

Feasibility and Safety of Combining Therapeutic Hypothermia with Magnesium Sulfate Administration, in the Management of Neonates with Hypoxic Ischemic Encephalopathy - Randomized Control Trial

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Abstract

Objective: The aim of the study was to confirm the therapeutic effectiveness and no significant side effects of therapeutic hypothermia plus magnesium sulfate in the group of newborns with HIE.

Materials and methods: Neonates born at ≥ 36 GA, with perinatal asphyxia and confirmed moderate or severe HIE were randomized to study (TH+Mg) or control (TH) groups. Neonates in study group received three doses of MgSO₄ (250 mg/kg) as intravenous infusion on three consecutive days in addition to therapeutic hypothermia. Newborns in control group were treated only with therapeutic hypothermia. Primary outcome was risk of death during initial hospitalization, secondary outcome: short-term neurological status. Additionally the circulatory, respiratory and hematological parameters were assessed.

Results: 75 patients were included in this study. There were no statistically significant differences in the rate of death (OD 1.053; 95% CI 0.26-4.25; p=0.943). Neonates in study group had better neurological outcome after cooling procedure, their neurological assessment at 5 days of age (by Thompson scale) improved (4.75 vs. 7.03; p=0.032). They had shorter time to full oral feeding 14.5 vs. 16.5 days; p=0.006 and shorter hospitalization time 23.55 (± 8.0) vs. 27.77 (± 14.1); p=0.003. Coagulation parameters, number of blood products transfusions and incidence of intracranial bleedings were not affected by the magnesium administration. Risk of PPHN incidences was lower (OD 4.5; 95% CI 0.90-22.38; p=0.049, NNT=7).

Conclusion: Applied therapy was not associated with increased risk of death or other significant adverse effects. Faster normalization of short-term neurological functions in study (TH+Mg) group was observed.

Keywords: Newborn; Asphyxia; Hypoxic ischemic encephalopathy; Magnesium sulfate; Therapeutic hypothermia

Abbreviations aEEG: Amplitude-integrated Electroencephalogram; BE: Base Excess; HIE: Hypoxic-Ischemic encephalopathy; TH: Therapeutic Hypothermia; SEM, Standard Error of the Mean; SHC: Selective Head Cooling; WBC: Whole Body Cooling; HIES: Hypoxic-Ischemic Encephalopathy Score

Introduction

In the first decade of the 21st century therapeutic hypothermia became broadly used in the management of the newborns with hypoxic ischemic encephalopathy [1]. The neonatal resuscitation guidelines since 2010 recommend that therapeutic hypothermia be used as a standard treatment method in asphyxiated newborns diagnosed with moderate or severe hypoxic ischemic encephalopathy [2]. Although the efficacy of therapeutic hypothermia has been shown in several randomized trials its beneficial effect is in many cases viewed as modest only. Therefore most new research efforts tend to focus on finding the ways to enhance the effect that therapeutic hypothermia

produces. The currently considered potential new treatment modalities include: modifications of the cooling process, combining therapeutic hypothermia with potentially neuroprotective drugs, and stem cells therapy. These new potentially therapeutic drugs include, but are not limited to: topiramate, erythropoietin, xenon, melatonin, magnesium sulfate and cannabinoids [3]. Magnesium plays an essential role in numerous biochemical pathways, including the reactions which may protect the developing brain. This molecule is also an irreplaceable co factor in glycolysis reaction, oxidative phosphorylation, protein synthesis, DNA, and RNA aggregation as well as in the maintenance of plasmatic membrane integrity [4-6].

Magnesium is a physiological calcium channel blocker and calcium antagonist. It has been reported that magnesium defends tissues against free radicals and magnesium deficiency increases the risk of lipid peroxidation.

When administered peripherally, it has the ability to readily pass through to central nervous system and finally block NMDA receptors. The increase in extracellular level of Mg²⁺ from 0.5 to 1.0 mmol/L is adequate to induce synaptic and neuronal response. Numerous papers have already published on the neuroprotective efficacy of NMDA

receptor antagonists in disorders resulting from toxic glutamate impact. Potential-dependent blocking by magnesium ions is one of the most significant features of NMDA receptor (Mg^{2+}). NMDA receptor inhibition by Mg^{2+} ions increases along with postnatal age [7,8]. Magnesium sulfate administered antenatally to the mothers has recently been used as potentially neuroprotective management in preterm neonates [9]. In some centers, including ours, neuroprotective properties of magnesium sulfate are also exploited in term, asphyxiated newborns [10,11]. Taking into account our previous experience with magnesium sulfate administered to asphyxiated term neonates and the outcomes of several other clinical trials, we've designed a randomized controlled trial to study the effect of therapeutic hypothermia combined with magnesium sulfate [12-15]. The research project was titled "Hypothermia Enhanced by Magnesium in Encephalopathy of Newborn" (acronym "HEMEN").

Aim of the Study

The aim of our study was to investigate the safety of therapeutic hypothermia combined with magnesium sulfate and its effect on the clinical course (circulatory stability, level of respiratory support, inotropes use, anti-seizure medications, use of blood components) and short term outcomes (death, neurological assessment, time to achieve full enteral nutrition, duration of hospitalization) in the group of neonates with HIE.

Material and Methods

Prospective randomized controlled trial was conducted at three centers: two Neonatal Intensive Care Units located in level III perinatal centers and one Pediatric Intensive Care Unit. The RCT protocol has been approved by the local bioethical board - PMM Hospital - Research Institute Ethical Committee. Written informed consent was obtained from all the parents of the study subjects.

The eligibility criteria were similar to previously reported in other RCTs studying the effects of therapeutic hypothermia in newborns: gestational age above 36 weeks of gestation, acute perinatal sentinel event (perinatal asphyxia) confirmed by Apgar score of ≤ 5 at 10 minutes of life and/or umbilical cord blood or any blood during the first hour after birth with pH < 7.1 and/or base deficit ≥ 16 , need of mechanical ventilation for at least 10 minutes after birth, and admission within 6 hrs of birth. Neonates with major congenital malformations and extremely poor prognosis (Apgar score 0 at 15 minutes of life or later) were not included in the study.

All infants meeting the above listed criteria underwent the standardized neurological evaluation (Sarnat grading scale). Newborns which have fulfilled the clinical criteria of moderate or severe hypoxic ischemic encephalopathy were further screened with aEEG using Infant aEEG cerebral function monitor Olympic CFM™ 6000. Patients with moderate or severe abnormalities on aEEG were randomized to either the control (therapeutic hypothermia - TH) or study group (therapeutic hypothermia with magnesium sulfate - TH+ $MgSO_4$). Each of the participating centers received an individually designed randomization table prepared by the central coordinating board. The long-term evaluation of psychomotor development according to the scale BAYLEY III is still being conducted and will be the subject of further publications.

Therapeutic hypothermia protocol

Therapeutic hypothermia was induced and maintained for 72 hours. The studied neonates underwent either selective head cooling (SHC) or whole body cooling (WBC). Natus/Olympic Medical Cool Cap System or Inspiration Healthcare/Tecotherm Neo was used for the cooling process.

Rectal temperature was maintained at 34.0-35.0°C in the SHC group, and slightly lower at 33.0-34.0 C in the whole body cooling group. The cooling period was followed by a rewarming phase at the rate of 0.5°C/ hr.

Administration of $MgSO_4$ protocol

Neonates who were randomized to the study group (TH+Mg) received three 250 mg/kg doses of magnesium sulfate given as one-hour continuous infusion spaced 24 hours apart on three consecutive days. 20% Magnesium Sulfuricum (Polpharma), 2 g/10 ml were used.

Serum Mg level expressed as mmol/dL, was assessed on study day 1 just before and one hour after infusion of the drug was completed, and then on day 2, 3, and 4 randomly with other routine labs. Serum magnesium level was measured with dry chemistry system - colorimetric method using a formazon dye (Analyser: Vitros 5.1/350 Ortho Clinical Diagnostics or Roche Diagnostics, Cobas 6000 analyzer series).

Throughout the duration of the cooling and rewarming process, vital signs (heart rate, arterial blood pressure, rectal temperature) and parameters of respiratory support (FiO_2 , MAP, level of respiratory support) were monitored and recorded every hour for the first 6 hours, then every three hours, and again every hour during rewarming. Total of 36 measurements were taken. The vital signs were monitored using Philips IntelliVue MP70 Neonatal Monitors or Drager Infinity[®] Delta Monitors.

The use of inotropes, anticonvulsants, volume expanders as well as the number of blood components (platelets, fresh frozen plasma, red blood cells) transfusions during first 7 days of life were also recorded. Parameters of coagulation were followed closely. Additionally clinical data such as number of seizure episodes, incidence of IVH detected by cranial USG, rate of PPHN confirmed by ECHO exam and feeding difficulties were analyzed.

Assessment of the neurological status of the studied infants was performed by trained personnel daily during the cooling and rewarming phase using the Hypoxic-Ischemic Encephalopathy Score described by Thompson-HIES [16].

Statistical analysis

Chi-square or Fisher exact test were used to compare the percent accuracy between groups. The difference between the groups and over time changes for continuous data were analyzed with one-way or two-way analysis of variance (ANOVA). Fishers LSD (least squares difference) post hoc comparison tests were applied to examine if significant differences between means exist. Differences in mean ranks values between groups were analyzed with the Student's t-test or Mann-Whitney U test. Statistical analysis was carried out using STATISTICA (StatSoft, Tulsa, OK, USA, version 6.0 PL). The p-value < 0.05 was considered statistically significant.

Results

Study population demographic data and clinical characteristics are presented in Table 1.

There were no statistically significant differences between the groups according to birth body weight, gestational age and Apgar score. Umbilical cord blood pH, BE, core temperature at admission and neurological assessment at the time of initiation of the cooling were also not different between the groups. 29 (78.4%) of patients in the control group and 30 (78.6%) patients in the study group were outborn and transferred to the one of the three study centers by specialized regional neonatal transport. The rate of cesarean sections,

which were performed mainly for the emergency reasons, was not different between the groups. Time to initiation of cooling procedure didn't exceed 6th hours of life and was (mean, \pm SD) 337.9 (\pm 108.2) and 305.0 (\pm 134.2) minutes in the control and study group respectively. The difference was not statistically significant.

We did not consider the outcome with regard to the cooling method. There were 49 subjects in the SHC and 26 in the WBC group. Due to randomization conducted separately for each center the groups were equal according to magnesium administration: WBC (TH-13; TH+Mg-13) and SHC (TH-24; TH+Mg-25).

	Control group (TH) 37	Study group (TH+Mg) 38	p-value
Gestational age (weeks); mean (\pm SD)	38.58 (\pm 1.9)	38.73 (\pm 1.7)	0.71
Female gender, number (%)	28/14 (43)	20/16 (36)	0.48
Birth body weight (g); mean (\pm SD)	3173.8 (644.7)	3321.9 (524.0)	0.28
Cesarean section (%)	28 (75.7)	24 (64.2)	0.25
Apgar score:			
@ 5 minutes	2.52 (\pm 1.71)	3.08 (\pm 1.74)	0.16
@ 10 minutes	3.08 (\pm 1.71)	3.71 (\pm 1.79)	0.13
Umbilical blood pH ^a (\pm SD)	6.87 (0.148)	6.89 (0.157)	0.47
Outborns n (%)	29 (78.4)	30 (78.6)	0.87
Base deficit	-20.50 (\pm 5.8)	19.8 (\pm 5.6)	0.83
Core temperature at admission ($^{\circ}$ C)	33.9 (\pm 1.69)	33.8 (\pm 1.85)	0.83
Sarnat grading scale at <6			
-Stage II	22	24	
-Stage III	15 (42.9)	14 (37.8)	0.67
Seizures on admission (n; %)	13 (35.1)	15 (44.1)	0.45
Time to initiation of hypothermia (minutes of life)	337.9 (\pm 108.2)	305.0 (\pm 134.2)	0.21
Infusion start time of MgSO ₄	-	227.8 (\pm 143.2)	-
The p-value <0.05 was considered statistically significant. ^a umbilical cord blood or any blood sampling during the first hour after birth			

Table 1: Demographic data and clinical characteristic of the study subjects.

Serum magnesium concentration in both groups during the first 4 days of the study is presented in Figure 1. At the study entry (time 0), mean serum Mg concentration was 0.89 mmol/L in the study group, and 0.95 mmol/L in the control group. Values were not significantly different. After the first infusion magnesium sulfate serum magnesium concentration increased to mean 2.21 mmol/L (ranged 1.43 to 3.77 mmol/L). In the next two days the average Mg concentrations ranged between 1.82 and 1.69 mmol/L, and then decreased to the mean values of 1.32 mmol/L on the study day 4. During the same time period in the control group serum magnesium concentration remained stable and ranged from 0.94 to 0.98 mmol/L. The difference between the groups was statistically significant (p-values <0.05).

Figures 2 and 3 presents the mean heart rate and mean arterial blood pressure values in each of the 36 points of measurement in the control and study groups during cooling and rewarming phase and additionally the averaged values over each day of the study. Time 0 in these and subsequent figures marks the initiation of the cooling procedure. In the study group (TH+Mg) there was a slight decrease in heart rate (by 7.6%) and mean arterial pressure (by 7.2%) on the first day of the study, but the difference between the groups was not statistically significant and of minor clinical significance.

Selected neonatal outcomes in the control and study group are presented in Table 2. Time to full oral feedings (bottle feeding or

breastfeeding) was significantly reduced in the treatment group (16.5 vs. 14.5 days). Furthermore, 33 of 34 (97%) patients in the treatment group were discharged from the hospital on the full oral feedings compared with only 27 of 36 (75%) in the control group. Also the duration of hospitalization time was shorter in the group neonates receiving magnesium sulfate (27.7 vs. 23.5 days). Number of patients requiring anticonvulsants were not different between the groups, but number of doses per patient was significantly lower in the study group.

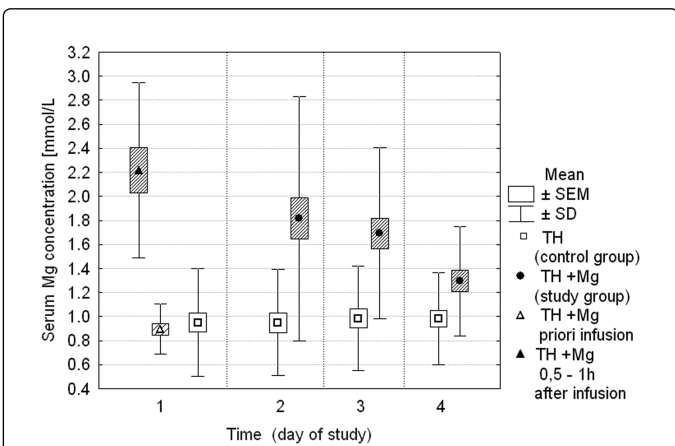


Figure 1: The mean serum magnesium concentrations during the maintenance and re-warming phase.

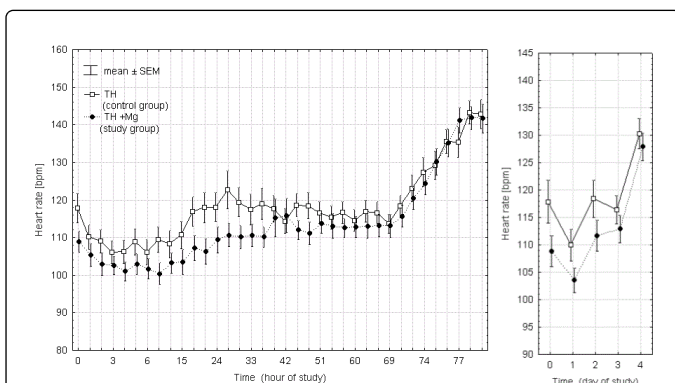


Figure 2: The mean heart rate in the control and treatment group during cooling and re-warming phase.

Number of days on mechanical ventilation and number of days on oxygen were not significantly different between the groups. During the cooling phase neonates receiving magnesium required a somewhat longer support with non-invasive ventilation (Figure 4), but again the difference did not reach statistical significance.

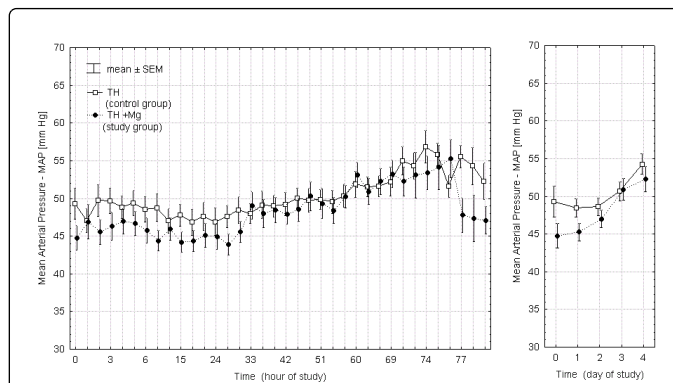


Figure 3: The mean arterial blood pressure values during the cooling and re-warming phase in the study and treatment group.

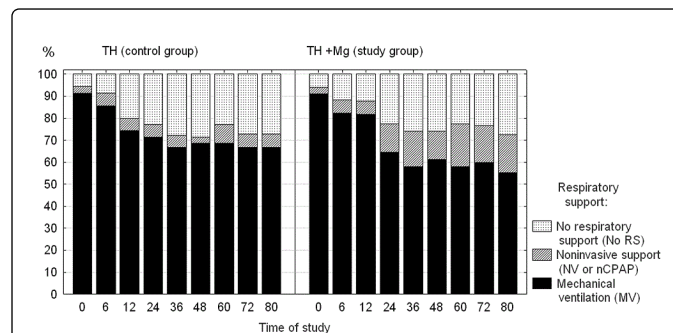


Figure 4: The level of respiratory support in the control and treatment group during cooling and re-warming phase.

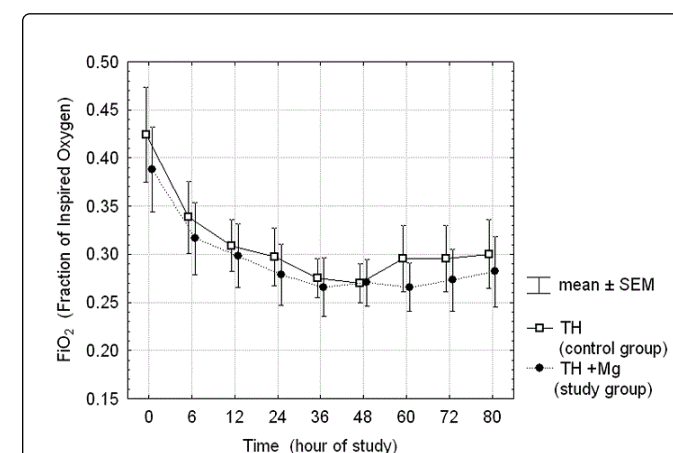


Figure 5: Fraction of inspired oxygen (FiO₂) values during the cooling and re-warming phase in the study and treatment group.

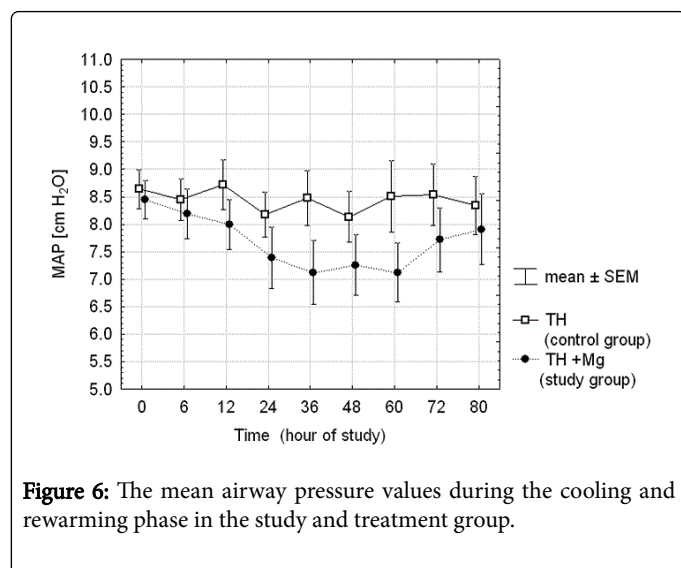


Figure 6: The mean airway pressure values during the cooling and rewarming phase in the study and treatment group.

There were analyzed together of any type of mechanical ventilation (SIMV, PC, HFOV) and any type of noninvasive respiratory support (noninvasive ventilation, nasal CPAP). There was a three-fold reduction of PPHN rate and need for INO in the treatment group, but the differences did not reach the statistical significance. Fraction of inspired oxygen (Figure 5) and mean airway pressure (Figure 6) were also not different between the groups.

Duration of antibiotic therapy was not affected by the treatment with magnesium sulfate. Inotropes use was slightly higher in the group of patients receiving magnesium sulfate, but the difference was not statistically significant. The overall death rate was 6.7%. The mortality did not differ between the groups.

	Control group (TH) 37	Study group (TH+Mg) 38	Statistical significance
Respiratory support [days] mean (±SD) ^a	5.3 (±4.0)	4.67 (±4.8)	NS
Noninvasive support [days] mean (±SD) ^{aa}	0.63 (±1.1)	1.85 (±4.1)	0.08
Oxygen supplementation [days] mean (±SD)	4.67 (±4.8)	4.51 (±6.1)	NS
Diagnosis of PPHN	6 (16.2)	3 (7.9)	0.049
iNO treatment	3 (8.1)	2 (5.3)	0.21
RBC transfusion (No of patients)	5	5	NS
No/patient	0.17	0.24	
FFP transfusion	13	11	NS
No/patient	0.72	0.4	
PLT transfusion	4	3	NS
No/patient	0.14	0.08	
Catecholamine use:			
- None	18	11	
- 1	19	27	0.08
- ≥2	15	15	NS
Use of anticonvulsant (No of patients)	31	28	NS
No of doses/patient	4.4	2.6	0.0025
Intracranial hemorrhage (grade)	I-3	I-0	NS
	II-2	II-2	
Antibiotic therapy [days] mean (±SD)	14.81 (±7.6)	13.63 (±6.8)	NS
Full enteral feeding	14.5 (±5.9)	14.2 (±5.1)	NS
Oral feedings (sucking) [days] mean (±SD)	16.5 (±9.4)	14.5 (±5.5)	0.0057

Age at discharge/DOL mean (±SD)	27.77 (±14.1)	23.55 (±8.0)	0.0026
Death during hospital stay n (%)	2 (5.4)	3 (7.9)	NS
The p value of < 0.05 was considered statistically significant			
ªany type of mechanical ventilation (SIMV, PC, HFOV), ªªany type of noninvasive respiratory support (Noninvasive ventilation, nasal CPAP).			

Table 2: Neonatal outcomes in the study population.

Coagulation parameters were evaluated as clinical indicated. Although more often controlled in the study group (34 vs. 25 results,) the values were similar in both groups (Table 3).

	Control group (TH)	Study group (TH+Mg)	p-value
No of results	25	34	
Prothrombin time	27.6 (±18.6)	23.3 (±15.6)	0.334
INR time	60.5 (±20.9)	67.4 (±21.5)	0.351
aPTT	54.9 (±27.1)	54.7 (±29.7)	0.981
The p-value of <0.05 was considered statistically significant.			

Table 3: Coagulation parameters in the study and treatment group.

The mean platelet count in the group receiving magnesium sulfate was slightly lower than in controls, but the difference was not statistically significant (Figure 7). The rate of thrombocytopenia $\leq 100,000/\text{mm}^3$ was 40.5 vs. 50.0% in TH vs. TH+Mg group respectively; p-value 0.336. Number of blood components transfusions needed was not significantly different either (Table 2).

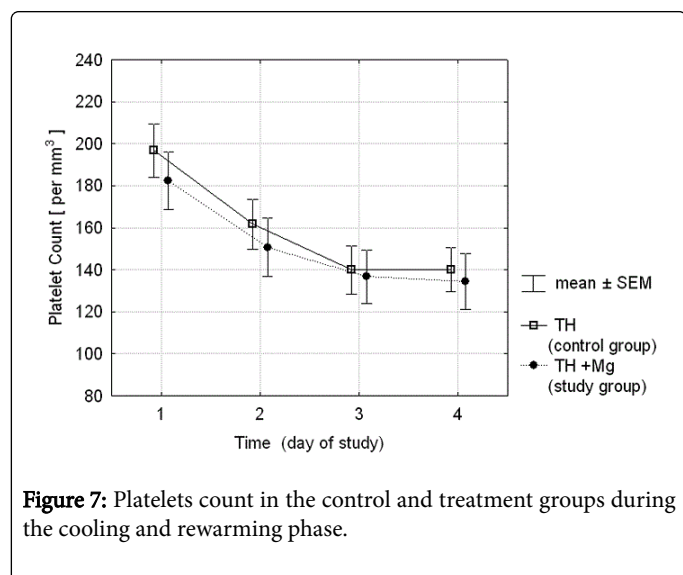


Figure 7: Platelets count in the control and treatment groups during the cooling and rewarming phase.

Hypoxic-ischemic encephalopathy scores (Thompson scale) performed daily during the cooling and rewarming procedure in both groups of patients are presented in Figure 8. On day 5 of the study after the cooling procedure was completed the score was significantly lower in the treatment group (4.75 vs. 7.03; p-value 0.03).

Discussion

Since the therapeutic hypothermia is considered a standard of care for the patients with HIE, clinical trials investigating the effects of new treatment options for this group of patients, especially drugs with potential neuroprotective effects will have to include hypothermia as a part of a study protocol. Until now there are several published studies evaluating the effectiveness of magnesium sulfate in the group of asphyxiated neonates, including two randomized controlled trials [17,18]. Results are promising. However, all of these studies were conducted before the era of therapeutic hypothermia. Furthermore, irrespective of the potential benefits, safety of using magnesium sulfate during therapeutic hypothermia in the group of term and late preterm neonates was not studied. It is particularly important in the light of the results presented by Mittendorf, et al. They studied the effects of prenatal aggressive treatment with magnesium sulfate on the outcome of the neonates born with very low birth weight and showed that patients exposed to high doses of magnesium were at higher risk for developing severe intracranial bleeding [19,20]. Other known side effects of high serum magnesium levels are: vasodilatation, hypotension, cardiac arrhythmias, coagulopathy, and gastrointestinal disturbances. Some of the potential side effects of magnesium administration are similar to the symptoms observed in the newborns with asphyxia or side effects of therapeutic hypothermia: bradycardia, hypotension, thrombocytopenia [21].

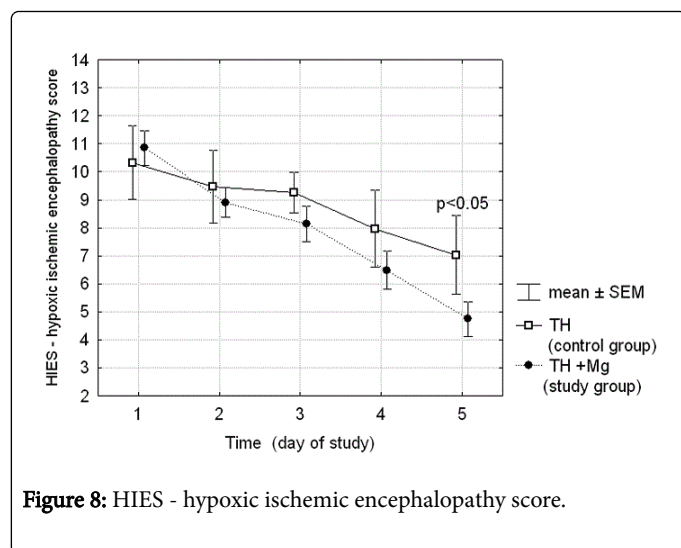


Figure 8: HIES - hypoxic ischemic encephalopathy score.

Although the therapeutic window for magnesium sulfate to act as a neuroprotective agent is not known it seems reasonable to start magnesium therapy as soon as possible after the hypoxic insult. In our study, the magnesium treatment was started on average 75 minutes before the initiation of hypothermia. In the study group expected therapeutic range of serum magnesium level was reached with the first dose of $MgSO_4$ and then maintained for 3 days of treatment [7,22,23]. According to Levene and co-workers serum magnesium concentration in the range between 1.5-2.8 mmol/dL should have the neuroprotective effect. Previously published studies have also shown that the target concentration range can be achieved with magnesium sulfate administered at the dose of 250 mg/kg.

We have observed that magnesium sulfate given to hypoxic newborns undergoing hypothermia does not significantly increase the occurrence of bradycardia or hypotension. The small drop in mean blood pressure in the group receiving magnesium was not associated with an increased need for vasopressors. This observation is consistent with the results reported by other authors using magnesium sulfate in similar dosage but without hypothermia [24]. Since, before the era of inhaled nitric oxide, magnesium sulfate was used for the treatment of persistent pulmonary hypertension of newborn observed in our study mild vasodilation may be perceived as an additional beneficial effect of magnesium administration [25]. It may also explain observed three-fold reduction of PPHN rate and need for iNO in our treatment group.

Because of its platelets inhibiting properties, antenatal exposure to high doses of magnesium sulfate may be associated with increased risk of intracranial bleeding. In our study magnesium sulfate administration was not associated with the increased risk of intracranial bleeding, and had no significant effect on the number of blood products transfusion or coagulation test results [19,20,26-28].

Side effects might depend on the dose of the drug. Optimal dose of magnesium sulfate for the neonates with hypoxic-ischemic encephalopathy is yet not determined. Levene and Blennow evaluated two different doses of magnesium sulfate ($MgSO_4$) in a group of full term infants with Apgar scores of <6 at 10 minutes [22]. With the larger dose (400 mg/kg) they observed hypotension at one hour post infusion and respiratory depression lasting three to six hours. EEG readings and heart rate were not affected. Mean serum Mg^{2+} increased from 0.79 to 3.6 mmol/L at one hour after infusion. After administration of the lower dose of $MgSO_4$ (250 mg/kg) mean serum

level Mg^{2+} increased from 0.71 to 2.42 mmol/L at one hour and blood pressure, heart rate, muscle tone and EEG were unchanged. The authors concluded that dose of 250 mg/kg $MgSO_4$ was not associated with hypotension although respiratory depression can occur. Because of the potential risk of hypotension, some experts suggest starting dopamine infusion with magnesium sulfate administration [23]. Others, however, didn't observe any significant effect on blood pressure, heart rate or respiratory rate. Respiratory depression was previously reported by some authors especially when using higher doses of $MgSO_4$ [21]. In this study, magnesium administration did not have a significant effect on the duration and level of respiratory support or oxygen requirement. Ichiba, et al conducted randomized control trial studying the effect of postnatal administration of $MgSO_4$ (250 mg/kg, 3 doses administered daily as continuous infusion) [23,24]. Analyzing the clinical outcomes they've noticed significantly higher survival rate with normal results of cranial computed tomography, electroencephalography and establishment of oral feeding by 14 days of age in the treated group compared with the control group. They've concluded that magnesium sulfate administered postnatally may improve neurodevelopmental outcome in infants with severe birth asphyxia. Those studies however were performed before the era of therapeutic hypothermia.

The presented research was carried out in the first three hypothermia centers established in the country. The studied neonates underwent either selective head cooling (SHC) or whole body cooling (WBC) depending on the availability. So far we have a several studies comparing the two aforementioned methods of therapeutic hypothermia of newborns [29]. The stability of vital parameters: ABP and HR at hourly intervals were previously assessed by Hoque et al. [30]. The authors did not find differences in mean arterial blood pressure or mean heart rate between groups (SHC vs. WBC) during the maintenance phase of cooling. The risk of adverse effects and early outcome depending on the therapeutic hypothermia method used were assessed by another group of researchers [31]. The authors did not reveal differences in terms of hypotension, bradycardia, thrombocytopenia, abnormality in the coagulation tests, renal dysfunction, need for mechanical ventilation and weaning from respiratory support while cooling or PPHN. Likewise, research conducted at University of Michigan confirmed that important components of multi-organ system dysfunction and hypotension treated with inotropes for >24 h, commonly required in newborns with HIE, were similar for both methods of TH [32]. The requirement for invasive mechanical ventilation, ability to perform extubation during the period of cooling and risk of PPHN were analyzed in two studies [31,33]. No differences were found between groups in regard to the need of mechanical ventilation during cooling or ability to be extubated during the cooling. Ventilatory parameters during the cooling including maximum peak inspiratory pressure (PIP), maximum positive end-expiratory pressure (PEEP), maximum FiO_2 (fraction of inspired oxygen), and highest $PaCO_2$ (partial pressure of carbon dioxide) were not different between whole body and selective head cooling either [33]. Finally, also Sarkar et al. who conducted an observational study examining organ function in whole body cooling and selective head cooling, stated that indicators of organ dysfunction were similar between group including coagulopathy treated with fresh frozen plasma (FFP), thrombocytopenia treated with platelet transfusion, hypotension treated with vasopressors for >24 hours [32]. The above mentioned group of authors published results of long term follow-up in prospective randomized, small-scale pilot study, when the rate of death, severe disability, composite outcome of death or

neuromotor development delay was evaluated. The authors did not reach statically significant differences [34]. Thus, neither SHC nor WBC has been proven superior in terms of safety and efficacy. At present, both methods are considered as appropriate and equivalent [35] and therefore in this study we did not consider the outcome with regard to the cooling method.

Easy availability makes magnesium sulfate a very attractive option as a neuroprotective drug. It can be administered to the patient in the birth hospital while neonate is being prepared for the transport to the center with therapeutic hypothermia. Timing of the intervention is very important in the management of the neonates suffering from perinatal asphyxia. Both, therapeutic hypothermia, as well as administration of potentially neuroprotective drug should be started during so called "therapeutic window". It is the initial potentially reversible phase of hypoxic insult lasting about 6 hours followed by the irreversible phase of apoptosis and destruction of neurons [36]. If the long terms follow up shows that magnesium sulfate has an additive neuroprotective effect and no significant side effects in the group of asphyxiated neonates treated with therapeutic hypothermia this relatively simple and not expensive intervention may be introduced into clinical practice.

Conclusion

- Combining therapeutic hypothermia with MgSO₄ administration is not associated with significant adverse effects. Coagulation parameters, number of blood components transfusions and incidence of intracranial bleedings were not affected by the magnesium sulfate administration.
- Magnesium sulfate administration improved the neurological status at 5 days of age and shortened hospitalization time. The study group was also more likely to be on full oral feedings at the time of discharge.
- Potential beneficial effects of the magnesium sulfate on the neurological status need to be confirmed by a long term follow-up.

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Competing Interests

None declared

Ethics Approval

The RCT protocol has been approved by the local bioethical board – PMMH-Research Institute Ethical Committee.

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