Dear Dr.,
This enclosed our Study Protocol with Statistical analysis plan (SAP) .

Official title:
Comparative study between nebuliezed and intravenous magnesium sulfate for treatment of persistant pulmonary hypertension in neonates

NCT number: NCT06603766

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Study Protocol with SAP

After obtaining approval from the medical ethical committee of Benha University Hospital.

This study was conducted in Benha University Hospitals at Neonatal Intensive Care Unit (NICU) on neonates who were admitted for PPHN after delivery. An informed consent was obtained from parents after explaining the procedure.

Community design and sampling:

A non-randomized controlled trial on neonates who met inclusion criteria. It was carried on 40 mechanically ventilated neonates with severe PPHN who was divided into two groups:

(A) Nebulized magnasium (NebMag) group (n= 20): received nebulized isotonic magnesium (64 mg/mL) and intravenous placebo.

(B) Intravenous magnesium (IVMag) group (n= 20): received intravenous magnesium and nebulized placebo.

The study time frame was 24h.

Duration of the study:

The study was conducted for two years from October 2021 to septmber 2023.

Ethical consideration:

- The whole study design was approved by the local ethics committee, Faculty of Medicine, Benha University **(Approval Number: 39-9-204)**.

- Confidentiality and personal privacy were respected in all levels of the study.

- Guardians felt free to withdraw from the study at any time without any consequences.

- Collected data was not and will not be used for any other purpose.

Inclusion criteria:

1) Newborns with documented PPHN as confirmed by ECHO.

2) Neonates born at ≥ 35 weeks' gestation with a birth weight of 2.5–4 kg

3) Neonates have to be connected to mechanical ventilation with an oxygenation index (OI) > 30 on two occasions at least 15 minutes apart.

4) The ECHO had to show a predominant right-to-left or bidirectional shunt (through ductus arteriosus and/or foramen ovale) and/or tricuspid regurgitant jet with a pressure gradient ≥ 2/3 of the systemic systolic blood pressure.

Exclusion criteria:

1) Infants of mothers who received magnesium sulfate within 48h before labor.

2) Congenital heart diseases other than PDA and patent foramen ovale.

3) Major congenital anomalies, including congenital diaphragmatic hernia and lung hypoplasia.

4) Prior need for cardiopulmonary resuscitation.

5) Mean arterial blood pressure (MABP) < 35 mmHg despite therapy with volume infusions and vasoactive inotropes.

6) Impaired kidney function; and prior administration of pulmonary vasodilators or prior administration of surfactant.

Methods of the study:

Patients included in the study were subjected to the following:

Complete history taking: Full antenatal and postnatal histories.

were obtained from all cases including:

- Demographic data (gestional age, sex, mode of delivery, birth weight).
- Associated condition (asphyxia, infection, polycythemia).

Clinical examination:

Full clinical examination: including general examination included vital signs, anthropmetric measurements, APGAR score in the 1st and 5th minutes (7).

systematic examination including chest and abdominal examination with emphasize on the cardiac examination including the apex site,1st, and 2nd heart sounds and manifestations of heart failure (tachycardia, tachypnea and enlarged liver), musculoskeletal and central nervous system examination.

Radiological investigation

- Chest X-ray
- ECG: The ECG may provide suggestive or supportive evidence of pulmonary hypertension by demonstrating RV hypertrophy and strain, and right atrial dilatation
- ECHO: measures pulmonary artery pressure, low ventricular output and low ejection fraction and fraction shortening.

Laboratory analysis

- Pulse oximeter monitoring in the four limbs:
- Blood pressure monitoring in the four limbs.
- Serial arterial blood gases: Post ductal arterial blood samples were drawn before (baseline) and 6, 12, and 24 h following study drug administration for gas analysis PH, PaO2, PaCO2, HCO3).
- Ventilatory parameters were recorded from ventilator (PIP, PEEP, MAP, fiO2, rate/min).
- serum magnesium level was measured before and 12 h following study drug administration.

The protocol of administration of magnesium sulfate:

- For intravenous administration a magnesium sulfate 10% solution was prepared by diluting an intravenous form of magnesium sulfate heptahydrate 50% with glucose 5%. Pharmacy designed 60mL aliquots of magnesium sulfate 10% and similar aliquots of glucose 5% as a placebo was prepared. Study participants received either active drug or placebo through an intravenous line a loading dose of 2 mL/kg over 30 min (equivalent to magnesium sulfate 200 mg/kg in case of the active drug), followed by a continuous infusion at a rate of 0.5 mL/kg/h (equivalent to magnesium sulfate 50 mg/kg/h in case of the active drug) for the 24 h study duration.

- For nebulization, an isotonic solution of magnesium sulfate (64 mg/mL) was prepared by diluting an intravenous form of magnesium sulfate heptahydrate 10% with distilled sterile water, 4-ml aliquots of isotonic magnesium sulfate (256 mg) and similar aliquots of isotonic saline as a placebo was designed. a 4-mL aliquot of active drug or placebo was loaded every 15 min to the jet nebulizer of ventilator.

The primary outcome in this study has been the change in oxygenation index (OI) from baseline to 12 and 24 h following study drug administration. OI: monitoring the oxygenation trend helps to guide when a baby might need to be referred for ECMO. It is a useful guide to monitor regularly in babies with PPHN**.**

OI: Mean Airway Pressure x FiO2 x 100 ÷PaO2 (in Pka) x 7.5

OI was preferred as it considers both oxygenation status and ventilatory support, over merely reporting individual values of SpO2, PaO2, FiO2, and ventilatory parameters.

Note: When you are calculating OI, be aware of which units of measurement you use for the PaO2 – kPa or mmHg (kPa x 7.5 converts to the equivalent PaO2 in mmHg).
FiO2 is the inspired fraction (e.g. 21% = 0.21, 100% =1.0)

The secondary outcomes have been the changes in (a) mean arterial pressure and VIS from baseline to 6, 12, and 24 h following study drug administration and (b) serum magnesium level from baseline to 12 h after study drug administration.

Statistical analysis

The data were coded, entered and processed on computer using SPSS (version 24). The results were represented in tabular and diagrammatic forms then interpreted. Mean, standard deviation, range, frequency, and percentage were use as descriptive statistics. **The following tests were done: Chi-Square test X^2** was used to test the association variables for categorical data. **Student's t-test** was used to assess the statistical significance of the difference between two population means in a study involving independent samples, with normal distribution. **The accepted level of significance was 0.05.** P value >0.05 is non-significant, P<0.05 is significant (*)