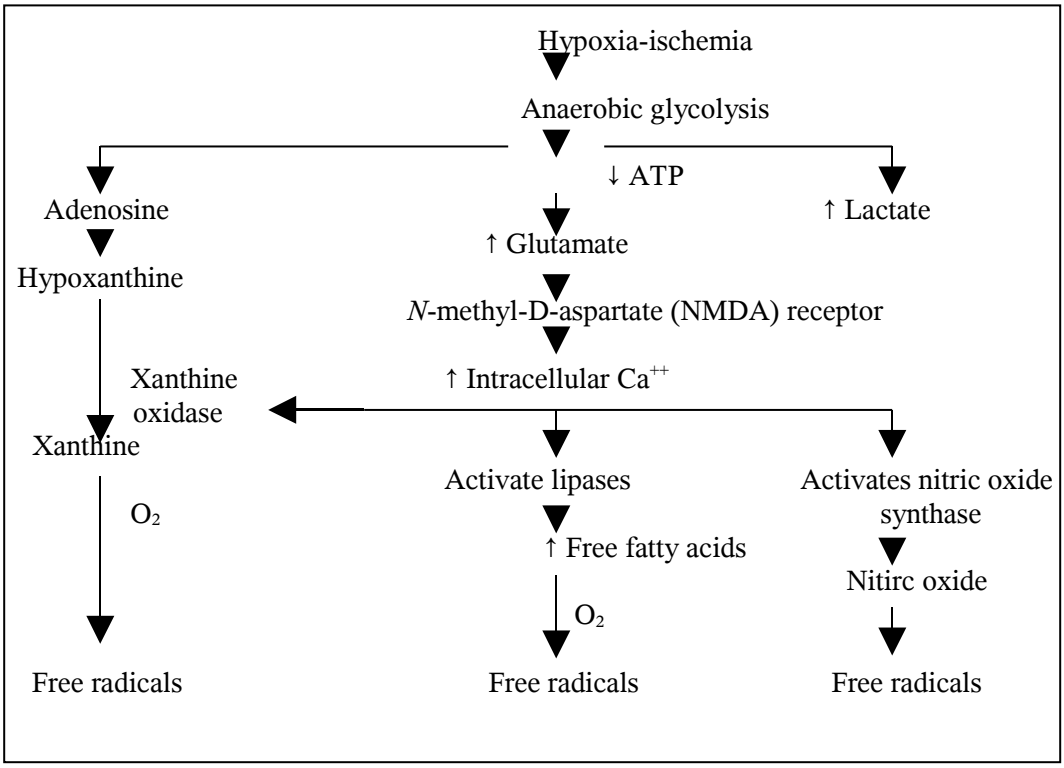


FIGURE 1: Potential pathways for brain injury after hypoxia-ischemia.

Primary energy failure is characterized by reductions in the cerebral blood flow and oxygen/substrates.[22] At the cellular level, the oxygen depletion results in a switch to anaerobic metabolism with rapid depletion of high energy phosphate reserves and accumulation of lactic acid resulting in tissue acidosis.[23] Transcellular ion-pump failure results in intracellular accumulation of Na^{+}, Ca^{++} and water (cytotoxic edema) causing osmotic dysregulation. Excitatory neurotransmitters, specifically glutamate, are released as a result of membrane depolarization from axon terminals.

Glutamate then activates specific cell surface receptors resulting in further influx of Na^{+} and Ca^{++} into postsynaptic neurons. The free fatty acid that accumulates in the cell secondary to membrane phospholipids turnover, undergo peroxidation by oxygen free radicals. Ca^{++} ions accumulate within the cytoplasm as a consequence of increased cellular influx as well as decreased efflux across the plasma membrane combined with release from mitochondria and endoplasmic reticulum. In selected neurons, the intracellular calcium induces the production of nitric oxide, a free radical that diffuses into adjacent cells that are susceptible to nitric-oxide toxicity. Elevation in intracellular Ca^{++} triggers a number of destructive pathways by activating lipases, proteases and endonucleases.[23] The combined effects of cellular energy failure, acidosis, glutamate release, intracellular Ca^{++} accumulation, lipid peroxidation, and nitric-oxide neurotoxicity serve to disrupt essential components of the cell, which ultimately lead to cell death. Many factors, including the duration or severity of the insult, influence the progression of cellular injury after hypoxia-ischemia.[23]

After adequate resuscitation, that may occur in utero or postnatally in the delivery room, cerebral oxygenation and perfusion are restored. Resolution of the hypoxia ischemia within a specific time interval reverses the fall in high energy phosphorylated metabolites and intracellular pH and promotes recycling of receptors. The duration of time for hypoxia ischemia to be successfully reversed and promote recovery will be affected by maturation, preconditiong events, substrate availablility, body temperature and simultaneous disease process.[23]

However, the process of cerebral energy failure recurs from 6 to 48 hours later in a second phase of injury. This phase is characterized by a decrease in the ratio of phosphocreatinine and adenosine triphosphate with an unchanged intracellular pH and stable cardio-respiratory status and contributes to additional brain injury.[25,26] The presence and severity of secondary energy failure depends on the extent of primary energy failure. The pathogenesis of the secondary energy failure is not as well understood as the primary energy failure, but likely involves multiple pathophysiologic processes including accumulation of excitatory neurotransmitters, oxidative injury, apoptosis, inflammation and altered

growth factors and protein synthesis.[23] Evidence suggests that circulatory and endogenous inflammatory cells and mediators also contribute to the ongoing brain injury. In human infants, the severity of the secondary energy failure is correlated with adverse neuro-developmental outcome at 1 and 4 years.[22]

The mechanism of neuronal cell death in animals and humans after hypoxia-ischemia includes neuronal necrosis and apoptosis. The intensity of the initial insult may determine the mode of death, with severe injury resulting in necrosis, whereas milder insults result in apoptosis. Because the mechanisms of neuronal necrosis versus apoptosis likely differ, strategies to minimize brain damage in an affected infant after hypoxia-ischemia likely will have to include interventions that target both processes.[24]

There is a latent phase in the time interval between the primary and the secondary energy failure that corresponds to the therapeutic window. The duration of the therapeutic window in near-term fetal sheep is approximately 6 hours. Initiation of therapies in the therapeutic window has been successful in reducing brain damage.[9,10]

Current therapies for neonatal hypoxic-ischemic encephalopathy

The management of neonates with HIE has been limited to supportive intensive care that includes correction of hemodynamic and pulmonary disturbances, correction of metabolic disturbances, treatment of seizures if present and monitoring for other organ dysunction.[18] Brain specific therapies have been developed over the last 15 years with the objective to attenuate components of the cascade of events triggered by hypoxia ischemia.[23] For a therapy to be applicable in the clinical field, the neuroprotective agent should have the following characteristics: It should target specific mechanisms mediating pathogenesis of tissue injury. It should be initiated with ease within a limited interval of time after the onset of injury. The therapy should be maintained for the duration needed to optimize the neuroprotective effect and it should be free of adverse effects.[23]

Pharmacologic agents that are under experimental investigation

1. Oxygen free radical inhibitors and scavengers

Oxygen-free radicals are generated during and after hypoxia–ischemia in several ways. First, free radicals are produced within mitochondria when cytochrome oxidase is not fully saturated with oxygen, thereby liberating free radicals at more proximal steps. These oxygen-free radicals cannot be consumed further and leak out into the cytoplasm. Other sources of oxygen-free radicals during hypoxia–ischemia and especially during the reperfusion interval of recovery are as by-products in the synthesis of prostaglandins from arachidonic acid and the conversion of hypoxanthine to xanthine and uric acid.[22]

Free radicals and reactive oxygen species (superoxide and hydrogen peroxide) cause tissue injury only when the radicals exceed the brain's endogenous antioxidant defenses. The newborn human infant, especially the premature infant, might be particularly susceptible to free radical injury because of a relative deficiency in the brain's antioxidants, including the enzymes superoxide dismutase and glutathione peroxidase.[22] Recent evidence also suggests that circulating and endogenous inflammatory cells act as mediators of hypoxic–ischemic injury in the immature brain, presumably through the production of oxygen-free radicals.[27]

Antioxidant enzymes superoxide dismutase and catalase conjugated to polyethylene glycol has been shown to reduce hypoxic-ischemic brain damage and maintain the stability of the blood brain barrier. However, these enzymes have a narrow therapeutic dosage range and are generally protective only when administered many hours after the hypoxic-ischemic insult. The prolonged time taken for the conjugated enzymes to penetrate the blood brain barrier into brain parenchyma precludes their clinical usefulness in reperfusion injury.[28]

Xanthine oxidase inhibitors /scavengers (allopurinol and oxypurinol) inhibit specific reactions in the production of prostaglandins and xanthine, the formation of which involves the generation of oxygen-free radicals. These drugs protect immature rats from hypoxic-ischemic brain damage if administered early during the recovery phase of resuscitation.[29] Randomized control trials comparing allopurinol with either no treatment[30] or placebo[31] showed no significant difference between treatments in the composite outcome of rate of death or developmental delay.

Studies done on *Miltiorrhizae* (a Chinese herb with antioxidative properties) and citicholine (also an antioxidant) showed that miltiorrhizae significantly reduced the combined outcome of mortality or neurological abnormality, the trial gave insufficient information to determine trial quality and validity of the findings. There was no information available on the adverse effects.[30,31]

Indomethacin, a cyclooxygenase and phospholipase inhibitor, has been shown to decrease brain damage in experimental animals by reducing the formation of free radicals.[32]

21 aminosteroids (lazeroids) have been shown to be neuroptrotective in experimental animal models of hypoxia-ischemia by scavenging the peroxyl radicals thus preventing the Fe-dependant lipid peroxidation. Their site of action is within cerebral blood vessels, thereby reducing reperfusion injury.[33]

Platelet-activating factor, a potent phospholipid inflammatory mediator, is synthesized in the brain, and its concentration is increased during cerebral ischemia. Liu et al have shown in immature rats that the platelet-activating factor antagonist BN 52021 attenuates hypoxic–ischemic brain damage.[34]

2. Excitatory amino acid antagonists

Research in experimental animals has implicated a role for the excitatory amino acid glutamate in the production of hypoxic–ischemic brain damage in the immature and adult brain. First, glutamate is directly toxic to mature neurons in culture. Second, neurons in culture and hippocampal slices die on exposure to anoxia, but their death can be prevented by the presence of magnesium, which blocks glutamate receptors within the calcium ion channel, or by specific glutamate antagonists. Third, direct injection of glutamate or glutamate agonists into specific regions of brain in vivo produces neuronal injury identical to that seen after hypoxia-ischemia. Fourth, deafferentation of the glutaminergic excitatory input into the hippocampus reduces the damage produced by hypoxia–ischemia. These studies provide convincing evidence that excessive exposure of neurons to glutamate, leads to morphologic alterations characteristic of ischemic neuronal necrosis.[34]

Available excitatory amino acid antagonists include phencyclidine, dextromethorphan, ketamine, MK-801, and NBQX, among others. These compounds have been found efficacious in reducing the extent of hypoxic–ischemic brain damage in adult animals even when administered up to 24 hours after the metabolic insult.[34]

Magnesium acts as a glutamate receptor antagonist in that it blocks the neuronal influx of calcium within the ion channel. Magnesium sulfate has been shown to reduce the severity of hypoxic–ischemic brain damage in immature rats.[35] One systematic review identified no RCTs on the effects of magnesium sulphate in infants with asphyxia. Limited evidence from one small RCT found that, when assessing reductions in the composite adverse outcome of survival, abnormal cranial computerised tomography and electroencephalography results, and failure to establish oral feeding by 14 days of age, magnesium sulphate/dopamine combination was more effective than no drug treatment. The RCT was underpowered to detect significant differences between groups when outcomes were assessed separately. The RCT gave no report on adverse outcomes.[4]

3. Calcium channel blockers

Increase in the intracellular calcium ion concentration to dangerous levels can cause neurotoxicity and neuronal damage.[22] Calcium channel blockers like flunarizine and nimodipine, reduce the extent of hypoxic brain damage by inhibiting the calcium ion influx into the neurons.[35] However in the clinical setting, Levene et al[37] showed a mean increase in heart rate with a decrease in the mean blood pressure in newborn infants after the administration of nicardipine thus limiting their potential toxicity.

4. Inhibitors of nitric-oxide production

The free radical gas nitric oxide is involved in a cascade of metabolic events by reacting with other free radicals to form even more reactive species. These cause cellular damage including components of the mitochondrial transport chain.[35] L-NAME is being evaluated in animal models.[5]

5. Monosialogangliosodes

Monosialogangliosodes are found in high concentrations in the brain and are important constituents of cellular membranes. GM_1, when given systemically, incorporates into cellular membranes and enhances stabilization of membrane integrity and function, in experimental animal models.[5]

6. Growth factors

Nerve growth factor have been shown to reduce the severity of hypoxic-ischemic brain damage in immature experimental animal models.[38]

7. Glucocorticosteroids

Glucocorticoids have been used previously in human infants, children and adults to reduce the cerebral edema that arises from hypoxia-ischemia. Controlled clinical studies using low and high dose steroids suggest that the therapy is ineffective and that mortality in treated rat pups was greater than in control animals.[35]

Barks et al[35] have found a protective effect of dexamethasone in the immature rat when the drug was administered $\geq$ 24 hours before the metabolic insult. Chumas et al[39] showed that pretreatment of immature rats with dexamethasone 6 hours before hypoxia-ischemia also provided protection with no

infarction.

8. Phenobarbital

The mechanism of neuroprotection using short-acting barbiturates relates predominantly to an overall suppression of cerebral oxidative metabolism. Barbiturates also blunt cerebral excitotoxicity by depressing glutamate responses within the brain. Goldberg et al[40] demonstrated in a clinical study that thiopental did not improve neonatal mortality or neurologic morbidity at 12 months of age. A recent clinical trial by Hall et al demonstrated beneficial effect with high dose phenobarbital therapy to severely asphyxiated newborn infants and is associated with significant improvement in neurologic outcome at 3 years of age.[35]

9. Fluid restriction

The rationale is that fluid restriction may limit cerebral oedema, which may be important in the pathogenesis of brain damage after perinatal asphyxia. However, there is concern that excessive fluid restriction may cause dehydration and hypotension, resulting in decreased cerebral perfusion and further brain damage.[4] There are no RCTs on the effect of fluid restriction in newborn infants with perinatal asphyxia

10. Hyperbaric oxygen treatment

Hyperbaric oxygen is widely used for treating infants with hypoxic–ischaemic encephalopathy in China but is not a standard practice in other countries.

One systematic review found lower rates of mortality and adverse neurological outcomes in infants treated with hyperbaric oxygen. However, the RCTs included in the review used poor methods, and potential publication bias was reported. Therefore, the results should be interpreted with caution.[4]

11. Others

Vitamin C and Vitamin E[5]

Nonpharmacologic interventions[35]

1. Hyperglycemia
2. Carbon Dioxide
3. Hypothermia
4. Hypoxic preconditioning

Gene therapy[5]

1. Apoptosis inhibition using proto-oncogene bcl-2, originally isolated from the follicular lymphoma chromosomal translocation t(14:18)
2. Calbindin-D_{28k} is an intracellular EF-hand calcium binding protein, which acts as a mobile, cellular calcium buffer. Bicistronic defective herpes simplex virus vectors that encode the calbindin-D_{28k} gene are used.

Hypothermia

Mechanism of action of Hypothermia

Brain hypothermia is an example of non-specific neuroprotective therapy signifying that it affects multiple processes in the events leading to brain injury. A relatively small reduction in brain temperature (1-6°C) of neonatal animals is associated with better maintenance of cerebral energy state during and immediately after ischemia, attenuation of the release of excitatory neurotransmitters, and decreased caspase-3 activation and morphologic evidence of apoptosis.[23] Other neuroprotective effects of hypothermia include normalization of the decreased protein synthesis, reduction of free radicals and modulation of microglial activation and cytokine production.[23] The net effect of modest hypothermia is an attenuation of secondary energy failure with histopathologic evidence of neuroprotection. Neuroprotection can be achieved when cooling is initiated at an interval of up to 5.5 hours after an hypoxic event.[9,10]

Hypothermia – History and animal trials

The first reports of hypothermia in newborns with birth asphyxia was reported by Westin[41,42] in 1959 and later by Kopachev[43] in Russia in the 1970's but there was no controlled evaluation. Renewed interest in the use of hypothermia to reduce brain injury after ischemia was rekindled in the 1980s when experimental studies in dogs and rats showed that moderate levels of hypothermia (32-34 degrees C) during or following ischemia could be neuroprotective and improve neurological outcome.[43] In the 1990's the principle was applied to fetal sheep model of cerebral ischemia by Gunn et al[5] and randomized controlled studies were performed later on newborn infants.

Studies done in young adult dogs demonstrated that the success of neuroprotection with short durations of cooling during resuscitation whereas a delay of 15 minutes in initiation of cooling after reperfusion may not improve functional outcome although it may slightly decrease tissue damage.[44] Studies done on the gerbil by Colbourne and Corbett clearly showed that mild postischemic hypothermia (34 degrees C, 1-25 hour postischemia) showed decreased neuronal damage histologically.[45] The critical duration of

cooling was also unknown. In the fetal sheep model of cerebral ischemia, Gunn et al demonstrated that moderate hypothermia between 30 and 33 degrees C for a period of 72 hours showed neuroprotection. There was an increase in damage if the hypothermia was started after 6 hours of the cerebral ischemia and there was no added benefit shown if the hypothermia was extended for beyond 72 hours.[10] Investigations using newborn swine compared the difference in the brain temperature during head and body cooling.[11] Head cooling performed with a constant rectal temperature increased the temperature gradient across the brain with the deep brain warmer than the superficial brain. However, brain hypothermia induced via body cooling to a rectal temperature of 34 degrees C, resulted in homogenous cooling of the cerebral cortex. The model demonstrated that surface cooling reduces deep brain temperature only when core body temperature is lowered. Rectal temperature was found to be a valid index of brain temperature in view of the small temperature gradients. Whole body cooling also consistently reduced the cerebral blood flow and the merabolic rate, the latter corretlated with the degree of temperature drop. The effect of selective brain cooling on cerebral blood flow is less consistent.[46]

The cardiovascular changes that occur during mild therapeutic hypothermia include minor cardiac arrhythmias, hypotension, hemoconcentration, sinus bradycardia and peripheral vasoconstriction.[47] Inadvertent reduction in core temperature below the target range occur from active cooling and/or anticonvulsant/ sedative drugs necessitating active re-warming. Phenobarbitone or midazolam reduces the infant's spontaneous activity and the decrease in muscular activity, which is an important source of heat production, causes the decrease in body temperature. Midazolam accentuated this tendency still further by impairing thermoregulatory vasoconstriction. There was a decrease in the mean arterial blood pressure associated with re-warming but not associated with any adverse clinical event. Benzodiazepine drugs facilitate peripheral vasodilatation and reduce the cardiac output. Rapid re-warming leads to peripheral vasodilatation lowering the blood pressure. Re-warming should be conducted slowly at not >0.5°C per hour.[48] Rapid increases in temperature during rewarming can cause

cerebral edema with neurological deterioration.[49] Increasing oxygen requirements during hypothermia was probably attributable to pulmonary hypertension or impairment of pulmonary surfactant by hypothermia. Sinus bradycardia during hypothermia did not seem to have any clinical consequences, and no episodes of arrhythmia were detected. The heart rate rose to during passive re-warming to the normal range. Electrocardiographic changes noted are prolonged QT interval, a prolonged PR interval and Osborn waves.[47] The low heart rate combined with increase stroke volume may be adequate for the reduced metabolic rate during hypothermia.[48] The cardiac output is reduced to 67% of the posthypothermic level. Skin perfusion was decreased but tissue perfusion was adequate as reflected by normal blood lactate levels.[47]

Coagulation abnormalities induced by mild hypothermia include platelet dysfunction and sequestration and enhanced fibrinolytic activity and physicochemical delay of the clotting cascade.[47] The series of enzymatic reactions of the coagulation cascade are strongly inhibited by hypothermia, as demonstrated by the dramatic prolongation of prothrombin time and partial thromboplastin time tests at hypothermic deviations from normal temperature in a situation where factor levels were all known to be normal. This coagulopathy has a multifactorial origin.[50]

Thrombocytopenia has been reported in moderate hypothermia in adults with stroke. This was asymptomatic and there were no bleeding tendencies in any of the patients. [51]

In newborn rabbits, a 2°C decrease in temperature was associated with a marked decrease in renal blood flow and glomerular filtration rate.[52] In studies done in adults with stroke, moderate hypothermia caused no change in urine output or creatinine clearance before, during and after the induction of hypothermia.[51] Mild hypokalemia occured during hypothermia and is thought to be caused by an intracellular shift of potassium. Excessive potassium supplementation must be avoided because of the risk of hyperkalemia during rewarming.[53] There were no changes in the serum sodium concentrations in studies done on adults.[51] Hypothermia also affects the pH value, and for every 1°C decrease in body temperature, there is an increase of 0.016 points in the pH value.

Immunologic effects including impaired leukocyte mobility and phagocytosis are reported during mild hypothermia with a trend to increased sepsis, especially pneumonia in studies done on adults.[51] Though there is a decrease in the total white cell count, there are no reports of neutropenia after induced hypothermia.[49]

Gut motility is impaired during hypothermia and the combined effect of hypothermia and sedation may lead to paralytic ileus and patients may require total parenteral nutrition and delayed start of enteral nutrition. Because metabolic rate decreases by 7% for each 1°C decrease in body temperature, caloric intake needs to be adjusted accordingly.[49] Hypothermia also decreases plasma insulin levels, and it has been hypothesized that this is secondary to catecholamine release. As a result, there is concomitant hyperglycemia, and exogenous insulin should be administered in patients undergoing induced hypothermia.[54]

Review of the human trials with therapeutic hypothermia for newborn infants with hypoxia-ischemia

A Cochrane meta-analysis in October 2007, and republished in October 2008[55], had reviewed eight randomized control trials, comprising 638 term infants with moderate or severe encephalopathy with evidence of intrapartum asphyxia. Therapeutic hypothermia resulted in a statistically significant and clinically important reduction in the combined outcome of mortality or major neurodevelopmental disability to 18 months of age [typical RR 0.76 (95% CI 0.65, 0.89), typical RD -0.15 (95% CI -0.24, -0.07), NNT 7 (95% CI 4, 14)]. Cooling also resulted in statistically significant reductions in mortality [typical RR 0.74 (95% CI 0.58, 0.94), typical RD -0.09 (95% CI -0.16, - 0.02), NNT 11 (95% CI 6, 50)] and in neurodevelopmental disability in survivors [typical RR 0.68 (95% CI 0.51, 0.92), typical RD -0.13 (95% CI -0.23, -0.03)].

In the outcome of mortality, meta-analysis of the four trials (Gunn 1998[52] ; Akisu 2003[56] ; Gluckman 2005[14]; Lin 2006[15]) that used selective head cooling with mild systemic hypothermia did not show a statistically significant effect on mortality [typical RR 0.83 (95% CI 0.59, 1.16), typical RD -0.05 (95% CI -0.14, 0.04)]. However, meta-analysis of the four trials that used whole body cooling (ICE 2002[58]; Shankaran 2002[11]; Eicher 2005[59,60] Shankaran 2005[17]) demonstrated a significant reduction in mortality in the hypothermia groups [typical RR0.66 (95% CI 0.47, 0.93), typical RD -0.13 (95% CI -0.23, -0.02); NNT 8 (95% CI 4, 50)].

On assessment of major neurodevelopmental disability among survivors, meta-analysis of the two trials that used selective head cooling with mild systemic hypothermia (Gunn 1998[52]; Gluckman 2005[14]) failed to show a statistically significant effect [typical RR 0.77 (95% CI 0.51, 1.17), typical RD -0.09 (95% CI -0.24, 0.05)]. However, meta-analysis of the two trials that used whole body cooling (Eicher 2005[59,60] Shankaran 2005[17]) demonstrated a significant reduction in major neurodevelopmental disability among survivors in the hypothermia groups [typical RR 0.60 (95% CI 0.40, 0.92), typical RD -0.17 (95% CI -0.31, -0.03)].

Cardiovascular side effects

Five trials reported effect on heart rate (Gunn 1998[52]; Akisu 2003[56]; Eicher 2005[59,60] Gluckman 2005[14] ; Shankaran 2005[17]). There were a total of 552 infants, of whom 26 had sinus bradycardia below 80/minute. Meta-analysis of the five trials demonstrated significantly increased sinus bradycardia in hypothermia groups [typical RR 5.96 (95% CI 2.15, 16.49), typical RD 0.07 (95% CI 0.04, 0.11)] (TABLE 01.16). Five trials reported the effect of hypothermia on the need for blood pressure support with inotropes (Gunn 1998[52]; ICE 2002[58]; Shankaran 2002[11]; Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 505 infants, of who 266 required inotrope support for hypotension. Meta-analysis of the five trials demonstrated an increase in hypotension treated with inotropes in hypothermia groups that was of borderline significance [typical RR 1.17 (95% CI 1.00, 1.38), typical RD 0.08 (0.00, 0.17)]. Six trials reported effect of hypothermia on cardiac arrhythmia requiring medical intervention and/or cessation of cooling (Gunn 1998[52]; ICE 2002[58]; Akisu 2003[56]; Eicher 2005[59,60] Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 569 infants, of whom 2 had an arrhythmia requiring medical intervention. Meta-analysis of the six trials failed to demonstrate a significant effect of hypothermia on arrhythmia requiring medical treatment in hypothermic groups [typical RR 1.04 (95% CI 0.07, 16.39), typical RD 0.00 (-0.02, 0.02)].

Haematological adverse effects

Three trials reported the effect of hypothermia causing anaemia that required blood transfusion (Gunn 1998[52]; Eicher 2005[59,60]; Gluckman 2005[14]). There were a total of 322 infants, of whom 39 were transfused for anaemia. Meta-analysis of the three trials failed to show significant effect of hypothermia in causing anaemia requiring blood transfusion [typical RR 1.16 (95% CI 0.67, 2.04), typical RD 0.02 (95% CI -0.05, 0.09)] (TABLE 01.19). Two trials reported the effect of hypothermia on white cell count (Gunn 1998[52]; Gluckman 2005[14]). There were a total of 254 infants, of whom 6 had leukopenia with a white cell count below 5 x 10^9/L. Meta-analysis of the two trials failed to show significant effect of hypothermia on the incidence of leukopenia [typical RR 0.97 (95%CI 0.22, 4.33),

typical RD0.00 (95%CI -0.04, 0.04)].

Four trials reported the effect of hypothermia on platelet count (Gunn 1998[52]; Eicher 2005[59,60]; Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 531 infants, of whom 124 were thrombocytopenic with platelet count below 150 x 10^9/L. Meta-analysis of the four trials showed statistically significantly increased thrombocytopenia in the hypothermia groups [typical RR 1.55 (95% CI 1.14, 2.11), typical RD 0.09 (95% CI 0.03, 0.15)].

Four trials reported the effect of hypothermia on coagulopathy resulting in major thrombosis or haemorrhage (Gunn 1998[52]; ICE 2002[58]; Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 486 infants, of whom14 had severe coagulopathy. Meta-analysis of the four trials failed to show significant effect on severe coagulopathy in cooled infants [typical RR 0.83 (95% CI 0.31, 2.24), typical RD -0.01 (95% CI -0.03, 0.02)].

Metabolic adverse effects

Four trials reported the effect of hypothermia on glucose homeostasis (Gunn 1998[52], Akisu 2003[56]; Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 490 infants, of whom 73 were hypoglycemic with a blood glucose below 2.6 mmol/L. Meta-analysis of the four trials failed to show significant hypoglycemia in hypothermic groups [typical RR 0.83 (95% CI 0.54, 1.27), typical RD -0.03 (95% CI -0.09, 0.03)] (TABLE 01.23).

Three trials reported the effect of hypothermia on serum potassium (Gunn 1998[52]; Eicher 2005[59,60] Gluckman 2005[14]). There were a total of 323 infants, of whom 175 had hypokalemia with a serum potassium below 3.5 mmol/L. Meta-analysis of the three trials showed no statistically significant difference in the incidence of hypokalemia in cooled infants [typical RR 1.03 (95% CI 0.85, 1.25), typical RD 0.02 (95% CI -0.09, 0.12)].

Renal impairment

Five trials reported the effect of hypothermia on urine output (Gunn 1998[52]; ICE 2002[58]; Shankaran 2002[11]; Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 505 infants, of whom 147 had

oliguria with urine output below1mL/kg/hour.Meta-analysis of the five trials showed no statistically significant difference in rate of oliguria in cooled infants [typical RR 0.81 (95% CI 0.59, 1.12), typical RD -0.05 (95% CI -0.12, 0.03)].

Sepsis

Five trials reported the effect of hypothermia on sepsis (Gunn 1998[52]; Akisu 2003[56]; Eicher 2005[59,60] Gluckman 2005[14]; Shankaran 2005[17]). There were a total of 552 infants, of whom 28 had culture proven sepsis. Meta-analysis of the five trials failed to show a significant effect of hypothermia on sepsis [typical RR 0.86 (95% CI 0.42, 1.76), typical RD -0.01 (-0.04, 0.03)].

Implications for practice and policy

Most of these trials were conducted in advanced neonatal centers in industrialized countries with considerable experience in therapeutic hypothermia and the results might not be replicable to other settings. The extremely high cost of the equipment and the need to rigorously monitor and control the temperatures further limits the use of this model for most settings[61] especially in resource poor countries.

METHODOLOGY

Population

Babies who were recruited into the trial included inborn (born at the Christian Medical College and Hospital) and outborn (born in a place other than the Christian Medical College and Hospital) admitted into the neonatal unit of the hospital.

Inclusion and exclusion criteria for Inborn babies

Inclusion criteria

- Gestational age ≥35 wks
- pH < 7.0 or base deficit ≥ 12 in umbilical cord arterial blood sample or postnatal ABG within first hour of life AND
- Any two of the following
    - Apgar score ≤ 5 at 5 minutes
    - Ventilation initiated at birth and continued for at least 10 minutes
    - History of acute perinatal event (any one)
        - Intrapartum fetal distress
        - Cord prolapse
        - Placental abruption
        - Maternal respiratory arrest
        - Uterine rupture / dehiscence

Exclusion criteria

- Inability to start cooling the baby by 5.0 hours of age
- Small for gestational age babies (less than 10^{th} centile for age)
- Chromosomal abnormality

- Major congenital anomaly
- Refusal of consent for study participation

Inclusion and Exclusion criteria for Outborn babies

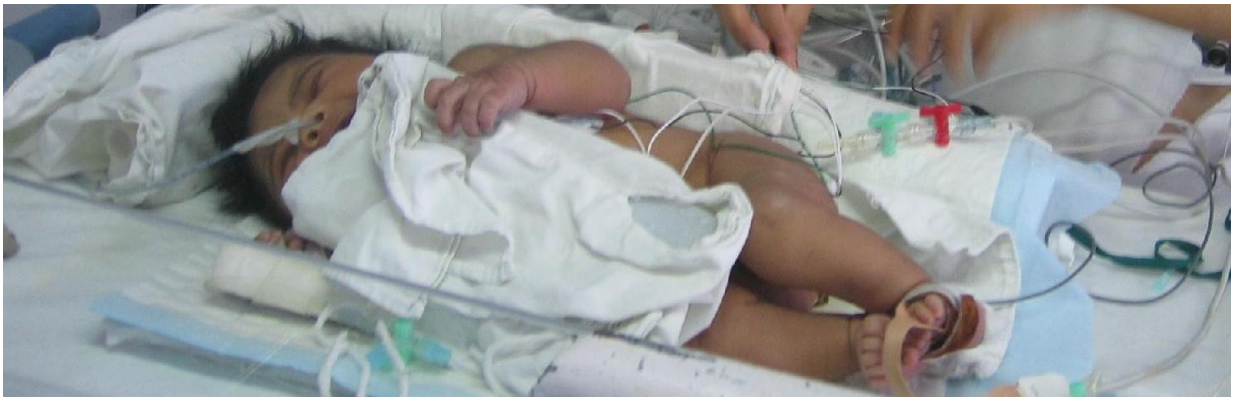
Inclusion criteria (all 3)

- Gestational age ≥35 wks
- Babies who did not cry immediately after birth with any or all of the following features:
    1. Not breathing normally at five minutes of birth
    2. Given assistance for breathing during or soon after birth
    3. Limp or flaccid since birth
    4. Not sucking well at the breast since birth without any oro-facial abnormality if documented by a pediatrician
    5. Apgar score of 5 or less at 5 minutes
- Evidence of encephalopathy

Exclusion criteria

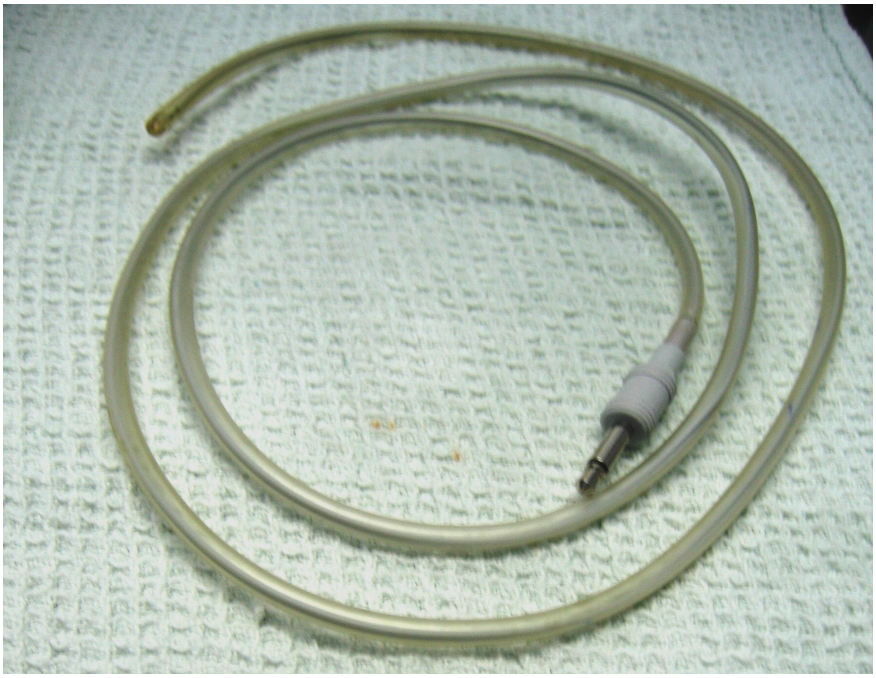
- Inability to start cooling the baby by 5.0 hours of age
- Small for gestational age babies (less than 10^{th} centile for age)
- Chromosomal abnormality
- Major congenital anomaly
- Refusal of consent for study participation

NEONATAL INTENSIVE CARE UNIT



NEWBORN INFANT UNGERGOING WHOLE BODY COOLING

WBC MATERIAL: COOLING-GEL PACKS



RECTAL THERMOMETER PROBE

After obtaining informed consent from the parents, a neurological examination was done on infants included in the trial and they were categorized as having moderate or severe encephalopathy, using the modified Sarnat criteria. The infant was placed supine on cloth covered cooling gel-packs after the overhead warmer was switched off. A rectal probe to monitor temperature was inserted to 5 cm within the rectum and rectal temperature of 33 $\pm$ 0.5°C was targeted. All infants had a central venous line and an arterial access. The temperature was continuously monitored and recorded. The peripheral skin temperature was measured simultaneously. When the infant's rectal temperature increased to 33.5°C, more cloth covered cooling-gel packs were placed and they were removed when the rectal temperature reached 33°C. The room temperature was monitored.

The vital parameters including heart rate (electrocardiogram), blood pressure, respiratory rate and transcutaneous oxygen saturation were monitored simultaneously and continuously. Blood gas analyses were done from the arterial line. The infant was sedated with phenobarbitone and fentanyl, if ventilation was required and sedation withdrawn 8 hours before rewarming was started. Inotropic agents were used if the mean arterial pressure was less than the gestational age, after volume expansion. The infants were catheterized to monitor hourly urine output.

At the end of 72 hours of induced hypothermia, the radiant warmer was turned on to raise the body temperature by 0.5°C/hr to reach a target temperature of 36.5°C. The temperature probe was removed after monitoring the temperature for 80 hours after study initiation.

All treatment modalities including medications (antibiotics, anticonvulsants, paralytics), ventilation and blood products were used as the clinical condition demanded.

The following laboratory parameters were monitored at regular intervals

Labs	Baseline				24 hours	48 hours	72 hours
S. Electrolytes	√				√	√	√
Blood urea	√				√	√	√
S. Creatinine	√				√	√	√
Blood lactate	√				√	√	√
SGOT/SGPT	√				√	√	√
Blood sugar	√				√	√	√
ABG	√ **0h**	√ **2h**	√ **8h**	√ **12h**	√	√	√
PT/aPTT	√				√		√
CBC	√				√	√	√
Neurological examination	√				√		√
Urine output	**Monitored hourly**						
ECG	**When clinically indicated** (drop in the heart rate <80/mt)						

Additional blood gas analyses were done if there was a clinical indication during this period. Any deviation from the protocol was immediately informed to the investigator.

The following were the adverse events that were monitored and reported to the investigator within 6 hours of its occurrence

1. Cardiac arrhythmia (bradycardia of <80/minute, prolonged PR interval, prolonged QT interval, atrial fibrillation and Osborn or J waves) requiring medical treatment
2. Persistent hypoxemia (transcutaneous oxygen saturation of 85% or a PaO_2 <50 mm Hg inspite of a FiO_2 of 1 on mechanical ventilation)
3. Hypotension despite full inotropic support (dopamine at an infusion rate of 10 μg/kg/min and dobutamine at an infusion rate of 20 μg/kg/min)
4. Skin changes (sclerema, apostatonecrosis)
5. Thrombocytopenia (<100,000/cumm)
6. Life threatening coagulopathy (pulmonary and/or gastric and/or umbilical cord bleeding)
7. Arterial thrombosis
8. Hepatic and renal failure
9. Electrolyte disturbances
10. Death

Statistical analysis

The sample size was calculated using the design of Gehan (1961). With a 10% precision and a 20% desired effectiveness, the sample size was calculated as 20. The analysis of the data was done using the SPSS 16.0 software. Mean, median, mode, standard deviation, frequency were calculated. Tests for significance used were Pearson's co-efficient and Mann-Whitney U test. The Kaplan-Meier graph was used to study the time-event survival analysis.

ANALYSIS AND RESULTS

The study was done in the neonatology unit of the Christian Medical College and Hospital, Vellore over a period of 11 months. Twenty newborn infants were recruited into the study (9 inborns and 11 outborns) after informed consent was obtained from the parents in the presence of the investigator. The babies were admitted to the neonatal intensive care unit and the cooling was started as per the protocol mentioned above.

TABLE 1: MATERNAL DEMOGRAPHICS

Gravida – No. (%)	
One	18 (90%)
Three	02 (10%)
Complications of pregnancy – No. (%)	
Gestational diabetes mellitus	1 (5%)
Pregnancy induced hypertension	2 (10%)
None	17 (85%)
Peripartum complications – No. (%)	
Fetal Heart Rate Deceleration	7 (77.7%)
Hemorrhage	1 (11.1%)
Meconium Stained Amniotic Fluid	1 (11.1%)
Details incomplete	11
Onset of labour – No. (%)	
Induced	2 (10%)
Spontaneous	18 (90%)
Duration of labour (in hours)	13 ± 10
Mode of delivery – No. (%)	
Normal	9 (45%)
LSCS	8 (40%)
Forceps	2 (10%)
Vacuum	1 (5%)
Inborn - No. (%)	9 (45%)
Outborn - No. (%)	11 (55%)

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

PERINATAL DEMOGRAPHICS

The perinatal demographics are mentioned in Table 1. Ninety percent of the newborns were born to primigravida mothers. All the mothers had regular antenatal care and only 3 (15%) had complications during pregnancy. Most of them went into spontaneous labour and 9(45%) had intrapartum complications. The details of intrapartum complications in outborn deliveries were incomplete in 11 cases. The mean duration of labour was 13 hours and 9 (45%) mothers had a vaginal delivery.

TABLE 2: NEONATAL DEMOGRAPHICS

Mean gestational age (wks)	38.5 ± 1.3
Age at starting cooling (hrs)	3.4 ± 1.2
Inborn	3 ± 1
Outborn	3.5 ± 1
Gender	
Male	8 (40%)
Female	12 (60%)
Male (inborn:outborn) - No. (%)	4 (20%):4 (20%)
Female (inborn:outborn) - No. (%)	5 (25%):7 (35%)
Birth weight (gms)	3034 ± 518
Cord blood pH (inborn only)	6.945 ± 0.118
Cord blood BE (inborn only)	-19.1 ± 3.2
Moderate encephalopathy - No. (%)	16 (80%)
Severe encephalopathy - No. (%)	4 (20%)

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

NEONATAL DEMOGRAPHICS

The neonatal demographics are mentioned in Table 2.

All the babies were born at term with a mean gestational age of 38 weeks (range: 36.8-39.2 weeks). The mean age for starting cooling after birth was 3.4 hours (range: 2.2-4.6). Initiation of cooling inborn babies was faster than the outborns. There were 12 (60%) female infants and 8 (40%) male infants. The mean birth weight was 3034 gms (range: 2514-3538 gms). Cord blood pH and base excess details were available only for inborn babies. The mean cord blood pH was 6.945 (range: 6.827-7.063) and the base excess was -19.1 (range: -15.9 to -22.3). Majority of the infants recruited had moderate encephalopathy according to the modified Sarnat staging.

TABLE 3A: TEMPERATURE CHARACTERISTICS OF THE NEWBORNS

Rectal temperature at start of cooling (°C)	36.0 ± 0.8°C
Skin temperature at start of cooling (°C)	35.8 ± 0.97
Time taken to reach target rectal temperature after initiation of cooling (minutes)	52 ± 25
No. who achieved target temperature within 1 hour of start of cooling (%)	13 (65%)
Time taken from birth to reach target temperature (minutes)	206 ± 63

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

TABLE 3B: MANN-WHITNEY U TEST OF SIGNIFICANCE

(Comparison between time to achieve cooling vs other parameters)

Parameters	p Value
Birth weight	<0.05
Gestational age	0.877
Gender	0.536
Inborn vs Outborn	0.393
Induced labor vs Spontaneous	0.817
Mode of delivery	0.115

TEMPERATURE CHARACTERISTICS OF NEWBORNS

The temperature characteristics of the newborns are shown in Table 3. The mean rectal temperature at the start of cooling was 36°C (range: 35.2-36.8°C) while the mean skin temperature was 35.8°C (range: 34.8-36.8°C). The mean time taken to reach target rectal temperature (32.5-33.5°C) was 52 minutes (range: 27-77 minutes). Seven babies (35%) achieved target rectal temperature after one hour of initiation of cooling. The average room temperature was maintained between 28-30°C. (Table 3A)

TESTS OF SIGNIFICANCE

Pearson's correlation looking at the linear relationship of birth weight and time taken to achieve target temperature showed a positive moderate correlation ($r=0.545$; $p<0.05$) which was statistically significant suggesting that the larger baby takes a longer time at school.

There was no statistical significance (using the Mann-Whitney U test) between time taken to cool and gestational age, gender, inborn or outborn, induced or spontaneous labor and mode of delivery. (Table 3B)

FIG. 1: TREND OF MEAN SKIN AND RECTAL TEMPERATURE DURING WBC

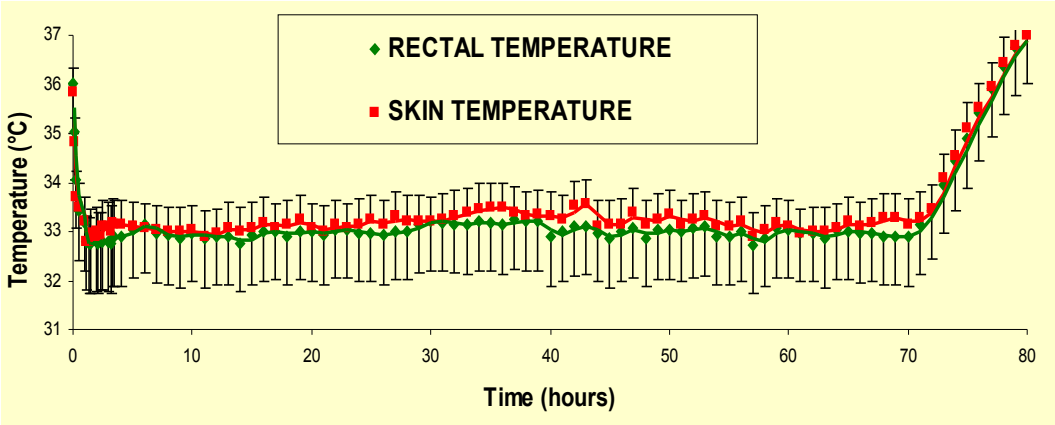
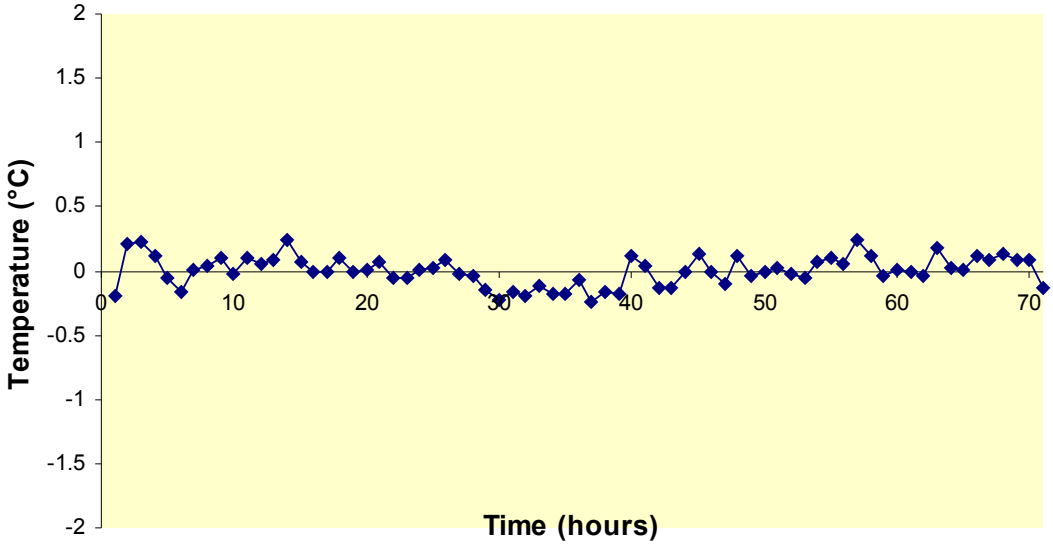


FIG. 2: TREND OF VARIATION IN MEAN RECTAL TEMPERATURE FROM TARGET TEMPERATURE DURING THE PERIOD OF COOLING



TREND OF MEAN SKIN AND RECTAL TEMPERATURE DURING WBC

The mean average rectal temperature during the period of cooling was between 32.8 and 33°C. The mean average skin temperature during the period of cooling was between 33 and 33.2°C. The mean average difference in the rectal and skin temperature was -0.15 ± 0.13°C. (Fig 1.)

TREND OF VARIATION IN MEAN RECTAL TEMPERATURE FROM TARGET TEMPERATURE DURING THE PERIOD OF COOLING

The variation in the mean rectal temperature from target temperature during the period of cooling was 0.001 ± 0.11°C

FIG. 3: MEAN VARIATION IN SKIN TEMPERATURE FROM THE TARGET TEMPERATURE DURING THE PERIOD OF COOLING

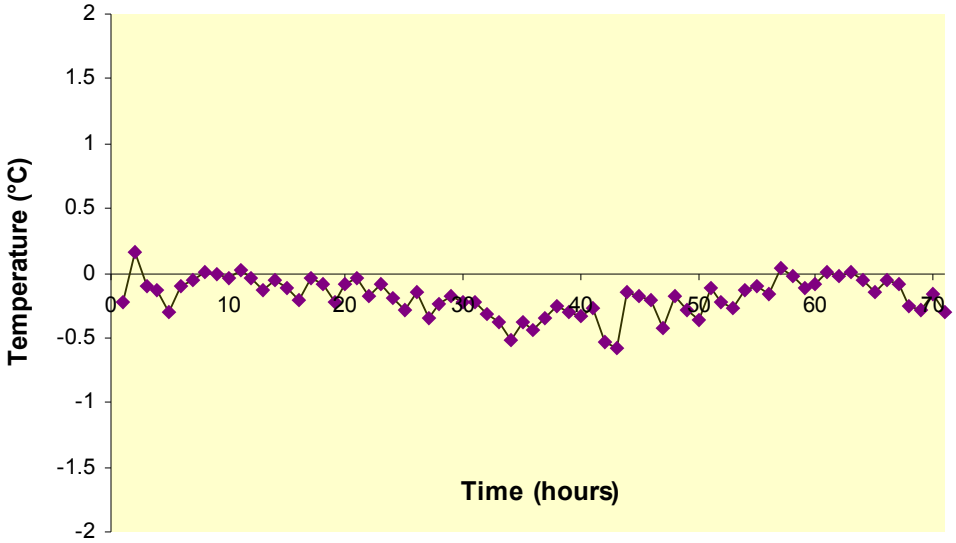
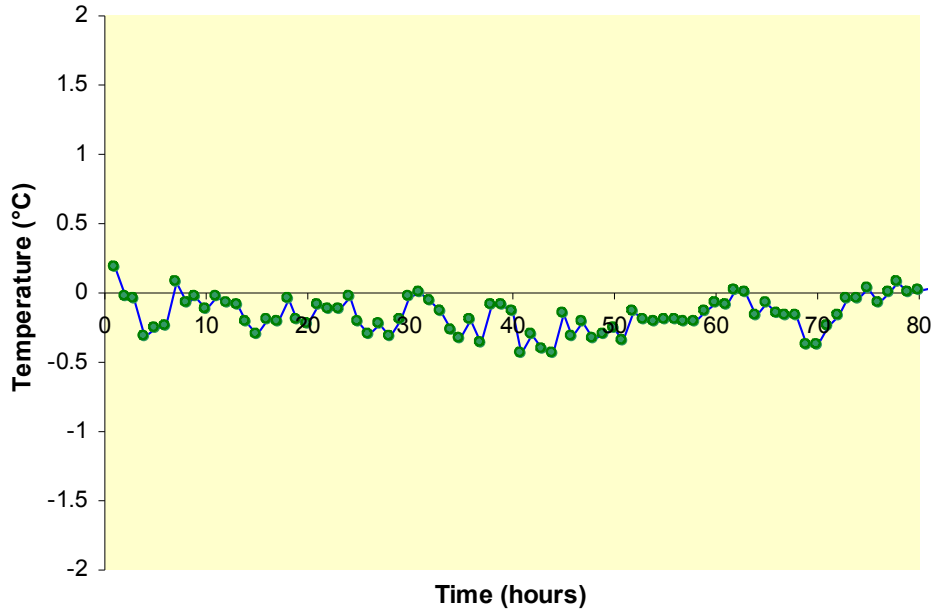


FIG. 4: MEAN VARIATION IN DIFFERENCE BETWEEN RECTAL AND SKIN TEMPERATURE DURING WBC



MEAN VARIATION IN SKIN TEMPERATURE FROM THE TARGET TEMPERATURE DURING THE PERIOD OF COOLING

The mean average difference between the target temperature and skin temperature during the period of cooling was -0.18 ± 0.14°C. (Fig. 3)

MEAN VARIATION IN DIFFERENCE BETWEEN RECTAL AND SKIN TEMPERATURE DURING WBC

The mean variation in the difference between rectal and skin temperature during the period of cooling is 0.18 ± 0.11°C. (Fig. 4)

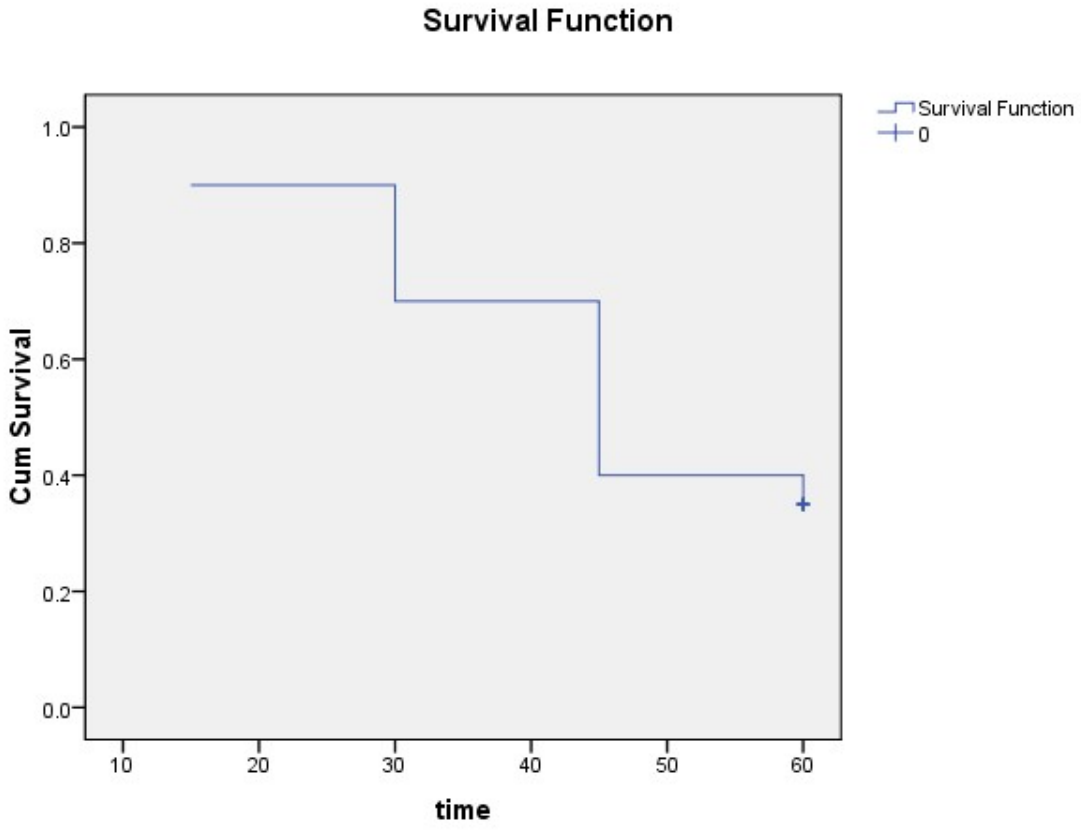


FIG. 5: KAPLAN-MEIER SURVIVAL ANALYSIS (TIME TO "EVENT"*)

* "Event" – achieving temperature of 32.5 – 33.5 °C

Cum survival – Cumulative proportion of sample that did achieve event

TIME TO EVENT ANALYSIS

The event of interest is rectal temperature of 32.5-33.5°C. The event was measured for every 15 minutes for the first 4 hours and every one hour up to 80 hours. At the end of the first hour from commencement of cooling, only 35% of the newborn infants did not achieve the target temperature (ie did not attain the event). (Fig.5.)

At the end of 15 minutes, 30% achieved the target temperature; the median time to event was 45 minutes (95% CI 34.265-55.735).

FIG. 6: TREND OF MEAN HEART RATE DURING WBC

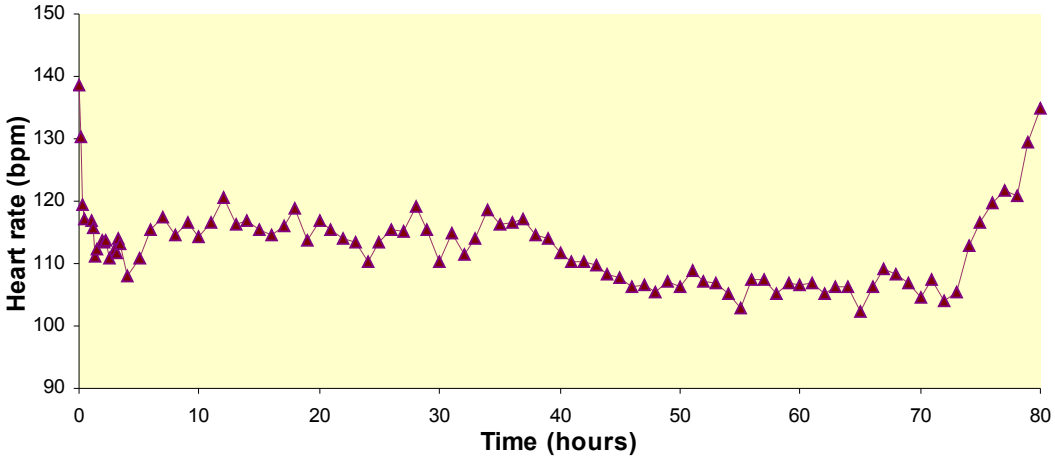
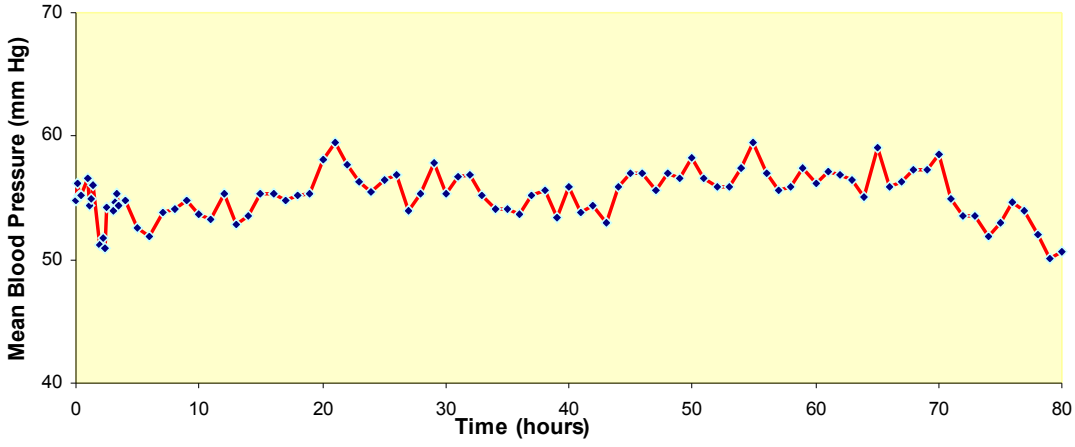


FIG. 7: TREND OF MEAN BLOOD PRESSURE DURING WBC



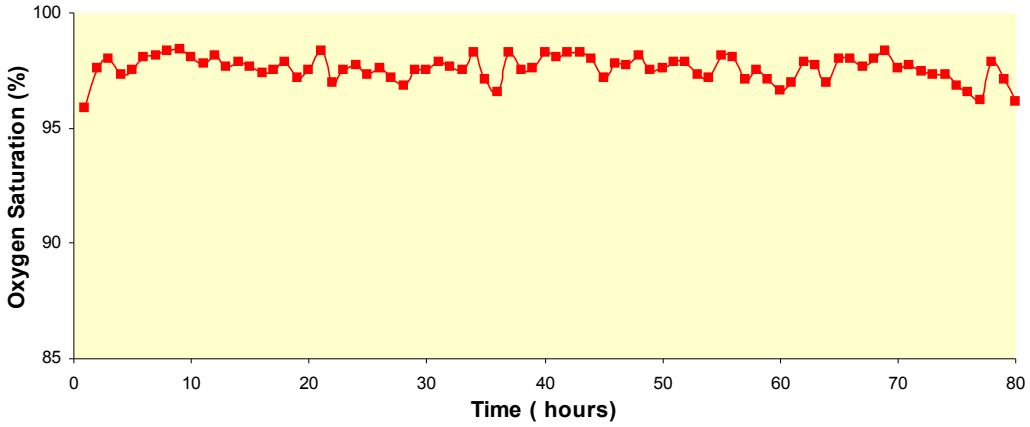
HEART RATE

The mean heart rate at the start of cooling was 138 per minute and the mean average heart rate during the period of cooling was 111 ± 5 per minute. (Fig. 6)

MEAN BLOOD PRESSURE

The mean blood pressure at the start of cooling was 54.7 mm Hg and the mean average mean blood pressure during the period of cooling was 55.6 ± 1.7 mm Hg. (Fig. 7)

FIG. 8: TREND OF MEAN OXYGEN SATURATION DURING WBC



OXYGEN SATURATION

The mean oxygen saturation was within the normal range through out the period of cooling and rewarming. (Fig. 8)

TABLE 4: ARTERIAL BLOOD GAS TREND OVER 72 HOURS

	2 HRS	8 HRS	12 HRS	24 HRS	48 HRS	72 HRS
pH	7.23±0.08	7.29±0.06	7.33±0.07	7.37±0.08	7.40±0.03	7.39±0.04
pCO_2	38.6±11.3	36.8±8.7	37.3±5.4	36.2±12.5	38.2±6.4	38.9±8.6
pO_2	89.9±38.9	86.3±48.3	108.9±95	96.9±47.8	107.7±66	93.1±24.8
HCO_3^-	15.6±4.7	17.3±4	19.1±2.8	20.5±5	22.5±3.2	22.5±2.5
BASE EXCESS	-11.1±5.9	-7.3±5.9	-5.77±3.8	-3.1±6.36	-1.28±2.9	-1.1±3.8
O_2 SATURATION	90.3±13.1	90.1±9.9	93±9.2	94.8±5.3	96.6±2	95.9±3

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

ARTERIAL BLOOD GAS TREND

Table 4 shows the arterial blood gas trend over 72 hours of WBC. There was significant acidosis among inborn babies who had the cord blood pH and base excess done. The acidosis corrected within 12 hours of the start of the procedure, though 2 (10%) had persistent metabolic acidosis requiring sodabicarbonate infusion. The serum bicarbonate levels also did not worsen on cooling but in fact increased to within normal limits within 2 hours and the base excess also corrected in 2 hours after the start of cooling.

TABLE 5A: METABOLIC PARAMETER TREND OVER 72 HOURS

	0 HRS	24 HRS	48 HRS	72 HRS
S. SODIUM (mEq/L)	134.5±4.8	133.7±4.8	134.8±4	135.9±6.7
S. POTASSIUM (mEq/L)	4.7±0.6	4.19±0.6	4.15±1	4.15±0.7
S. BICARBONATE	12.9±5.0	19.2±5.1	20.1±2.7	19.6±3.7
BLOOD UREA	19.7±5.7	26.4±11.8	23.3±11.7	19.6±12.2
S. CREATININE	0.9±0.1	0.8±0.4	0.6±0.2	0.5±0.1
BLOOD LACTATE	7.2±4.3	4.1±2.8	2.8±2.9	1.9±1.1
BLOOD SUGAR	103.1±53	153.8±143.2	102.4±65.8	90±34.1

Plus–minus values are means ±SD.

rcentages are based on the number of infants for whom data were available.

TABLE 5B: HEPATIC ENZYME TREND OVER 72 HOURS

	0 HRS	24 HRS	48 HRS	72 HRS
AST (IU/L)	129.4±112.3	155.8±188	142.1±260	68.5±31.8
ALT (IU/L)	47.8±67.3	64.5±69.3	85.4±88	56±65.2

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

METABOLIC PARAMETERS

Table 5A shows the biochemical profile of the newborn infants during WBC. The electrolytes remained within normal limits during the cooling procedure. There was a mild increase in the blood urea after 24 hours of cooling, but this showed a down trend in the following hours. Serum creatinine and blood lactate showed a downward trend as the WBC progressed. The average blood sugars were within the normal range. However, 2 (10%) had hypoglycemia and 3 (15%) had hyperglycemia requiring insulin. Liver enzyme levels were within normal limits (Table 5B).

TABLE 6: HEMATOLOGY TREND OVER 72 HOURS

	0 HRS	24 HRS	48 HRS	72 HRS
HB (g/dL)	17±1.8	16.1±3.4	16.2±2	15.8±2.1
PCV	52±5.1	49.6±5.3	43.5±11.9	47.5±4.8
TOTAL WBC COUNT(/cmm)	23015.7±9861	14818±5527	11351±3117	9669±3021
NEUTROPHIL (%)	69±15	85±9	76±9	67±10
PLATELET COUNT (/cmm)	200850±53817	165055±45304	145263±50675	127894±45077
PT (sec)	20.59±10.5	24±23.4		12.76±1.9
INR	2±1.9	1.9±1.9		1±0.1
PTT (sec)	63.2±43	58.8±30.9		49.8±9.8

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

HEMATOLOGY TREND

ble 6 show the hematology trend over 72 hours of WBC. The hemoglobin and the packed cell volume dropped marginally during the WBC. The total white cell count showed a decreasing trend and there was no neutropenia. The platelet count also showed a decreasing trend during the procedure. The PT and the INR which was prolonged at the start of cooling got worse at 24 hours and subsequently improved. The aPTT which was impaired at the initiation of cooling showed an improving trend over 72 hours.

TABLE 7: MEDICATIONS USED DURING WBC

MEDICATION	NO (%)
Anticonvulsants	12 (60%)
Saline bolus	11 (55%)
Inotropes	16 (80%)
Insulin	3 (15%)
Fresh Frozen Plasma	6 (30%)
Sodabicarbonate	2 (10%)
Ranitidine	1 (5%)

Plus–minus values are means ±SD.

Percentages are based on the number of infants for whom data were available.

MEDICATIONS USED

Medications used during WBC are shown in Table 7. Twelve (60%) infants required anticonvulsants for seizure control. Phenobarbitone, phenytoin and midazolam were the anticonvulsants used. Saline boluses were given to 11 (55%) babies for acidosis and hypotension. Sixteen (80%) infants required inotropic support for which dopamine and dobutamine were used. Fresh frozen plasma for prolonged coagulation profile was required in 6 (30%) of newborns. Hyperglycemia requiring insulin was seen in 3 (15%) babies. Persistent acidosis requiring sodabicarbonate infusion was started in 2 (10%) infants. Ranitidine for altered blood through the nasogastric feeds was required for 1 (5%) baby.

TABLE 8: SERIOUS ADVERSE EVENTS DURING WBC

ADVERSE EVENTS	NO (%)
Cardiac arrhythmias	Nil
Hypoglycemia[Φ]	2 (10%)
Hyperglycemia requiring insulin[Δ]	3 (15%)
Thrombocytopenia[¶] (< 100000/cmm)	5 (25%)
Bleeding[§]	1 (5%)
Skin changes[Ψ]	3 (15%)
Hypoxemia[⌂]	1 (5%)
Hepatic dysfunction[#]	1 (5%)
Oliguria[*]	1 (5%)
Persistent acidosis[†] requiring sodabicarbonate infusion	2 (10%)
Death	1 (5%)

Φ Hypoglycemia defined as plasma sugar < 45mg/dL or blood sugar <50 mg/dL

Δ Hyperglycemia defined as plasma sugar >150 mg/dL or blood sugar > 175mg/dL

§ Bleeding was defined as overt bleeding with a platelet count of less than 40,000 per liter with abnormal results on coagulation studies

¶ Thrombocytopenia did not cause clinical bleeding, except in one baby who had DIC

Ψ Aposteatonecrosis was seen in 3 babies that resolved spontaneously

⌂ Hypoxemia defined as transcutaneous oxygen saturation of 85% or a PaO_2 <50 mm Hg inspite of a FiO_2 of 1 on mechanical ventilation

Hepatic dysfunction defined as AST >200 and ALT >100

* Oliguria defined as <0.5 ml/kg/hr

† Persistent acidosis is defined as acidosis persisting 3 hours after the start of intervention

Percentages are based on the number of infants for whom data were available.

SERIOUS ADVERSE EVENTS

Serious adverse events that occurred during WBC are seen in Table 8. There were no cardiac arrhythmias recorded during WBC. There were 5 (25%) babies who developed transient sinus bradycardia, defined as heart rate <80 beats/minute. There were no systemic effects and no medication was required. ECG was done to look for the presence of J waves and there were none. Hypoglycemia, defined as blood sugar < 50mg/dL, was seen in 2 (10%) of babies which required a higher infusion of carbohydrates. Hyperglycemia requiring insulin, defined as plasma sugar > 150 mg/dL, was seen in 3 (15%) babies. Thrombocytopenia, defined as platelet count < 100000/cmm, were seen in 5 (25%) of newborn infants during the WBC. Infants who had thrombocytopenia at the start of WBC have not been included, as the thrombocytopenia was attributed to the bone marrow involvement secondary to hypoxia. Bleeding requiring transfusions was seen in 1 (5%) infant. Skin changes (sclerema or aposteatonecrosis) were noted in 3 (15%) of infants and there was spontaneous resolution of the same during the recovery period. Hypoxemia requiring ventilatory support was seen in 1 (5%) infant. Hepatic dysfunction, defined as AST >200 and ALT >100, was seen in 1 (5%) infant. Oliguria, defined as urine output < 0.5 ml/kg/hr, was seen in 1 (5%) infant. One infant died during WBC. This infant had prolonged bleeding parameters and a subgaleal bleed that worsened with cooling. Neurosonogram was done on all infants who underwent WBC, within the first week. One (5%) showed periventricular white matter changes while the rest were normal.

DISCUSSION

Hypothermia has been widely studied as a neuroprotective strategy in newborn and adult animals after ischemia and hypoxia-ischemia. In perinatal animals (sheep, rats and piglets), hypothermia was found to be beneficial when implemented within 5.5 hours of brain ischemia.[10] Studies done in many of the developed countries using expensive equipment for whole body cooling or selective head cooling was found to be beneficial in terms of the reduction of mortality and long term morbidity.[13,17] This trial was done in a developing country to look at the feasibility of obtaining consent and achieving the target temperature within one hour of initiation and within 6 hours of birth, maintaining the target temperature for 72 hours and to look at adverse events and complication that could occur in our population and setting.

The sample size was calculated using the design of Gehan (1961). With a 10% precision and a 20% desired effectiveness, the sample size was calculated as 20. The analysis of the data was done on the SPSS 16.0 software. Mean, median, mode, standard deviation, frequency, Pearson's co-efficient, student t test and the Kaplan-Meier graph were used.

Inclusion criteria

This trial was designed to include infants with moderate or severe encephalopathy based on the modified Sarnat staging. Twenty newborn infants were recruited into the trial over a period of 11 months after fulfilling the eligibility criteria. Most other studies on hypothermia for perinatal asphyxia included babies with similar clinical profile to ours.[13-15,17] Some studies in addition have included aEEG as part of the inclusion criteria hoping to improve the specificity of brain injury.[62] A control arm was not included in this study as this was a feasibility trial and it was felt that the efficacy of the intervention had already been proved in randomized control trials and published data make it unethical to deny cooling to any infant.

Method and duration of cooling and rewarming

Cloth covered ice-gel packs were used as the cooling agent with the skin and the rectal temperature being monitored closely. This was an innovative method as trials in most of the developed countries

used expensive equipment. Continuous monitoring of the vital parameters was done using multi-parameter monitors. The target temperature in this trial was a rectal temperature of 32.5-33.5°C. The target temperature in other trials and the methods of cooling are mentioned in Table 9. The temperature range used in this trial is termed moderate hypothermia. The duration of hypothermia was 72 hours in our study as it was in all the other trials. Four studies used head cooling devices in conjunction with whole body cooling (Gunn 1998[52]; Akisu 2003[56]; Gluckman 2005[14]; Lin 2006[15]), while the other four used whole body cooling alone (Shankaran 2002[11]; ICE 2002[58]; Eicher 2005[59,60] Shankaran 2005[17]). The duration of hypothermia was 72 hours in all but one study that cooled infants for 48 hours (Eicher 2005[59,60]). The rewarming period was by 0.5°C per hour as in other trials. Six studies rewarmed infants by 0.5 degrees Celsius per hour with the rewarming period of four hours (Akisu 2003[56]; Eicher 2005[59,60];Gluckman 2005[14] ; Gunn 1998[52] ; Shankaran 2002[11]; Shankaran 2005[17]), one study rewarmed infants by 0.5 degrees Celsius every second hour with a duration of 8 hours for rewarming (ICE 2002[58]) and one study allowed infants to rewarm spontaneously at room temperature, such that rewarming took up to 12 hours (Lin 2006[15]).

The cost of whole body cooling apparatus with a reservoir and blanket was US$ 13,185.00 (Hemotherm dual reservoir (400 MR). Nicola JR et al[63] published data of their trial done in a low resource setting in Uganda between July and October 2007. They used low cost resources to achieve whole body cooling, including a mattress made of commercially available water bottles laid sideways in the cot and filled with cool water from the tap in the neonatal unit. The target temperature for this trial was a rectal temperature of 33-34°C. The material use in our study was available free of cost as part of the packing material of vaccine transport.

TABLE 9: IMPORTANT CHARACTERISTICS OF OTHER TRIALS TILL DATE

	Method of cooling	Target temperature	Rewarming protocol	Duration of cool
Akisu 2003[56]	Servo controlled Cooling Cap	Rectal temp: 36-36.5°C		72 hours

		Left EAC temp: 33-33.5°C		
icher 2005[59,60]	Ice to head and body for 2 hours and servo controlled cooling blanket	Rectal temp: 32.5-33.5°C	0.5°C/hour	48 hours
uckman 2005[14]	Servo controlled cooling cap	Rectal temp: 34-35°C	0.5°C/hr	72 hours
Gunn 1988[52]	Cooling cap	3 groups with rectal temp in (i) 36-36.5°C (ii) 35.5-35.9°C (iii) 34.5-35.4°C		72 hours
ICE 2002[58]	Hot/Cold Gel packs	Rectal temp: 33.5-34.5°C	0.5°C/2hours	72 hours
Lin 2006[15]	Cooling cap	Rectal temp: 34-35°C	Rewarm spontaneously to room temperature	72 hours
nkaran 2002[11]	Servo controlled cooling blanket	Oesophageal temp: 34.5°C	0.5°C/hour	72 hours
nkaran 2005[17]	Blanketrol II Hyper-Hypothermia System	Oesophageal temp: 33-34°C	0.5°C/hour	72 hours
cola JR 2008[63]	Water bottles with cool tap water	Rectal temp: 33-34°C		72 hours

It was also possible to obtain an informed consent and initiate treatment within 5 hours of birth in both inborn and outborn infants. The mean time to achieve target temperature in our study was 52 ± 25 minutes (range 15 to 105 minutes). In a similar study done in China it took 55 ± 20 minutes to reach target temperature.[15] Although many of the studies do not give the data on how long it took to cool the infants to the target temperature, available data suggest that upper limit of 90 minutes is more feasible.[17] Though only 65% were cooled in our trial within one hour, using this criterion of 90 minutes, 95% of our newborn infants reached the target rectal temperature.

An undulating pattern of temperature may not only increase the adverse effects of cold exposure when at the lowest point of temperature fluctuation but also compromise the degree of neuroprotection at the peak temperature because even small changes (as little as 1-2°C) in brain temperature may modulate the extent of hypoxic ischemic damage. In this trial the mean variation in the rectal temperature was only 0.001 ± 0.18°C from the mean target temperature of 33°C.

The mean skin temperature was 33.1 ± 0.14°C during the period of cooling as compared to the mean temperature during the trial by Shankaran of 31.9 ± 1.4°C.[17] The mean average difference between the

target temperature and skin temperature during the period of cooling was -0.18 ± 0.14°C. The relative constant skin temperature in relation to the rectal temperature implies that the skin temperature is comparable with the rectal temperature.

In developing countries where there are technical difficulties in a constant monitoring of rectal temperature, monitoring of the skin temperature may be adequate. During the initiation of the cooling, multiple cloth covered ice-gel packs were used till the target temperature was achieved. After attaining the target temperature, the number of ice-gel packs used to maintain the target temperature for the next 72 hours were very minimal. The babies tend to maintain the temperature without any difficulty. However, large for date babies required more cloth covered ice-gel packs to maintain target temperature. In developing countries which cannot afford high technology equipment, this study proves that simple cloth covered ice-gel packs with good nursing care and monitoring of the skin temperature, is practical. There was no requirement for servo-controlled blankets, manual adjustments of the water temperature of the cooling cap and output of the overhead heater or complex adjustments of the air temperature. The inability to cool 7 infants within the target time was attributed to the birth weight, nursing protocol and the absence of the investigator on site. The 7 babies had a mean birth weight exceeding 3000 gms and took a mean time of 82 minutes to cool. The investigator was not available on site for 4 of these babies. Two of the babies were started on the cooling protocol during the nursing shift resulting in inadequate monitoring. This was seen half way through the study and required additional protocols were implemented to rectify the same.

Two inborn babies who fulfilled the eligibility criteria could not be recruited into the trial as the investigator was informed after the target time of 5.0 hours of birth. Three outborn babies who fulfilled the eligibility criteria could not be included in the trial as they arrived at the hospital after 5.0 hours of life.

TABLE 10: COMPARISON OF SERIOUS ADVERSE EVENTS WITH OTHER PUBLISHED TRIALS

Adverse events	***Gluckmann 2005[14] (n = 112)***	***Gunn 1998[52] (n=13)***	***Shankaran 2002[11] (n =9)***	***Shankaran 2005[17] (n=103)***	**WBC 2008**
Cardiac arrhythmias	0 (0%)	1 (7.6%)	0	2 (1.9%)	**0 (0%)**
Hypoglycemia	14 (13%)	3 (23%)	-	12 (11.6%)	**2 (10%)**
Hyperglycemia	-	-	-	-	**3 (15%)**
Bleeding	1 (0.8%)	4 (31%)	1 (11.1%)	3 (2.9%)	**1 (5%)**
Skin changes	1 (0.8%)	-	-	4 (3.8%)	**3 (15%)**
Hypoxemia	-	4 (31%)	3 (33.3%)	25 (23.1%)	**1 (5%)**
Hepatic dysfunction	42 (38%)	0	1 (11.1%)	20 (19.4%)	**1 (5%)**
Persistent acidosis	22 (20%)	0		2 (1.9%)	**2 (10%)**
Death	36/108 (33%)	1 (7.6%)	2 (22.2%)	45 (44%)	**1 (5%)**

TABLE 11: COMPARISON OF MINOR ADVERSE EVENTS WITH OTHER PUBLISHED TRIALS

Adverse events	*Gluckmann 2005*[14] *(n = 112)*	*Gunn 1998*[52] *(n=13)*	*Shankaran 2002*[11] *(n =9)*	*Shankaran 2005*[17] *(n=103)*	*ICE 2002*[58] *(n=7)*	*Akisu 2003*[56] *(n=11)*	WBC 2008 (n=20)
Sinus bradycardia	10 (8.9%)	1 (7.6%)	-	1 (0.9%)		0 (0%)	**5 (25%)**
Hypotension (requiring inotropic support)	74 (66%)	3 (23%)	5 (28.8%)	55 (53%)	3 (42.8%)	-	**17 (35%)**
Anemia requiring transfusion	5 (4.4%)	2 (15%)	-	-	-	-	**0 (0%)**
Leukopenia	2 (1.7%)	1 (7.6%)	-	-	-	-	**0 (0%)**
Thrombocytopenia	39 (34.8%)	7 (53.8%)	-	3 (2.9%)	-	-	**5 (25%)**
Coagulopathy with hemorrhage	0 (0%)	2 (15%)	-	3 (2.9%)	1 (14.2%)	-	**1 (5%)**
Hypokalemia	71 (63.3%)	8 (61.5%)	-	-	-	-	**0 (0%)**
Sepsis	3 (2.6%)	1 (7.6%)	-	5 (4.8%)	-	1 (9%)	**0 (0%)**
Oliguria	24 (21.4%)	13 (100%)	3 (33.3%)	24 (23%)	3 (42.8%)	-	**1 (5%)**

The results of this feasibility trial agreed with other pilot studies that hypothermia is not associated with adverse events like cardiac arrhythmias, prolonged acidosis, life threatening bleeding or thrombosis. The fall in the heart rate when the rectal temperature was lowered to the target temperature was consistent with other reports and the physiologic effects of cold. The mean blood pressure increased after the initiation of whole body cooling and decreased to normal levels after the baby was re-warmed.

The biochemical and hematological parameters were monitored over the 72 hours of cooling. There was no evidence of anemia requiring transfusions or hemoconcentration in any of the newborn infants. There was thrombocytopenia in five of the infants that could be attributed to cooling. However, there was no significant clinical bleeding in 4 of the babies. One baby who had deranged coagulation profile before the start of cooling had developed disseminated intravascular coagulation with a subgaleal bleed to which he succumbed. There was no leucopenia or neutropenia that was noted. Though immunologic effects due to impaired leucocyte mobility and phagocytosis were noted in adult studies, there was no evidence of sepsis in any of the newborn infants who underwent whole body cooling. There was no

evidence of hypokalemia and persistent metabolic acidosis. Hypoglycemia was seen in two infants requiring higher carbohydrate content. They returned to normal levels after re-warming and did not require any further interventions. Hyperglycemia requiring insulin was seen in three babies. This was attributed to the decrease in insulin secretion during hypothermia. This effect has not been seen in any of the other trials involving newborn infants. Hepatic dysfunction was noted in most babies before the initiation of cooling and this was attributed to the hypoxic ischemic damage. However, one infant who had normal liver enzymes at the start of WBC, showed a worsening trend as the cooling progressed. This was not clinically significant and returned to normal levels prior to discharge. Blood lactate levels showed a downward trend suggesting that moderate hypothermia does not increase anaerobic metabolism in the cells.

The skin changes were noted specifically as cloth covered ice-gel packs were used in the initiation and maintenance of cooling. During the start of cooling, there was hyperemia in the areas where the ice-gel packs were placed and this required frequent rotation of the packs to different areas of the body. However, during the maintenance phase, the number and duration of application of the ice-gel packs was very minimal and there were no skin changes noted during this phase. The aposteatonecrosis that occurred in three babies occurred during the start of cooling. These changes subsequently resolved without any intervention and without scarring.

Death occurred in one infant who was born with a large subgaleal hemorrhage. The baby also had prolonged coagulation parameters at birth with thrombocytopenia that occurred after the initiation of cooling. The bleed into the subgaleal space worsened during the whole body cooling. The condition of the child was informed to the investigator who met with the committee monitoring adverse events. The baby was started on the rewarming protocol after 27 hours of hypothermia. The small sample size precludes any definite conclusion on adverse events. We demonstrated a good correlation between the rectal temperature and the skin temperature in babies who were cooled. However, this may not be a true correlation as the numbers studied were small.

This feasibility trial in a developing country using low cost cooling techniques and careful monitoring of temperature, establishes that this neuroprotective strategy can be used in peripheral hospitals that have a neonatal care unit. This neuroprotective strategy will help decrease the mortality and morbidity of neonatal infants born with perinatal asphyxia and will improve the quality of life of these infants.

CONCLUSIONS

The conclusions of this feasibility trial are

1. In a low resource setting, it is feasible to
    - Obtain an informed consent and start the procedure within the acceptable time-frame of 6 hours following birth.
    - Achieve a target rectal temperature within 6 hours of birth and 90 minutes of commencement of whole body cooling.
    - Maintain the target rectal temperature for 72 hours.
2. Low-cost, easily available and reusable materials like cloth covered ice-gel packs are sufficient for achieving and maintaining the target rectal temperature.
3. Constant and careful monitoring of the skin temperature may be adequate in low resource centers.
4. There were only minimal serious adverse events during this procedure.
    - Newborn infants who have deranged coagulation parameters with obvious signs of bleeding should probably be excluded from the cooling process.

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