A RANDOMIZED CONTROLLED TRIAL COMPARING TWO DOSES OF CAFFEINE FOR APNOEA IN PREMATURITY

DR. ANIS MUNIRAH BINTI MOHD KORI

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CHAPTER 1:

THE PRELIMINARIES

Acknowledgement

Firstly, thanks to GOD ALMIGHTY for his bestowed upon the strength and good health in order to complete this research. I would like to express my sincere gratitude to Prof. Dr Hans Van Rostenberghe for his expertise, assistance, guidance and patience throughout the process of this thesis becomes a reality. I would like to thank Associate Professor Dr Ariffin Nasir, Dr NorRosidah Ibrahim, Mr. Sabri and Dr Najib Majdi for their advice and contribution for this thesis. I am highly indebted to my fellow lecturers, colleagues, supporting staffs, friends and family for the encouragement and supports along the walks.

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LIST OF ABBREBIATION AND NOMENCLATURE

AOP	Apnoea of prematurity
BPD	Bronchopulmonary dysplasia
CAP	Caffeine for apnoea in prematurity
IQR	Interquartile range
IVH	Intraventricular haemorrhage
NEC	Necrotizing enterocolitis
NICU	Neonatal Intensive Care Unit
NIV	Non-invasive ventilation
PVL	Periventricular leukomalacia
RCT	Randomized controlled trial
ROP	Retinopathy of prematurity

ABSTRACT

Caffeine is the most used methyl xanthine for the prevention of apnoea in prematurity, but its ideal dose was uncertain. This study compared two doses of caffeine for prevention of apnoea in prematurity. A clinical trial was conducted on 78 preterm infants ≤32 weeks in NICU at tertiary center hospital. They were randomly allocated to receive the intervention (loading 40 mg/kg/day and maintenance of 20 mg/kg/day) or the control (loading 20 mg/kg/day and maintenance of 10 mg/kg/day) dose of caffeine. The primary outcome of the study was the frequency and total days of apnoea per duration of treatment for both groups. The frequency of apnoea range from zero to fourteen in the intervention and zero to twelve in control group. There was no statistically significant different between the groups with *p* value 0.839. The number of days of apnoea was also similar between both groups with *p* value 0.928. There was also no significant difference of adverse events between both regimens. This study did not support the use of higher dose caffeine as a prevention for apnoea in prematurity.

Keywords: apnoea; prematurity; caffeine; neonatal morbidities.

ABSTRAK

Kafein adalah methyl xanthine yang paling banyak digunakan untuk pencegahan apnoea dalam pramatang, tetapi dos yang ideal tidak pasti.Kajian ini membandingkan dua dos kafein untuk pencegahan apnoea dalam pramatang.Percubaan klinikal telah dijalankan ke atas 78 bayi pramatang ≤32 minggu di NICU, Hospital USM, Kubang Kerian, Kelantan, Malaysia. Mereka diperuntukkan secara rawak untuk menerima campur tangan (memuatkan 40 mg/kg/hari dan penyelenggaraan 20 mg/kg/hari) atau kawalan (memuatkan 20 mg/kg/hari dan penyelenggaraan 10 mg/kg/hari) dos kafein. Hasil utama kajian adalah kekerapan dan jumlah hari apnoea setiap tempoh rawatan bagi kedua-dua kumpulan.Kekerapan apnoea berkisar dari sifar hingga empat belas dalam campur tangan dan sifar hingga dua belas dalam kumpulan kawalan.Tiada statistik yang signifikan berbeza antara kumpulan dengan nilai p 0.839.Bilangan hari apnoea juga sama antara kedua-dua kumpulan dengan nilai p 0.928.Tiada perbezaan ketara peristiwa buruk antara kedua-dua rejimen.Kajian ini tidak menyokong penggunaan kafein dos yang lebih tinggi sebagai pencegahan untuk apnoea dalam pramatang.

Kata kunci: apnoea; pramatang; kafein; morbiditi neonatal.

CHAPTER II:

THE TEXT

2.1 Section A:

Introduction

Introduction

Apnoea of prematurity is a significant clinical problem reflecting the immaturity of respiratory control systems [1,2]. The incidence is widely variable depending on the gestational age. Uncontrolled episode of apnoea maybe associated with prolonged ventilation, failed extubation and prolonged hospital stay [3]. A Cochrane review showed that caffeine was superior to other methylxanthines for the prevention and treatment of apnoea in prematurity in view of its lower toxicity and wider therapeutic index [4].

The pharmacological effects of caffeine in AOP include: (1) stimulation of the respiratory centre in the medulla; (2) increased sensitivity to carbon dioxide; (3) increased skeletal muscle tone; (4) enhanced diaphragmatic contractility; (5) increased minute ventilation; (6) increased metabolic rate; and (7) increased oxygen consumption [1,2]. Caffeine also stimulates the central nervous and cardiovascular systems, enhances catecholamine secretion, has a diuretic effect, and alters glucose homeostasis [2].

Cytochrome P450 1A2 (CYP1A2) is known to be the major enzyme involved in the metabolism of caffeine. Therefore, caffeine has the potential to interact with drugs that are substrates for CYP1A2, inhibit CYP1A2, or induce CYP1A2 [2]. Few data exist on drug interactions with caffeine in preterm neonates [2]. Lower doses of caffeine may be needed following coadministration of drugs which are reported to decrease caffeine elimination (e.g., cimetidine and ketoconazole) and higher caffeine doses may be needed following coadministration of drugs that increase caffeine elimination (e.g., phenobarbital and phenytoin) [2]. Interconversion between caffeine and theophylline has been reported in preterm neonates. The concurrent use of these drugs is not recommended[2].

The caffeine for apnoea in prematurity (CAP) trial has shown that the benefits of caffeine therapy outweigh any potential risk up to the corrected age of 18 to 24 months [5]. A subsequent follow-up of the CAP trial has shown that caffeine therapy reduces the severity of motor impairments at 5 years corrected age [6]. Caffeine is now widely used as a standard treatment for apnoea in prematurity. Side effects of caffeine are relatively rare but include tachycardia, jitteriness, feeding intolerance, increase oxygen consumption and transient decrease in the rate of growth [2,7]. Despite the establishment of caffeine therapy in preterm infants, variable information exist on the optimal loading and maintenance dose in different neonatal departments. A commonly used dose for caffeine citrate is 20 mg/kg loading followed by 5 to 10 mg/kg per day as maintenance [8].

There are no clinical trials to support decisions about when to discontinue caffeine therapy in preterm infants. However, because AOP is not common past 34 weeks gestation, caffeine therapy should be continued until preterm infants are 34 to 36 weeks corrected gestational age and free of any apnoea episodes for at least 8 days [8]. According to the previous study, the mean duration of caffeine use varied depending on the gestational age and indication of caffeine use. The duration of caffeine use is not stated in all studies. Mc Pherson et al used the shortest mean duration of caffeine, which was just 1.5 days. Mohamed et al used caffeine for 31.1 days, which was the longest mean time of caffeine duration.

The optimum caffeine dose in preterm infants has not been well-studied in terms of benefits and risks. There are two meta-analyses conducted comparing different dosages of caffeine. Even though a few trials showed benefits of giving higher doses of caffeine without causing adverse side effects, the results of the meta analyses were inconclusive

[3,9-10]. The reason of conducting this study is to determine the efficacy and safety of a high versus a lower dose of caffeine for the prevention of apnoea in prematurity.

2.2 Section B:

Study protocol

2.2.1 Documents submitted for ethical approval

Dissertation proposal

School Of Medical Science
University Science Malaysia
Prepared in partial requirement fulfilment
For the Degree of Master of Medicine (Paediatric)
2017/2021

Randomized controlled trial (RCT) comparing between High versus Standard dose caffeine citrate for apnoea in prematurity

Research title:

Randomized controlled trial comparing between High versus Standard dose caffeine citrate for apnoea in prematurity

Principal investigator (MMC No. if applicable):

Dr Anis Munirah Mohd Kori MMC 61637

Co-researchers: (MMC No. if applicable)
Professor Dr. HansVan Rostenberghe MMC 31842

Associate Professor Dr. Ariffin Nasir MMC 31850
Dr Najib Majdi bin Yaacob MMC 41754
Dr Rosidah Ibrahim MMC 35070

Introduction

Caffeine is now used as a standard treatment for apnoea in prematurity due to its higher therapeutic index, better enteral absorption, and longer half-life than other methylxanthines.

Despite the establishment of caffeine therapy in preterm infants, variable information exist on the optimal loading and maintenance dose in different neonatal departments aiming to balance benefits and risks. The usual standard dosing for caffeine citrate is 20 mg/kg loading followed by 5 to 10 mg/kg per day as maintenance. The optimum caffeine dose in preterm infants has not been well-studied in terms of benefits and risks. A few trials conducted showed benefits of giving higher dose of caffeine without causing adverse side effects.

Caffeine therapy is associated with the following side effects: tachycardia, jitteriness, feeding intolerance, increase oxygen consumption and transient decrease in the rate of growth of preterm baby which are reversible and safely used as a standard clinical practice.

Mohammed, S., et al. (2015). "High versus low-dose caffeine for apnea of prematurity: A randomized controlled trial." European journal of pediatrics **174**(7): 949-956

Problem statement

- Available literatures regarding high versus standard/low dose caffeine for apnoea in prematurity is not conclusive due to heterogeneity of the doses, gestational age, indication of starting caffeine and sample size
- However, some data suggest that the high dose caffeine is beneficial without causing adverse effects except for mild tachycardia
- The reason of conducting the study is to determine the efficacy and safety of a high dose of caffeine compared to standard dose for the prevention of apnoea in prematurity

Study rationale(s)

Apnoea in prematurity causes complications to the premature baby that leads to prolonged hospital stay. With the introduction of high dose caffeine, the incidence of apnoea may be reduced and the dose that been used as a high dose is likely to be saved from adverse side effects

Mohammed, S., et al. (2015). "High versus low-dose caffeine for apnea of prematurity: a randomized controlled trial." European journal of pediatrics **174**(7): 949-956

Steer, P., et al. (2003). "Periextubation caffeine in preterm neonates: a randomized dose response trial." Journal of paediatrics and child health **39**(7): 511-515

Steer, P., et al. (2004). "High dose caffeine citrate for extubation of preterm infants: a randomised controlled trial." Archives of Disease in Childhood-Fetal and Neonatal Edition **89**(6): F499-F503

Vliegenthart, R., et al. (2018). "High versus standard dose caffeine for apnoea: a systematic review." Archives of Disease in Childhood-Fetal and Neonatal Edition: fetalneonatal-2017-313556

Research Question(s)

Is a high dose caffeine more efficacious and equally safe compared to a standard dose of caffeine for the prevention of apnoea in premature babies?

Objective

General:

To compare the efficacy and safety of high versus standard dose of caffeine citrate for prevention of Apnoea in prematurity

Specific:

Primary Objectives:

1. To compare the frequency of apnoea per duration of treatment in prematurity between high versus standard dose of caffeine
2. To compare total number of days of apnoea in prematurity between high versus standard dose of caffeine

Secondary Objective:

1. To determine respiratory and non-respiratory outcomes and side effects of caffeine in prematurity for high and standard dose of caffeine

Literature review

Several studies on pharmacodynamics and pharmacokinetics have investigated if a higher dose of caffeine would augment the previously reported beneficial effects without increasing the risk of adverse effects (Charles BG et al, 2008). A systematic review on high versus standard dose caffeine was conducted for apnoea in prematurity and published in 2017 by Roos et al. The objective of this study was to identify, appraise and summarise studies investigating the effect of a higher versus a standard dose of caffeine in preterm infants. A systematic review identified all randomised controlled trials (RCTs) comparing a high versus a standard caffeine treatment regimen

in infants with a gestational age <32 weeks, by searching the main electronic databases and abstracts of the Pediatric Academic Societies. Primary outcomes were BPD and mortality at 36 weeks postmenstrual age. Secondary key-outcome was neurodevelopmental outcome at 12 and 24 months corrected age. Six RCTs including 620 infants were identified. Meta-analysis showed a significant decrease in BPD, the combined outcome BPD or mortality, and failure to extubate in infants allocated to a higher caffeine dose. No differences were found in mortality alone and neurodevelopmental impairment. Although this review suggests that administering a higher dose of caffeine might enhance its beneficial effect on death or BPD, firm recommendations on the optimal caffeine dose cannot be given due to the low level of evidence. A large RCT is urgently needed to confirm or refute these findings and determine the optimal dose of caffeine.

Zhao Ying et al has compared two group; high (first dose 20 mg/kg, daily maintenance after 15 h was 15 mg/kg) versus low dose (first dose 20 mg/kg, daily maintenance dose 5 mg/kg after 24 h) caffeine group which involve 164 premature <32 weeks in Tianjin Central Hospital of Obstetrics and Gynecology from October 2013 to December 2014. The outcome includes the number of apneas in the high-dose group was significantly lower than that in the low-dose group with *p value = 0.009.* There were no significant differences in the incidence of adverse reactions related to caffeine treatment including tachycardia, irritability, feeding difficulties, hyperglycemia, hypertension, digestive disorders and electrolyte imbalance (P >0.05). There were no significant differences in clinical outcomes between the two groups, including in-hospital death, chronic lung disease, and other comorbidities (P >0.05).

Sameh Mohammed et al have conducted a study at the Neonatal Intensive Care Unit (NICU) of Mansoura University Children's Hospital, Mansoura, Egypt between

July 2011 and July 2012 that involve 120 premature baby <32 weeks. The outcomes include high, compared to low, dose caffeine was associated with a significant reduction in extubation failure among mechanically ventilated preterm infants (*p=0.02*). High dose caffeine was associated with a significant reduction in both apnea frequency (*p<0.001*) and days of documented apnea (*p<0.*001) in all infants without causing significant side effects. They proposed a large well powered randomized control trial to prove the efficacy of high-dose caffeine in decreasing frequency of apnea and increasing chances for successful extubation in preterm infants.

PA Steer et al have conducted a study published in 2003. The study compare the effectiveness of three different dosing of caffeine in the periextubation period in premature <32 weeks and ventilated at least 48 hours in Queensland, Australia. The doses were (3, 15 and 30 mg/kg) respectively. The caffeine was commenced 24 hours prior to a planned extubation or within 6 hours of an unplanned extubation. The outcomes include no significant difference in the number of failure extubation between the three dosing groups, although there was a trend to decreased failure of extubation in the two highest dose groups (p=0.06); low dose group had significantly more documented apnoea per group over 7-day than the other two dose groups (p=0.01); No statistically significant for adverse effects; Infants on the two highest doses of caffeine recorded a significantly higher mean heart rate and mean SpO2 (p<0.01); The highest dose group also was noted to have significantly less time spent with low SpO2 recordings(p=0.03).

Randomized clinical trial compare the efficacy of theophylline (7.5mg/kg Loading Dose and 3mg/kg thrice daily Maintenance Dose-group C) with caffeine administered in 2 different doses (12.5mg/kg Loading Dose and 3mg/kg daily Maintenance Dose-Group A versus 25mg/kg Loading Dose and 6mg/kg daily Maintenance Dose-Group B) to a

group of very preterm infant with frequent and clinically significant apnoeic attacks. The participants involved 36 babies of Premature <31 weeks gestation and had ≥10 apnoea in eight hours or 4 apnoea episodes in 1 hour. The outcomes include successful treatment defined as a >50% reduction in the number of apnoeas (as measured at 8 hours period):

- Group B>group A p=0.036
- Group C>group A p=0.01

There was significant reduction in the number of apnoea between day 0 and day 1 in Group A ($p<0.05$), Group B and C ($p<0.01$). There was a mean rise in heart rate of 3bpm and 5 bpm in group A and B ($p<0.05$ on paired *t* test), mean rise in heart rate of 12 bpm in group C ($p<0.01$).

A multicentre RCT published in 2004 by P steer et al, was conducted in four Australian neonatal intensive care units (Mater Mothers' Hospital, Brisbane, Queensland; Royal Prince Alfred Hospital, Sydney, New South Wales; Mercy Hospital for Women, Melbourne, Victoria; Royal Hobart Hospital, Hobart, Tasmania). The purpose of the study was to determine if a higher dose of caffeine citrate in the periextubation period was more effective than a standard dose regimen in facilitating successful extubation in very preterm infants without an increase in adverse effects. The two groups were Loading Dose (80mg/kg vs 20mg/kg) over 15 minutes and Maintenance Dose (20mg/kg vs 5mg/kg) 0D. 238 infants were enrolled into the study with gestational age < 30 weeks who had received or who were expected to receive at least 48 hours of mechanical ventilation. The outcomes include significant different in extubation failure and number of documented apnoea ($p<0.01$); no significant difference of adverse effect except longer duration of gaining birth weight in higher dose ($p<0.01$); No significant different of major morbidity and death; No significant different for

developmental assessment at 12 months corrected age. The continuation of the trial was conducted by Peter H gray et al published in 2011; the study compare two dosing regimens for caffeine citrate for prematurity in term of development and temperament at 1 y.o and behaviour at 2 y.o corrected age. 97 of 109 (89%) survivors from high dose group and 93 of 110 (85%) survivors from standard dose group. The result showed no significant different for cognitive assessment (p=0.075); non-significant trend for benefit in the high dose caffeine group for death or major disability(15.4 % vs 24.2% ; RR 0.75 (95% CI 0.49 -1.14); no difference in the mean value for temperament and behaviour.

One trial has been conducted to compare between high versus standard loading dose with same maintenance dose by Mc Pherson et al to neurodevelopmental outcome. The study involved 74 infants premature < 30 weeks in NICU at St. Louis Children's Hospital,Washington over 20 months study period. They compare the effect of high versus standard loading dose of caffeine with same maintenance dose (80mg/kg total over 36 hours vs 30mg/kg/36 hours with Maintenance Dose: 10mg/kg OD after 48 hours initial caffeine dose) on brain structure, early neurobehaviour during the neonatal period, and developmental outcome at 2 years. Out of 74, 59 infants remained at term corrected age (TEA), 58 infants able to undergo MRI, 46 infants able to continue follow up till 2 years corrected age. The outcomes were Qualitative brain injury (MRI Brain done at TEA) showed there is statistically significant association between high-dose caffeine and cerebellar hemorrhage (p=0.02); there is no significant different for Brain Metrics, Volumes, and Diffusion based on MRI Brain; for neurobehavioral outcomes at TEA –There is association between high dose group and hypertonia based on NICU Network Neurobehavioral Scale (NNNS) (p=0.02) and lower deviant sign based on Dubowits score (p=0.03); for developmental assessment at 2 years corrected age using

Bayley Scale of infant and toddler showed no significant different for cognitive, language and motor development.

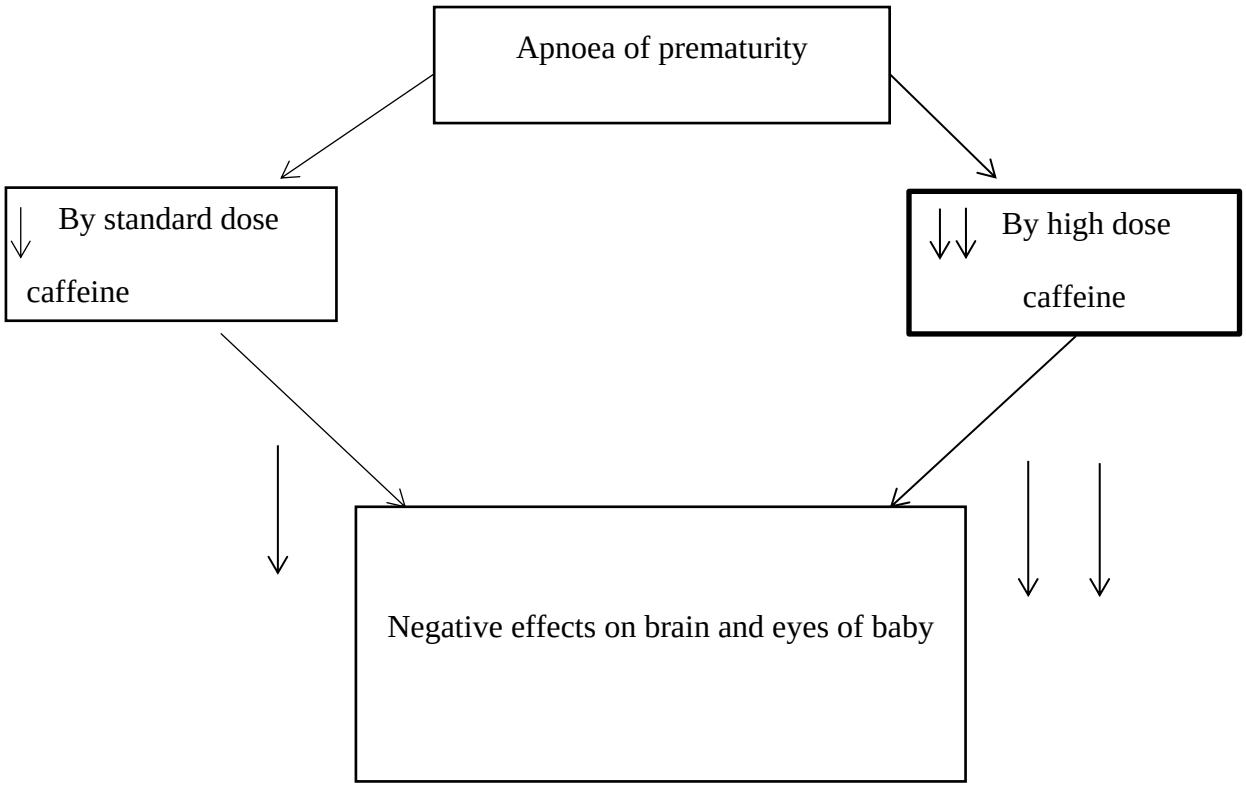
Zhao ying et al has conducted a RCT to evaluate and compare the clinical efficacy and safety of different doses of caffeine in the treatment of primary apnea in preterm infants. The loading dose were same with different maintenance dose (Loading Dose : 20mg/kg with Maintenance Dose:15mg/kg after 15 hours vs 5mg/kg after 24 hours). The study was conducted from October 2013 to December 2014 involved 164 children with primary apnoea of gestational age <32 weeks in Tianjin Central Hospital of Obstetrics and Gynecology,China. The results showed the number of apneas in the high-dose group was significantly lower than that in the low-dose group with P = 0.009; there were no significant differences in the incidence of adverse reactions related to caffeine treatment (P >0.05); there were no significant differences in clinical outcomes between the two groups, including in-hospital death, chronic lung disease, and other comorbidities (P >0.05).

The latest study published in 2018 conducted by Fatemah Taramarzi et al in Bu-Ali Sina Teaching Hospital, Sari,Iran. They compare the efficacy and safety of once versus twice-daily caffeine-dose in premature infants with RDS (same Loading Dose: 20 mg/kg and Maintenance Dose: 5 mg/kg/dose OD-group 1 vs 2.5 mg/kg BD-group 2). 40 patients were enrolled in the study period with Gestational Age of 26 to 37 weeks, with evidence of RDS that was diagnosed by a neonatologist. The outcomes showed reduction in failure of extubation and CPAP failure rate and length of NICU stay among preterm infants, but not statistically significant different; no significant differences in the duration of mechanical ventilation, CPAP therapy and mortality rate ; blood gas levels change during the course of caffeine therapy but no statistically significant different; the means of O2 saturations on the first three days of treatment with caffeine

were higher in group 2 compared to group 1 (p=0.049); no significant difference in term of side effects but lesser in group 2.

Regarding the safety of caffeine, only limited numbers of studies assessing caffeine effect on premature infant physiology. Most of this studies were observational studies. In order to assess the caffeine effects on renal calcium exretion, Zanardo et al in 1995 did a clinical trial involving 20 premature infants where 10 of them were given theophylline while the other half were given caffeine. Day 5 urinary calcium was measured and showed significantly higher calcium urinary excretion in patient treated with either caffeine and theophylline compared to control. t al in 2014 did a similar study involve large cohort of premature infants with gestation age 32 weeks or less and birth weight less than 1500gm. 125 infant divided into 2 groups with 1 group received loading dose of caffeine citrate 20 mg/kg intravenously followed by 10 mg/kg daily maintenance dose. Patients were followed until 45 days of life for evidence of osteopenia of prematurity (defined as serum phosphorus <5 mg/dl, alkaline phosphate > 900 IU/ L and radiological evidence of Osteopenia of Prematurity on wrist x-ray). 45.1% caffeine group has radiological evidence of Osteopenia of prematurity (OP) with total of 52% of infant from both groups developed OP. (Gharehbaghi, Taheri et al. 2016) Caffeine has also been shown to increase diuresis in premature infants. In a study by Bairam et al in 1987 involving small cohort of premature infant with mean gestational age of 30 weeks, both caffeine and theophylline caused significant increased up to 2 to 3 fold in urinary sodium excretion. (Bairam, Boutroy et al. 1987)

Conceptual framework



Research design

Randomised control trial (RCT), blind, parallel design with a one-to-one allocation

Study area/study period

The study will be conducted in Neonatal Intensive Care Unit, Hospital USM, Kubang Kerian Kelantan. The hospital is a teaching hospital for University Sains Malaysia and this is where the Health Campus located. Kubang Kerian is one of the major towns of Kelantan, a state in Malaysia where majority of its population are Malays from different socio-economic background. The Neonatal Intensive Care Unit provides level 1 to level 4 intensive care to neonate patient and received referral from all parts of Kelantan and all over the country. The study is planned to be conducted in the period between 1st January 2019 to 1st December 2019.

Study population

Reference population	Premature babies admitted to the NICU
Source population	Premature babies admitted to the NICU, HUSM
Target population	Premature babies 26 to 32 completed weeks admitted to the NICU, HUSM
Sampling frame	Premature babies 26 to 32 completed weeks admitted to the NICU, HUSM

Subject criteria

Inclusion criteria

1. Inborn prematurity ≤ 32 completed weeks admitted to NICU Hospital USM
2. 24 hours periextubation period if ventilated

Exclusion criteria

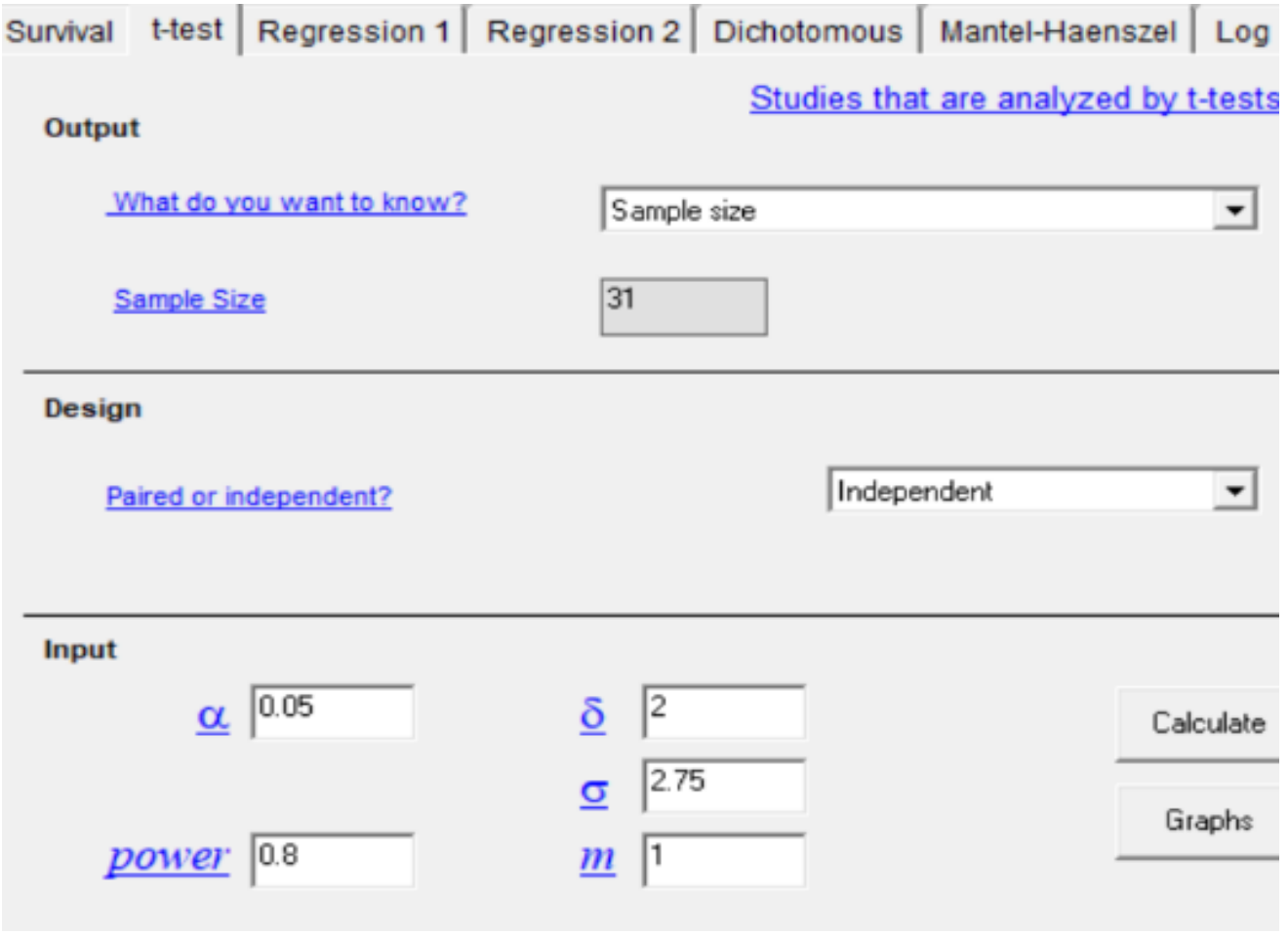
1. Hydrops fetalis
2. Lethal congenital anomaly
3. Major neurological condition; examples Grade 3 or 4 intraventricular haemorrhage
4. Contraindication on starting oral medication (Examples Necrotising Enterocolitis, bowel pathology like malrotation, Hirschprung disease)

Withdrawal criteria

1. Premature baby who developed Supraventricular tachycardia (SVT) or persistent tachycardia during the course of treatment.

Sample Size Estimation

Objective 1: To compare the frequency of apnoea per day in prematurity between high versus standard dose of caffeine



We are planning a study to compare the frequency of apnoea in high dose versus standard dose caffeine. It is a continuous response variable from independent control and experimental subjects with 1 control(s) per experimental subject. In a previous study by P steer et al in 2003, the response within each subject group was normally distributed with standard deviation 2.75. If the true difference in the experimental and control means is 2, we will need to study 31 experimental subjects and 31 control subjects to be able to reject the null hypothesis that the population means of the experimental and control groups are equal with probability (power) 0.8. The Type I error probability associated with this test of this null hypothesis is 0.05.

From the database, there is no study done to compare the total number of days of apnoea in prematurity between high versus standard dose of caffeine.

The estimated sample size is 31 participants in each arm. Therefore, the total number of the participants is about 78 including 20% dropouts.

Sampling method and subject recruitment

Before premature delivery and subject recruitment, medical officer or specialist will be subjected to do prenatal counselling to the mothers in antenatal ward HUSM. During the counselling, the study will be briefly explained to the mother in order to give them an ample time to think whether they want their babies to be included in the study or not. For mother who came in labour, the study will be explained and consent will be taken before recruitment.

Premature babies who fulfil inclusion and exclusion criteria will be assigned randomly into either two groups (Group A: High dose group, Group B: Standard dose group). Infants in group A received high dose oral caffeine citrate (loading dose of 40 mg/kg/day equivalent to 20 mg /kg/day of caffeine base and maintenance dose of 20 mg/kg/day equivalent to 10 mg /kg/day of caffeine base). Maintenance dose will be given 24 hours after loading. Infants in group B received standard dose oral caffeine citrate (loading dose of 20 mg/kg/day equivalent to 10mg/kg/day of caffeine base and maintenance dose of 10mg/kg/day equivalent to 5 mg/kg/day of caffeine base)

Oral caffeine loading dose will be given to the allocation group (Group A and Group B) within 24 hours periextubation period. If the patient is not intubated, oral caffeine loading dose will be given immediately. If patient has failure extubation, caffeine will be off and continue ventilation. Sameh Mohammed et al defined failure extubation as requiring reintubation within 72 hours. If successful extubation, maintenance dose (MD) will be continued 24 hours after starting caffeine and continue on daily basis. If patient

has feeding intolerance while the patient is on caffeine, the medication will be changed into intravenous aminophylline. Infant's oxygen saturation, heart rate, and respiratory rate were continuously monitored, preductal, using monitor, with the targeted oxygen saturation maintained between 91 and 95 %. Tachycardia is defined as heart rate greater than 180 beats per minute, without a substitutive explanation. Blood pressure will be checked, indirectly by oscillometry or Intra-arterial blood pressure if patient is on arterial line, and the reading was considered high if blood pressure was more than 95th percentile (Zubro et al 1995)

Every fluid intake will be measured including both enteral and parenteral fluid from feeding, parenteral nutrition, and medication or fluid boluses. Fluid output will also be indirectly measured by weighing the pampers. Patient will also be weighted twice weekly. The frequency of apnoea and bradycardia were taken from the monitors and validated by the attending qualified nurses and medical profession. Apnoea is defined as sudden cessation of breathing that lasts for at least 20 seconds accompanied by bradycardia or oxygen desaturation in infant younger than 37 weeks gestational age (Malaysia Paediatric Protocol 3rd edition). Urine and serum electrolyte including sodium, potassium, calcium, phosphate, chloride and creatinine will be measured at day 5 of caffeine treatment.

Outcomes

The primary outcome of the study is the mean frequency of apnea per duration of treatment and number of days with apneic episodes post caffeine loading dose throughout duration of caffeine therapy (usually until 34 weeks corrected age). The mean number of apneic episode per day and the number of days with apnea will be calculated and the mean between the two groups will be compared.

Secondary outcome are the respiratory and non-respiratory outcomes of caffeine. Respiratory outcomes include extubation failure, duration of non-invasive ventilation (Non invasive ventilation that includes optiflow, Bipap,Duopap and Cpap) , duration of oxygen therapy (Patient is on nasal prong oxygen), developmental of chronic lung disease (defined as the need for oxygen treatment at 36 weeks' postmenstrual age, according to the National Institute of Child Health and Human Development diagnostic criteria),and the need for post-natal steroid therapy for Chronic Lung Disease. Non-respiratory outcome includes tachycardia, hypertension, time to reach full enteral feeding, urine output (ml/kg/hour), weight gain(patient will be weighted biweekly), diuretic effect of caffeine, urinary calcium and sodium excretion, developmental of osteopenia of prematurity, length of hospital stay, neonatal mortality, necrotizing enterocolitis (any stage, according to modified Bell classification), intraventricular hemorrhage (IVH) in which bedside ultrasound cranium will be done by trained doctor according to protocol and grading accordingly ,periventricular leukomalacia (PVL),Retinopathy Of Prematurity (ROP) and the number of caffeine withhold for suspected side effects. Infants were weaned off mechanical ventilation if they fulfilled our NICU guidelines including spontaneous respiratory effort, gag or cough with suctioning, satisfactory blood gases (pH more than 7.25, pCO2 less than 60 mmHg, and base deficit less than 8mEq/L) on a mean airway pressure less than 8 cm H 2O and frequency less than 30 breaths per minute, saturation more than 90 % on FiO2 less than 30 % in the preceding 24 h, and approval of the attending physician. Infants were weaned off CPAP if they fulfilled our NICU guidelines including pressure of 3 to 6 cm H 2O for 24 to 48 h, FiO2 less than 30 % to keep target saturation in the preceding 24 h, respiratory rate less than 60 breaths per minute, no single apnea requiring bagging in the preceding 24 h, no more than 6 apneas requiring stimulation in the preceding 24 h, satisfactory blood gases (pH more than 7.25,

pCO2 less than 60 mmHg, and base deficit less than 8 mEq/L), infant tolerates time off CPAP during nursing care, and approval of attending physician.

Heart rate post caffeine treatment defined as mean heart rate recorded over 24 hours at day 7 of treatment recorded as beat per minute. Based on Malaysia paediatric protocol fourth edition, tachycardia in neonate is defined as heart rate more than 170. Supraventricular tachycardia (SVT) in prematurity is defined as heart rate more than 250 with absence of 'p' wave from ECG. Caffeine will be withheld if baby develop persistent tachycardia and SVT. Hypertension is defined as mean blood pressure above 95th percentile. The presence of hypertension will be determined at day 7 of treatment. Full enteral feeding is defined as oral feeding of at least 100ml/kg/day. Urine output at day 7 of treatment will be measured indirectly through non-invasive procedure by weighing the diapers. Weight gaining includes the total weight gain throughout the duration of caffeine treatment until completed treatment in g/kg/day and the number of days required to regain birth weight. Developmental of osteopenia of prematurity is determined through its surrogate biomarker ALP with cut off value of >500IU/L and serum phosphate level <1.8mmol/L throughout caffeine therapy.

Diuretic effects of caffeine will be determined by calculating urine sodium functional excretion at day 7 of treatment using formula:

$$\frac{\text{Urine Na}}{\text{Se Creatinine}} \times \frac{\text{Urine Creatinine}}{\text{Se Na}} \times 100\%$$

Calcium excretion will be determined by calculating urine calcium excretion using formula:

$$\frac{\text{Urine Calcium}}{\text{Urine Creatinine}} \times \frac{\text{Urine Creatinine}}{\text{Serum Calcium}} \times 100\%$$

Research tool

All the research tools that will be used in the study is routinely used for premature babies in Neonatal Intensive Care Unit. All the research tools have been proved its safety and reliability.

RANDOMISATION

Sequence Generation

No sampling will be used in this study. All premature infants admitted to NICU Hospital USM that fulfilled the inclusion and exclusion criteria will be included in the study. The random sequence will be generated by a researcher not involved in the recruitment of patients, neither in the data collection nor care of the NICU patients using block randomization known only to the randomizer. A designated pharmacist was responsible for the randomization of selected infants and the preparation of caffeine dose.

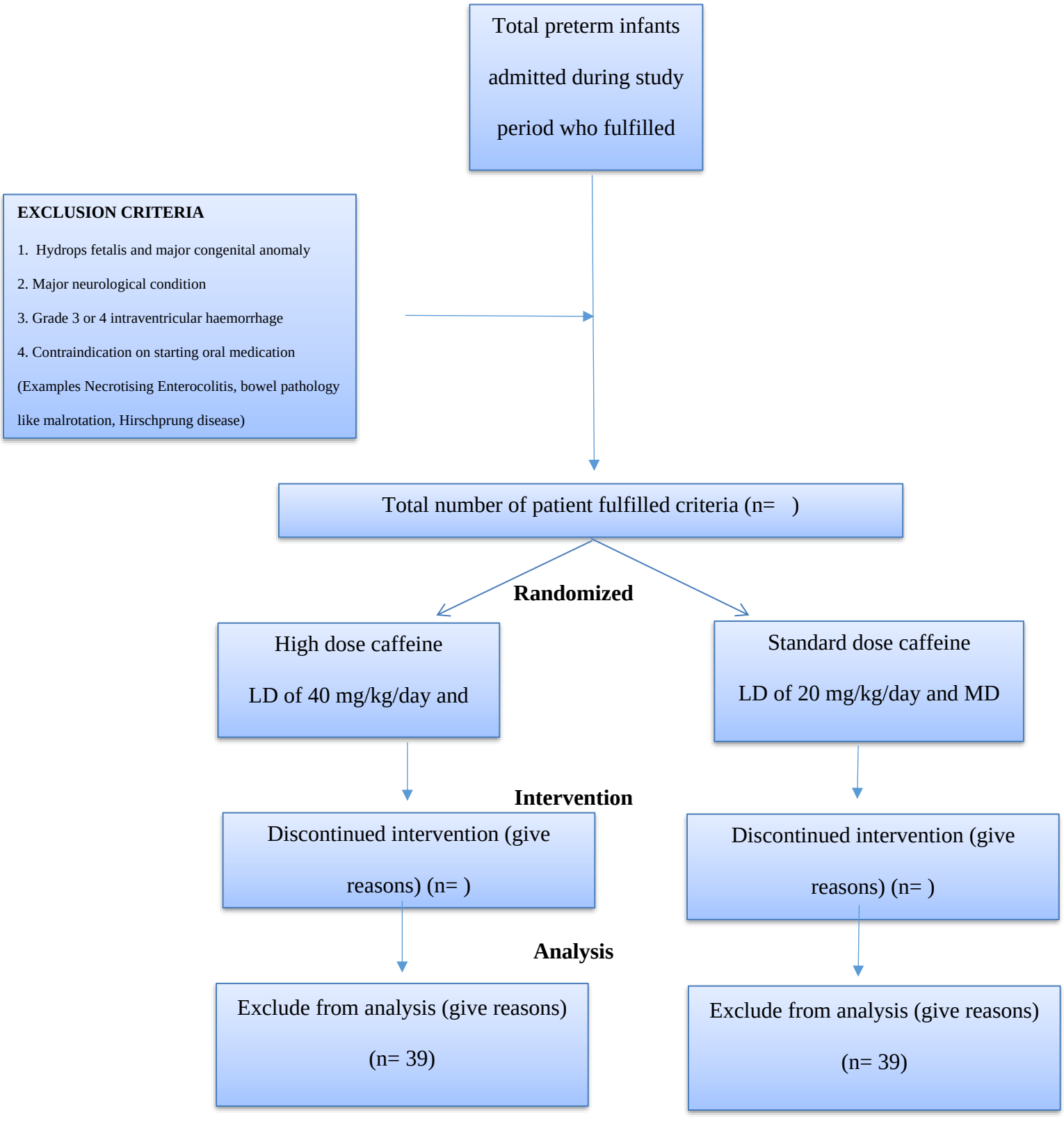
Allocation Concealment Mechanism and Implementation

Patients will be recruited by the main investigator and only after inclusion in the study, consecutively numbered, sealed and opaque envelopes, carrying the allocation will be opened.

Blinding

The investigators, nursing staff, and family will be blinded to patient's group.

Study flowchart



Data analysis

The data will be analysed using SPSS version 26. After data has been entered, it will be explored, checked and cleaned. P-value ≤0.05% was considered statistically significant. Number and percentage are represented the qualitative variables, meanwhile, mean±SD and median with Interquartile Range displayed the quantitative variables. Chi2 and fisher exact test will be used to compare categorical variables between the two groups for respiratory and non-respiratory outcomes.

Expected result(s)

Baseline characteristics

Characteristic	**High Dose (n=)**	**Low Dose (n=)**	**p-value**
Gestational age (weeks)			
Birth weight (grams)			
Male Sex			
Singleton			
Vaginal Delivery			
Caesarean Section			
Small for gestational age			
Maternal chorioamnionitis			
Antenatal steroid therapy			
Surfactant therapy			
Postnatal age at caffeine therapy (days)			
Duration of caffeine therapy (days)			

Primary Objective

Outcome	High Dose (n=)	Low Dose (n=)	p-value
Documented Days of Apnoea			
Frequency of Apnoea per day			

Respiratory Outcomes

Outcome	High Dose (n=)	Low Dose (n=)	p-value
Extubation Failure			
Chronic Lung Disease (BPD) at 36 Weeks Corrected Age			
Duration of Non-Invasive Ventilation			
Duration of Oxygen Therapy (days)			
Post Natal Steroid Therapy for BPD			

Non respiratory outcomes

Outcome	**High dose (n=)**	**Low dose (n=)**	**p-value**
Tachycardia			
Hypertension			
Time to reach full enteral feeding (days)			
Caffeine withhold for suspected side effect			
Weight gaining (g/kg/day)			
Urine flow rate at day 7 of treatment			
Urine Sodium excretion			
Urine calcium excretion			
Osteopenia of prematurity			
Intraventricular Haemorrhage (≥grade 3)			
Retinopathy of Prematurity			
Necrotising Enterocolitis			
Periventricular Leukomalacia			
Length of Hospital stay (days)			
Death before hospital discharge			