PEER REVIEW HISTORY

BMJ Paediatrics Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

This paper was submitted to a another journal from BMJ but declined for publication following peer review. The authors addressed the reviewers' comments and submitted the revised paper to BMJ Paediatrics Open. The paper was subsequently accepted for publication at BMJ Paediatrics Open.

ARTICLE DETAILS

TITLE (PROVISIONAL)	Hypothermia for Encephalopathy in Low and Middle-Income Countries: feasibility of whole body cooling using a low-cost servo controlled device
AUTHORS	Thayyil, Sudhin; Olivera, Vania; Kumaraswami, Kumutha; Narayanan, E; Somanna, Jagdish; Bankappa, Naveen; Bandya, Prathik; Chandrasekeran, Manigandan; Swamy, Ravi; Mondkar, Jayashree; Dewang, Kapil; Manerkar, Swati; Sundaram, Mangalabharathi; Chinathambi, Kamalaratnam; Bhardwaraj, Shruti; Bhat, Vishnu; Madhavan, Vijayakumar; Nair, Mohandas; Lally, Peter; Montaldo, Paolo; Atreja, Gaurav; Mendoza, Josephine; Basset, Paul; Ramji, Siddarth; Shankaran, Seetha

VERSION 1 - REVIEW

REVIEWER	Reviewer 1
REVIEW RETURNED	11-Nov-2017

GENERAL COMMENTS	1. In the introduction part of the manuscript, the authors need to provide rationale/justification for this study by briefly discussing the existing studies of cooling in LMIC set up. Are there no studies of servo-controlled cooling in LMIC set up? 2. The authors mention that it is a low cost servo-controlled cooling system, but have not given the cost details. 3. Upon reading the title, my initial impression was that it is a pilot RCT, with the same protocol as the HELIX study. The authors need to clarify in the title and abstract that it was an observational study. 4. How was encephalopathy graded? 5. How were seizures diagnosed? Based on clinical observation or EEG? 6. What was the definition of gastric bleeding? How severe was the gastric bleeding? Did they need red cell transfusions? Did they need treatment with proton pump inhibitors? How long did the bleeding last? 7. What was the definition of PPHN? How was it managed? 8. What was the definition of pulmonary haemorrhage and its severity? How was it managed? 9. Which anticonvulsants were used? Which sedatives were used? 10. In the discussion section, the authors need to compare the results of this study (especially the incidence of tachycardia, gastric and pulmonary haemorrhage) with those of other cooling studies in the LMIC set up. Was similar high incidence of these adverse outcomes noted in other LMIC studies? If not, what could be the reason? 11. The authors report that gastric bleeding was associated with

	higher risk of mortality (<p=0.001). Which statistical test was used to analyse this association? What were the odds ratios and confidence intervals? Which potential confounders were adjusted for this analysis? 12. The authors hypothesize that that a higher incidence of gastric bleeds could have been due to IUGR. But they have not given the incidence of IUGR. 13. Is it possible that majority of infants were IUGR and hence more susceptible to cold injury and hence pulmonary and gastric bleeding and tachycardia? If this feasibility study was done as a pilot RCT, it would have helped answer this question at least to some extent. I am not sure why the investigators chose an observational design instead of the scientifically more robust pilot RCT. 14. What implications these results have for the HELIX multicentre RCT in LMIC set up? Is it possible that a milder degree of hypothermia (e.g. 34.5) might have avoided /could prevent these complications? These details need to be covered in the discussion section. 15. Web figure-2 needs a legend and labelling of the two graphs.

- This paper received two reviews from its previous journal but only one reviewers gave the permission to publish their review.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Comments to the Author

1. In the introduction part of the manuscript, the authors need to provide rationale/justification for this study by briefly discussing the existing studies of cooling in LMIC set up. Are there no studies of servo-controlled cooling in LMIC set up?

Introduction is now expanded to include the study rationale. The HELIX trial (RCT using a servo controlled device in 408 babies in LMIC) is currently ongoing and will complete recruitment by 2018. This is included in the discussion.

Please see Introduction (second paragraph)

"There are several challenges in extrapolating the safety and efficacy data of cooling therapy from high income countries to low and middle-income countries (LMIC), which shoulder 99% of the disease burden[1]. Firstly, the population co-morbidities are very different, and there is far higher incidence of foetal growth restriction, meconium aspiration and perinatal sepsis in low and middle income countries and these may reduce the neuroprotective effect of cooling. Secondly, in high income countries, cooling is provided only in specialist cooling centres with facilities for optimal intensive care support and with high nursing to patient ratios. Such facilities and staffing resourcing levels are not available in most low and middle-income country neonatal units. Finally, for effective neuroprotection, it is essential to rapidly reduce the core body temperature and precisely maintain this within the target range (33 to 34^{o}C) for 72 hours. Deviations from these regimes with over or under-cooling, or prolonging the duration of therapy, may be potentially harmful [7]. Thus, in high-income countries, therapeutic

hypothermia is administered using sophisticated and expensive servo controlled devices[8], which are unaffordable in low and middle-income countries.

Despite the lack of evidence, TH is increasingly used routine clinical practice in LMIC. A recent survey of cooling practices in India[9] suggested that most units offering cooling therapy use various improvised local solutions including air conditioning, ice packs, and phase changing materials to manually achieve cooling, but the efficacy of these methods is still debatable and they may be associated with increased monitoring and staffing requirements[8]."

Please see discussion (last paragraph)

"A large pragmatic trial of hypothermia for neonatal encephalopathy is currently underway in India, Bangladesh and Sri Lanka [24]. A total of 408 babies with moderate and severe encephalopathy will be randomised to whole body cooling using a servo controlled cooling device or usual care. All babies have detailed infection screen and 3 Tesla magnetic resonance imaging and spectroscopy in addition to detailed neurodevelopmental assessment at 18 months. The study protocol of the HELIX trial is available online and may be useful for clinicians in LMIC who wish to offer therapeutic hypothermia in these settings."

2. The authors mention that it is a low cost servo-controlled cooling system, but have not given the cost details.

We have now included the cost information of the device. Please see second paragraph of discussion

"It is likely that the eventual costs (approximately $1000 USD) of this device would be one tenth of high-income country cooling devices"

3. Upon reading the title, my initial impression was that it is a pilot RCT, with the same protocol as the HELIX study. The authors need to clarify in the title and abstract that it was an observational study.

The title is now expanded to clarify. However, this is not an observational study, but rather a single arm interventional trial.

"Hypothermia for Encephalopathy in Low and Middle-Income Countries (HELIX): feasibility of whole body cooling using a low-cost servo controlled device"

4. How was encephalopathy graded?

Encephalopathy was graded using the NICHD NRN system. We have now provided the details of the system in Table 1.

5. How were seizures diagnosed? Based on clinical observation or EEG?

Seizures were purely clinical as EEG was not available. This is now clarified in the results (third paragraph)

*Sixty-four babies were male (65.4%), twenty-one (25.6%) had severe HIE, and 61 (74.4%) had moderate HIE. **Clinical seizures** were present within the first six hours in 68 babies (87.2%).*

6. What was the definition of gastric bleeding? How severe was the gastric bleeding? Did they need red cell transfusions? Did they need treatment with proton pump inhibitors? How long did the bleeding last?

Definition and details of the gastric bleeding is now included in the results (penultimate paragraph). We don't have the information on precise duration of the bleeds.

*Gastric bleeds **(defined as fresh blood > 5 ml from nasogastric tube**) was recorded in 25/49 (51%) and pulmonary haemorrhage (defined as copious bloody secretions with clinical deterioration requiring change(s) in ventilatory management) in 6/49 (12.2%) of the babies ……. **The bleeds were treated with blood product transfusions (fresh frozen plasma, packed cell transfusions and platelets)**.*

7. What was the definition of PPHN? How was it managed?

Definition and management of PPHN is now included in the results (last paragraph)

"Persistent pulmonary hypertension (defined as severe hypoxemia disproportionate to the severity of lung disease with a significant pre-and post-ductal saturation difference on pulse oximetry) occurred in 7/49 (14.2%) of the babies. PPHN was manged by vasopressors and by optimising conventional ventilation. Inhaled nitric oxide was not available."

8. What was the definition of pulmonary haemorrhage and its severity? How was it managed?

Definition and management of pulmonary bleeds is now included in the results (penultimate paragraph)

*"Gastric bleeds (defined as fresh blood > 5 ml from nasogastric tube) was recorded in 25/49 (51%) and **pulmonary haemorrhage (defined as copious bloody secretions with clinical deterioration requiring change(s) in ventilatory management)** in 6/49 (12.2%) of the babies (Table 3). There was no significant difference between those with and without gastric bleeds for any of birthweight,*

sedation or severity of encephalopathy. However, there was a highly significant association between gastric bleeds and mortality. No patients without gastric bleeds died, whilst almost half (44%) of patients with a gastric bleed died. ***The bleeds were treated with blood product transfusions (fresh frozen plasma, packed cell transfusions and platelets)"***

9. Which anticonvulsants were used? Which sedatives were used?

Details of anticonvulsants and sedation is now included in the results

Thirty-seven babies (46.3%) required invasive ventilation during first 24 hours. ***Most babies required treatment with anticonvulsive drugs (59 (76.6%) on day one to 52 (72.2%) on day four), using mainly phenobarbitone (52.8% on day 1 to 63.6% of the babies on day 4) or multiple drugs (13 to 18.1% of the babies).*** *Sixty-three (81.8%) babies required some inotropic support on day 1, decreasing to 48 babies (67.6%) on day four. Main inotropes used were a combination of dopamine and dobutamine. Requirement of inotropic support during the first 4 days was higher for non-survivors (mean 3.9 days vs 3.0 days, p=0.000, CI [-1.64, -0.3]) but only 25% (12/48) of babies requiring inotropic support on day four died.* ***Sedation was used in 22 (36.7%) babies. This corresponded to 14 (19.0%), 18 (25.3%), 17 (23.9% and 18 (26.1%) between days 1 to 4, respectively. When using sedation, choral hydrate and fentanyl were the preferred sedatives (53% and 41% respectively)***

10. In the discussion section, the authors need to compare the results of this study (especially the incidence of tachycardia, gastric and pulmonary haemorrhage) with those of other cooling studies in the LMIC set up. Was similar high incidence of these adverse outcomes noted in other LMIC studies? If not, what could be the reason?

We have now expanded the discussion to include these and have speculated reasons. Nevertheless, there is limited good quality data from LMIC, hence such comparisons are difficult.

Discussion (fourth paragraph)

"Coagulopathy is a well known complication of hypoxic ischemic encephalopathy and gastrointestinal bleeding has been reported in upto 25% of the babies undergoing therapeutic hypothermia in high-income countries[10,11]. Cooling studies from low and middle-income countries have not yet reported such bleeds as yet. In these settings, it is possible that gastric bleeds were not specifically looked for or recorded in the case record forms and hence were overlooked"

11. The authors report that gastric bleeding was associated with higher risk of mortality (<p=0.001). Which statistical test was used to analyse this association? What were the odds ratios and confidence intervals? Which potential confounders were adjusted for this analysis?

The details of the statistical analysis and confounders is now provided under methods (statistical analysis) and the results. Odd Ratios for gastric bleeds and mortality cannot be calculated as there

were no deaths in the group without gastric bleeds. Thus, mathematically it is not possible. Table 3 provides further details on gastric bleeds.

"The associations between clinical variables and mortality was examined. The unpaired t-test or Mann-Whitney test was used to compare the continuous variables between survivors and non-survivors, whilst Fisher's exact test was used to compare categorical variables between groups. We used IBM SPSS (v24; IBM Corp, New York) and STATA (v15; StataCorp LP, Texas) for all data analysis"

12. The authors hypothesize that that a higher incidence of gastric bleeds could have been due to IUGR. But they have not given the incidence of IUGR.

There was no association of birthweight and gastric bleeds. Details are provided in the results. Please see table 3.

13. Is it possible that majority of infants were IUGR and hence more susceptible to cold injury and hence pulmonary and gastric bleeding and tachycardia? If this feasibility study was done as a pilot RCT, it would have helped answer this question at least to some extent. I am not sure why the investigators chose an observational design instead of the scientifically more robust pilot RCT.

The aim of this study was to examine the feasibility of conducting a large phase III RCT and to inform its trial design. A pilot RCT may not be more scientifically robust for this purpose, and indeed false conclusions may be drawn from such underpowered randomised controlled trials. Any number of speculations can be made at this stage, however data from larger phase III HELIX trial would be required to provide a definite answer to these issues.

14. What implications these results have for the HELIX multicentre RCT in LMIC set up? Is it possible that a milder degree of hypothermia (e.g. 34.5) might have avoided /could prevent these complications? These details need to be covered in the discussion section.

The HELIX multi-centre RCT is currently ongoing and will complete recruitment by 2018. The implications are given in the discussion (last paragraph). There is no evidence to support the use of milder degree of hypothermia (34.5 C) in a phase III trial at present.

"The data presented in this feasibility study will inform the study design of future cooling trials in LMIC. Our inclusion criteria were purely clinical so as to increase the generalisability of the therapy in LMIC, where blood gas and aEEG are not routinely available. A large pragmatic trial of hypothermia for neonatal encephalopathy is currently underway in India, Bangladesh and Sri Lanka [24]. A total of 408 babies with moderate and severe encephalopathy will be

randomised to whole body cooling using a servo controlled cooling device or usual care. All babies have detailed infection screen and 3 Tesla magnetic resonance imaging and spectroscopy in addition to detailed neurodevelopmental assessment at 18 months. The study protocol of the HELIX trial is available online and may be useful for clinicians in LMIC who wish to offer therapeutic hypothermia in these settings."

15. Web figure-2 needs a legend and labelling of the two graphs.
Legends are provided

VERSION 2 – REVIEW

REVIEWER	Rao, Shripada Princess Margaret Hospital for Children and King Edward Memorial Hospital for Women, Perth, Western Australia Centre for Neonatal Research and Education, University of Western Australia Competing Interests: Nil
REVIEW RETURNED	15-Jan-2018

GENERAL COMMENTS	The authors have addressed previous reviewers' comments adequately. 1. Few minor grammatical corrections are required. Examples: a. "Bleeding problems, particularly gastric bleeds frequently occur encephalopathic babies who had cooling therapy in these settings and is associated with high mortality". It is better written as follows: Bleeding problems, particularly gastric bleeds frequently occur in encephalopathic babies undergoing cooling therapy in these settings and are associated with high mortality. b. "These hospitals were funded by the Indian government, and offered free healthcare to low income populations" This sentence is better written as follows: "These hospitals are funded by the Indian government, and offer free healthcare to low income populations". 2. The authors mention that babies who died were more likely to have tachycardia (93% vs. 51%; p=0.005). Please clarify the definition of tachycardia here because, from figure 2, it appears that mean heart rate was never>160 in the study infants.

REVIEWER	Koutoumanou, Eirini UCL, ICH Competing interests: None
REVIEW RETURNED	29-Jan-2018

GENERAL COMMENTS	This is a well written and presented report. Please see below some minor suggestions: Please report what NICHD stands for as soon as it appears on the report. Out of the 173 screened babies, were 82 the only eligible ones? If yes, please state so clearly. If not, how was the selection of the 82

	out of 173 made? Can the authors please double check if maternal age was normally distributed as based on the results presented I suspect it wasn't, i.e. moving either side of the mean of the survivors group 2 standard deviations (22.6±2* 3) takes you to a minimum maternal age of 22.6-6=16.6 – were mothers as young as 16 (and younger in fact) part of this sample? If not, the data is not normally distributed and should be summarised with a median and inter-quartile range. Please add confidence intervals in table 3 for the comparisons presented as these will provide more meaningful results of the significant and non-significant differences seen. I was not able to find a reference in the paper for the following statement that's seen in the abstract: "Fifteen (18%) babies died before discharge from hospital". This is one of the most important results from this data and it should be easily spotted in the core part of the paper. I do not think the paper finished with the right note. I do not think referring to another future study as the concluding remarks of your manuscript is appropriate. Correct: "designed clinical trials are trial are required"

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1

1. Few minor grammatical corrections are required.
Examples:

a. "Bleeding problems, particularly gastric bleeds frequently occur encephalopathic babies who had cooling therapy in these settings and is associated with high mortality".

It is better written as follows: Bleeding problems, particularly gastric bleeds frequently occur in encephalopathic babies undergoing cooling therapy in these settings and are associated with high mortality.

b. "These hospitals were funded by the Indian government, and offered free healthcare to low income populations"

This sentence is better written as follows: "These hospitals are funded by the Indian government, and offer free healthcare to low income populations".

We thank the reviewer for pointing out these grammatical errors. We have now corrected these.

2. The authors mention that babies who died were more likely to have tachycardia (93% vs. 51%; p=0.005). Please clarify the definition of tachycardia here because, from figure 2, it appears that mean heart rate was never>160 in the study infants.
The definition of tachycardia in babies undergoing therapeutic hypothermia is elusive. In our experience, most babies have heart rate of <120 bpm during cooling therapy, hence we considered heart rate >120bpm during cooling as elevelated. We have now made this explicit in the results.

*"The babies who died were more likely to have a **heart rate higher than 120bpm** (93% vs. 51%; p=0.005) and more **persistently elevated heart rate (>120bpm)**(median of 5 hours compared to a median of 1 hour) than those who survived; p=0.01). Duration of **heart rate above 120bpm** did not appear to be associated with sedation or sedation days, neither did sedation (lack of) seem to be associated with mortality" (page 6, last para)*

Reviewer: 2

#1. This is a well written and presented report. Please see below some minor suggestions:

Please report what NICHD stands for as soon as it appears on the report.

NICHD stands for the National Institute of child Health Health and Human Development (NICHD). This is now expanded at the first appearance. (page 4, methods, first para)

#2. Out of the 173 screened babies, were 82 the only eligible ones? If yes, please state so clearly. If not, how was the selection of the 82 out of 173 made?

Only 82 were eligible. We have now made this explicit in the results

*"Out of 173 babies screened, **91 did not meet the eligibility criteria (age>6 h; mild encephalopathy; non-availability of the device)**." (page 5 results, first para)*

#3. Can the authors please double check if maternal age was normally distributed as based on the results presented I suspect it wasn't, i.e. moving either side of the mean of the survivors group 2 standard deviations (22.6±2* 3) takes you to a minimum maternal age of 22.6-6=16.6 – were mothers as young as 16 (and younger in fact) part of this sample? If not, the data is not normally distributed and should be summarised with a median and inter-quartile range.

The data are normally distributed and the mean and median (IQR) 22(3) are similar, with a skewness of 0.790 and kurtosis of 2.1. Hence we have provided the mean values for all variables, except the Apgar score, which is now given as median (IQR) in Table 2.

#4. Please add confidence intervals in table 3 for the comparisons presented as these will provide more meaningful results of the significant and non-significant differences seen. We have now provided confidence intervals for mean and proportion difference in Table 3.

#5. I was not able to find a reference in the paper for the following statement that's seen in the abstract: "Fifteen (18%) babies died before discharge from hospital". This is one of the most important results from this data and it should be easily spotted in the core part of the paper.

We have now included this in the main text.

"Out of 173 babies screened, 91 did not meet the eligibility criteria (age>6 h; mild encephalopathy; non-availability of the device). We enrolled the remaining 82 into this study after informed parental consent (49 babies from Institute of Child Health (Madras Medical College, Chennai, 14 babies from

Indira Gandhi Institute of Child Health, Bangalore; 10 babies from Lokmanya Tilak Municipal General Hospital, Mumbai; 9 babies from Jawaharlal Nehru Institute of Postgraduate Medical Education and Research, Puducherry). Sixty-one (74%) babies had moderate and 21 (26%) had severe encephalopathy. ***Fifteen (18%) babies died before discharge from hospital (five (8%) babies with moderate encephalopathy and 10 (47%) with severe encephalopathy)****." (page 5, results, first para)*

#6. I do not think the paper finished with the right note. I do not think referring to another future study as the concluding remarks of your manuscript is appropriate. Correct: "designed clinical trials are trial are required"

The conclusion is not changed in view the editorial remarks