EFFECT OF BIRTH ASPHYXIA ON THE THYROID HORMONE IN TERM NEWBORN DELIVERED IN HOSPITAL UNIVERSITI SAINS MALAYSIA

DR. AHMAD ZAFRULLAH AFHAM BIN AHMAD FIKRI

DISSERTATION SUBMITTED IN PARTIAL

FULFILLMENT OF THE REQUIREMENT FOR

THE DEGREE OF MASTER IN MEDICINE

(PAEDIATRICS)

UNIVERSITI SAINS MALAYSIA

2023

Principal Investigator:

Dr. Ahmad Zafrullah Afham AHMAD FIKRI

Department of Paediatric, School of Medical Sciences,

Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan

Main supervisor:

Professor Hans Amin Van ROSTENBERGHE

Senior Consultant Paediatrician & Neonatologist,

Department of Paediatric, School of Medicine Sciences,

Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

Co-supervisor:

Associate Professor Noraida RAMLI

Senior Consultant Paediatrician & Neonatologist,

Department of Paediatric, School of Medicine Sciences,

Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

Dr. Nor Rosidah IBRAHIM

Senior Paediatrician,

Department of Paediatric, School of Medicine Sciences,

Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

Dr KUEH Yee Cheng

Medical Lecturer,

Department of Biostatistics and Research Methodology, School of Medical Science,

Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan.

ACKNOWLEDGMENT

I would like to express my sincerest gratitude to all those who have helped me complete this research. First and foremost, I would like to thank my main supervisor, Prof Hans Amin Van Rostenberghe for his guidance, support and invaluable advice throughout the process. Thank you to my co-supervisor, Associate Prof Noraida Ramli and Dr Nor Rosidah Ibrahim, who never failed to guide me during the work in Neonatal Ward Hospital Universiti Sains Malaysia. A special tribute to Dr Kueh Yee Cheng, who always helping me during statistical data analysis. I am also grateful for the support from my family- my mother and siblings who continuously believe in me to complete this Master Programme journey successfully. To my beloved wife, Dr Noran and my three daughters, thank you for always being there to share the joys and tears for every progress I made. Additionally, I would like to thank all my lecturers from Paediatric Department, peers and colleagues for their help and support in completing this research. Finally, I would like to thank the Universiti Sains Malaysia for providing me with the resources and facilities necessary until today.

TABLE OF CONTENTS

CHAPTER 1:
THE PRELIMINARIES

LIST OF TABLES AND FIGURES

LIST OF ABBREBIATION AND NOMENCLATURE

Hospital USM	: Hospital Universiti Sains Malaysia
TSH	: Thyroid Stimulating Hormone
FT4	: Free thyroxine
Cord TSH	: Cord Thyroid Stimulating Hormone
HIE	: Hypoxic Ischemic Encephalopathy
AS	: Apgar Score
IQR	: Interquartile range

ABSTRACT

Introduction

The objectives of this study are to compare thyroid hormone levels in term asphyxiated newborns versus healthy controls and to determine potential correlations between the severity of birth asphyxia and the thyroid hormone levels.

Methods

A prospective case-control study was performed by comparing cases of birth asphyxia with the healthy controls. The primary outcomes included cord blood Thyroid Stimulating Hormone (TSH), and TSH and Free Thyroxine 4 (FT4) levels at 24-36 hours after birth. Correlations were sought between the severity of birth asphyxia with the thyroid hormone levels for the cases.

Results

The study included 20 cases and 21 controls. Demographic data were similar for the two groups except for a significantly higher Caesarean section rate in the control group. There were no significant differences between the groups in the cord TSH and the 24-36 hours FT4 levels, but the median (IQR) TSH level at 24 to 36 hours was significantly lower in the case group than in the control group, (8.3 mIU/L(4.5-13.7) versus 14.1 mIU/L(9.4-17.0); $p<0.05$). The highest Thompson scores within the first six hours after birth was significantly correlated with lower TSH levels (r of -0.468; $p<0.05$). There was also a significant correlation between the Apgar score at 10 minutes and the level of FT4 at 24-36 hours after birth (r of 0.501; $p<0.05$).

Conclusion

This study showed that TSH at 24-36 hours after birth was significantly lower in asphyxiated newborns. Selected parameters of the severity of birth asphyxia correlated with thyroid hormone levels at 24-36 hours.

(Total: 250 Words)

Keywords

Birth Asphyxia, Term newborn, Thyroid hormone, Thyroid stimulating hormone, Severity

Abstrak

Tajuk: Kesan Kelahiran Asfiksia Kepada Hormon Tiroid Di Kalangan Bayi Matang Yang Dilahirkan Di Hospital Universiti Sains Malaysia (Hospital USM).

Pengenalan: Objektif kajian ini adalah untuk membandingkan tahap Hormon Tiroid in kalangan bayi matang yang mengalami kelahiran asfiksia dengan bayi sihat, dan untuk menentukan korelasi antara darjah keterukan kelahiran asfiksia dan tahap Hormon Tiroid.

Kaedah: Satu kajian secara *prospective* telah dijalankan. Bayi dengan kelahiran asfiksia telah dibandingkan dengan kumpulan bayi kawalan yang sihat. Hasil utama kajian ini adalah termasuk tahap darah Hormon Perangsang Tiroid yang diambil dari tali pusat semasa kelahiran (cTSH), dan Hormon Peransang Tiroid (TSH) dan Hormon Tiroksin (FT4) yang diambil 24-36 jam selepas kelahiran. Korelasi antara darjah keterukan kelahiran asfiksia dengan tahap hormon tiroid akan dikenalpasti di kalangan kes.

Keputusan: Kajian ini melibatkan 20 bayi kes dan 21 bayi kawalan. Maklumat demografik untuk kedua-dua kumpulan adalah serupa kecuali kadar kelahiran melalui pembedahan caesarean adalah lebih tinggi di kalangan bayi kumpulan kawalan. Tiada perbezaan signifikan antara kedua-dua kumpulan ini untuk tahap TSH yang diambil daripada tali pusat dan FT4 yang diambil semasa 24-36 jam selepas kelahiran. Walau bagaimanapun, tahap TSH yang diambil 24-36 jam selepas kelahiran adalah lebih rendah secara signifikan di kumpulan kes berbanding kumpulan kawalan (8.3 mIU/L (4.5-13.7) berbanding 14.1 mIU/L (9.4-17.0); $p<0.05$). Skor *Thompson* tertinggi dalam masa 6 jam pertama selepas kelahiran mempunyai korelasi signifikan dengan tahap TSH (r of -0.468; $p<0.05$). Terdapat juga korelasi signifikan antara skor *Apgar* pada 10 minutes dan tahap FT4 pada 24-36 jam selepas kelahiran (r of 0.501; $p< 0.05$).

Kesimpulan: Kajian ini menunjukkan TSH and FT4 pada 24-36 jam selepas kelahiran adalah lebih rendah secara significan di kalangan bayi dengan kelahiran asfiksia. Parameter darjah keterukan kelahiran asfiksia mempunyai korelasi dengan tahap Hormne Tiroid pada 24-36 jam selepas kelahiran.

Kata Kunci

Kelahiran Asfiksia, Bayi Matang, Hormone Tiroid, Hormone Perangsang Tiroid, Darjah Keterukan.

CHAPTER II:

THE TEXT

2.1 Section A:

Introduction

Introduction

Birth asphyxia remains one of the leading causes of neonatal morbidity and mortality in developing and developed countries. It is graded as the fifth cause of under five years old child deaths after pneumonia, diarrhoea, neonatal infections, and complications of preterm birth. Throughout the world, each year, an estimated 23% of 4 million neonatal deaths and 8.5% of all deaths < 5 years old are associated with signs of asphyxia at birth. (1) Birth asphyxia may affect multiple organ systems, including the brain, the respiratory system, the cardiac system, the kidneys and others.(2) Little is known about the impact of birth asphyxia on thyroid hormone levels.

Earliest study on the effect of birth asphyxia to the level of thyroid hormone was done in year 1985 by Marietta Borges in Venezuela. This study showed the level of thyroid hormone (consisted of FT4, FT3 and TSH) significantly decreased in newborn with transient low Apgar score compared to healthy control newborn with p value < 0.05. (3) Later, in year 1994, H.F Tahivoric from University of Tuzla, Bosnia and Herzegovina did a study comparing thyroid hormone level taken at birth and at day 5 of life, between asphyxiated term newborn delivered via emergency Caesarean Section with healthy term newborn delivered via elective Caesarean Section. The study showed significantly lower thyroid hormone level in cord blood among asphyxiated newborn but normalised at day 5 of life, demonstrating existence of transient hypothyroxinaemia at birth in asphyxiated newborn. (4)

Another study, done by DN Pereira in Brazil in year 2003, looking at the difference in Thyroid Hormone level at birth and between 18 to 24 hours after birth in asphyxiated versus healthy term newborn. This study showed no significant difference in Thyroid Hormone level at birth between two group, but all FT3, FT4 and TSH were lower

in asphyxiated group in blood test taken between 18 to 24 hours after birth. This study also looked at the correlation between the severity of birth asphyxia and mortality rate with the level of Thyroid Hormone. They found that the newborn with severe degree the asphyxia or passed away, had significantly lower Thyroid Hormone Level. (5) Nishant Prabhakar from India did a similar study in year 2016, comparing Thyroid Hormone level at birth and between 18-24 hours after birth in term asphyxiated newborn versus term healthy control. It found that asphyxiated newborn had significantly lower Thyroid Hormone level in blood test taken at 18-24 hours after birth but no significant difference in cord blood. (6) Up to this date, there is no study have been done in Malaysia to look at the effect of birth asphyxia to the level of Thyroid Hormone

Part of the management for newborns with birth asphyxia is to reduce the metabolism of the brain. Therapeutic cooling of the brain and whole-body cooling have been shown to effectively reduce brain metabolism and decrease the incidence of neurological sequelae of birth asphyxia. (2) This signifies that the energy provision to the brain cells may be impaired. Secondary energy failure in birth asphyxia occurred approximately at 6 to 48 hours after initial brain injury and was believed to be related to oxidative stress, excitotoxicity, and inflammation. (7) Thyroxine is essential for providing energy to the cells by its action on acid phosphatases, stimulating the intracellular Adenosine Triphosphate (ATP) production. Measuring thyroid hormone level in between 24- 36 hours after birth is done for study purposes because that is right within the period of secondary energy failure occurred in asphyxiated newborns, in which this is critical timing to have adequate thyroid hormone for a developing brain recovers from asphyxia. We choose blood taking to be coincided with clinical indication to reduce vulnerability of participants.

There is limited evidence that the administration of thyroxine in the cases of pending acute tubular necrosis (ATN) in asphyxiated newborns would be beneficial to the compromised renal cell. (8) Unfortunately, there is no study done yet to look at the effect of thyroxine treatment to the brain cell in asphyxiated newborn.

The objectives of this study were 1) to compare the level of thyroid hormones (cord TSH and serum FT4 and TSH levels at 24- 36 hours after birth) between term newborns with birth asphyxia and healthy controls and 2) to determine whether there was a correlation between the severity of birth asphyxia and the thyroid hormone levels.

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)

2019/2023

EFFECT OF BIRTH ASPHYXIA ON THE THYROID HORMONE IN TERM NEWBORN DELIVERED IN HOSPITAL UNIVERSITI SAINS MALAYSIA

DR. AHMAD ZAFRULLAH AFHAM BIN AHMAD FIKRI

P-UM0226/19

Hospital USM, Kubang Kerian, Kelantan

Supervisor:

Professor Dr Hans Amin Van Rostenberghe

Paediatric Department, Hospital USM, Kubang Kerian, Kelantan

Co Supervisor:

Associate Professor Noraida Binti Ramli

Paediatric Department, Hospital USM, Kubang Kerian, Kelantan

Dr Nor Rosidah binti Ibrahim

Paediatric Department, Hospital USM, Kubang Kerian, Kelantan

Dr Kueh Yee Cheng

Department of Biostatistics and Research Methodology, Hospital USM, Kubang Kerian Kelantan

Research Title : **Effect of Birth Asphyxia on Thyroid Hormone in Term Newborn Delivered in Hospital Universiti Sains Malaysia (Hospital USM)**

Principal investigator: Dr Ahmad Zafrullah Afham Bin Ahmad Fikri(MPM70739)

Co-researchers : **Professor Dr Hans Amin Van Rostenberghe (MPM31842)**

: **Associate Professor Dr Noraida Binti Ramli (MPM32514)**

: **Dr Nor Rosidah Binti Ibrahim (MPM35070)**

: **Dr Kueh Yee Cheng**

Introduction

Birth asphyxia is caused by lack of oxygen to organ systems due to hypoxic or ischemic insult that occur within close temporal proximity to labour (peripartum) and delivery (intrapartum). It causes multiple alteration to the fetus and newborn due to failure of normal physiology gas exchange. (1) Birth asphyxia will affect the metabolism in cells where the rational of therapeutic hypothermia come- by reducing metabolism and subsequently reduce energy requirement. (2)

The incidence of birth asphyxia is dependent on the definition used, the ability to accurately make the diagnosis and the quality of obstetrical care. Throughout the world each year, estimated 23% of 4 million neonatal death and 8% of all death < 5 years old, are associated with signs of asphyxia at birth. (3) In a Swiss study, it has recorded 622 clinically diagnosed cases of intrauterine or birth asphyxia based on ICD-10 codes, with the incidence of birth asphyxia (defined as fulfilling three of the following – five minutes Apgar score $\leq$ 5, pH < 7, base deficit $\geq$ 16 OR lactate > 12mmol) range from 5 to 8 per

1000 live birth over the study period from year 2004 to 2014. The incident of hypoxic-ischemic encephalopathy (HIE) was about 1 per 1000 life birth. (3)

Managing newborn with birth asphyxia with therapeutic hypothermia is a standard care which needs to be started within the first 6 hours after delivery as this intervention deemed to be neuroprotective. Certain inclusion and exclusion criteria have been set before starting therapeutic hypothermia as this could complicated issues- such as arrythmias, thrombocytopenia and coagulopathy. (2)

Problem statement & Study rationale

The effect of birth asphyxia is multisystem including respiratory, cardiac, circulation and neurological but little is known about its effect to the thyroid hormone. The need of studying the effect of birth asphyxia on thyroid hormone is undeniably important because thyroxine can increase the production of acid phosphatase that produces ATP in the cell. This provides energy to the energy-deprived asphyxiated cells. In other words, the suppression of thyroid hormone in an asphyxiated newborn prevents the cell from getting enough energy because the reduction ATP production leads to energy failure by the brain.

The studies about the effect of perinatal asphyxia to the thyroid hormone were scarce, the earliest was in year 1985. However so far, all the studies have come to conflicting results. The onset of the thyroid hormone involvement is different in each study leading to questionable timing to look into the level of thyroid hormone and to determine the best time for potential thyroxine treatment. Moreover, there is no similar study in the local setting in Malaysia/ South East Asia. The studies regarding correlation

of severity of perinatal asphyxia and the degree of thyroid hormone affected were also limited.

For this study, I wish to determine how the effect of birth asphyxia to the level of thyroid hormone at birth and after 24 to 36 hours of life. On top of that, I also want to determine the magnitude of thyroid hormone affected by the severity of birth asphyxia. Outcome from this study may benefit in the decision of potential treatment with thyroxine in the newborn with birth asphyxia.

Research Question(s)

1. How birth asphyxia could affect the level of thyroid hormones?
2. What is the correlation between severity of birth asphyxia to the level of thyroid hormones?

Objectives

General: To assess the effect of birth asphyxia on the level of thyroid hormones in term newborn delivered in Hospital Universiti Sains Malaysia (Hospital USM).

Specific:

1. To compare difference level of Thyroid Stimulating Hormone (TSH) and Thyroxine (T4) in asphyxiated term newborn versus control group using cord blood and sample taken between 24- 36 hours after birth.
2. To determine the correlation between severity of birth asphyxia (graded by cord blood pH, Apgar score at 10 minutes and highest Thompson scoring) with the level of thyroid hormone.

Hypothesis

1. Thyroid Stimulating Hormone (TSH) and thyroxine (T4) are lower in asphyxiated newborn.
2. The more severe the birth asphyxia, the lower the TSH, T4.

Literature review

1-DN Pereira et al; Effect of Perinatal asphyxia on thyroid stimulating hormone and thyroid hormone level, published in year 2003. (4) Prospective case control study in Hospital de Clinicas, Porto Alegre, Brazil involving 17 term newborns with Perinatal asphyxia and 17 term normal newborn as control group. Case group defined by newborn with Apgar score $\leq$ 3 in 1st minutes and < 5 in 5 minutes and cord blood gas pH < 7.15 who require bag and mask ventilation at least one minutes immediately after birth. Control group defined by newborn with Apgar score $\geq$ 8 at 1st minutes and $\geq$ 9 at 5 minutes with umbilical cord blood gas pH >7.2 Cord blood and arterial blood sample we collected immediately after birth and 18 to 24 hours respectively and were analysed for serum concentration of T4, T3, FT4, rT3 and TSH. All newborn were followed up till discharge or death. Result showed no difference in mean level of T4, T3, FT4, rT3 and TSH taken from cord blood between asphyxiated newborn with control group. In the sample collected between 18 to 24 hours after birth, mean level of T4, T3, FT4, rT3 and TSH were significantly lower in asphyxiated newborn compared to control group. This study also showed asphyxiated newborn with moderate to severe hypoxic ischemic encephalopathy had significantly lower mean level of T4, T3, FT4, rT3 and TSH and greater risk of death.

2-Nishant Prabhakar et al; Effect of perinatal asphyxia on level of thyroid hormone in term neonates, published in 2016. (5) This is prospective case control study done in a Tertiary Care paediatric centre in central India, involving 60 full term newborns with perinatal asphyxia and 60 full term normal newborn as control group. Case group defined by newborn with newborn who require bag and mask ventilation $\geq$ 1 minutes or Apgar score at 1 and 5 minutes $\leq$ 7 or require intubation. Control group is defined by newborn with apgar score > 7 at 1 and 5 minutes. Cord blood and thyroid hormone level (consisted

of TSH, T3 and T4) taken at18 to 24 hours after birth were compared between the 2 groups. Result show there was no significant difference observed in cord blood (P>0.05) but a significant lower level of thyroid hormone observed in asphyxiated group as compared to control group in venous sample 18 to 24 hour after birth. P value < 0.004 for T3, and P< 0.001 for TSH and T4)

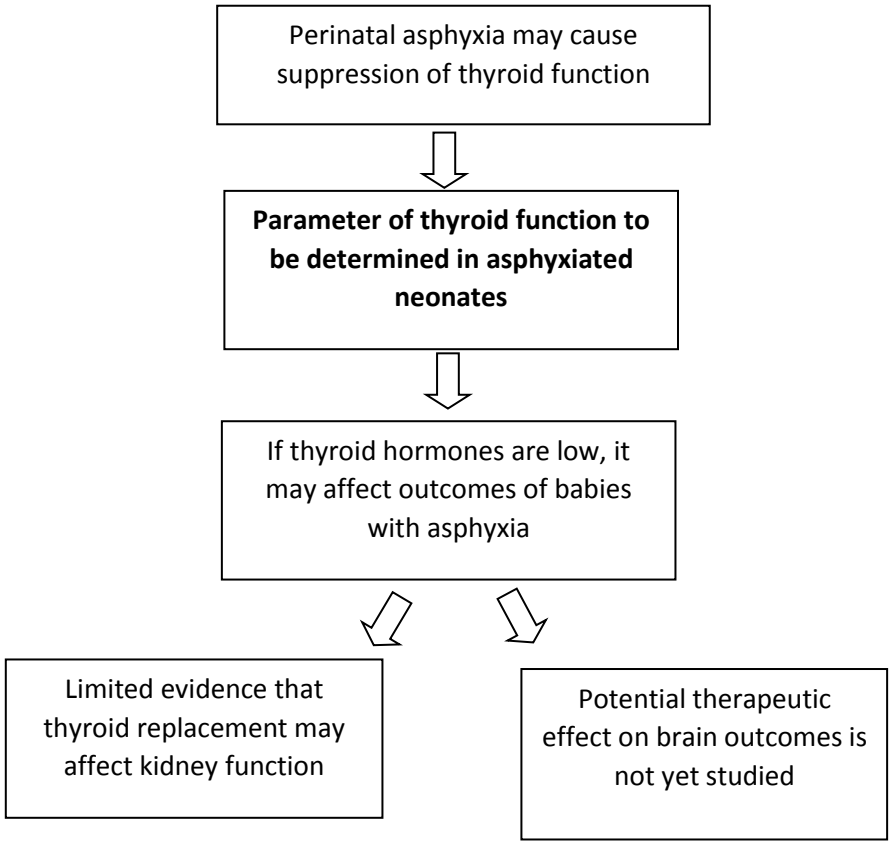
3-H.F Tahirovic et al; from Department of Paediatric, University of Tuzla-Bosnia and Herzegovina – investigated regarding transient Hypothyroxinaemia in term neonates with birth asphyxia delivered by emergency Caesarean Section. (6) The study was published in 1994. It involved 27 asphyxiated term newborns delivered by EMLSCS between week 40 to 42 gestation, and 30 healthy term newborns delivered by elective Caesarean Section. In both groups, the Caesarean Section were performed under general anaesthesia. Case group was defined by newborn with Apgar score < 6 at 1^{st} minutes and control group were the newborn with Apgar score $\geq$ 6 at 1^{st} minutes of life. T4,T3, FT4, rT3, TSH, TBG measured at cord blood and on day 5 of life. The mean value T4 and FT4 observed in cord blood of asphyxiated neonates were significantly lower compared to the matched control (p value <0.01 and 0.0005 respectively), while there were no differences in concentration of T3, rT3, TSH, and TBG. At the day 5 of life, serum concentration of T4 and FT4 become normal, demonstrating existence of transient hypothyroxinaemia at birth in hypoxic babies delivered by emergency caesarean section.

4-Marietta Borges-Effect of Asphyxia of Free Thyroid Hormone Level in Full Term Newborn, published in 1985. (7) This is prospective study done in Hospital Central Carlos Arvelo, Caracas, Venezuela involving 3 groups of newborn-Group 1 (control): term healthy newborn with normal pregnancy delivered by spontaneous vaginal delivery (n=7). Group 2: Term newborn with transient low Apgar score at birth (n=7). Group 3: Term newborn to mother with toxaemia and hypertension. FT3, FT4 and Thyroid

stimulating hormone were taken from cord blood, then serially at 3, 24, and 48 hour after delivery in all patient. Cord blood of TSH, FT4, and FT3 were not statistically different in the three groups. Control group had increased significantly level of FT4 and FT3 from cord to 48 hour after delivery ($p< 0.001$ /$p< 0.001$ respectively). However, group 2 and 3 failed to increase the FT4 and FT3 concentration above baseline during first 48 hour. Level of FT4, FT3, and TSH at 3, 24, and 48 hours were significantly higher in control group than group 2 and 3 ($p< 0.001$ at 3, 24 and 48 hours). They also noted rapid increase in TSH value by 5 minutes of delivery, followed by a progressive TSH decline to level similar to baseline over 48 hours in all three group.

Conceptual framework

Diagrammatic illustration of the study framework based on literature review with some text to explain the diagram.



Research design

The study will be done as prospective case control study.

For the objective 1 which is to compare the level of TSH and T4 in asphyxiated newborn with control group using cord blood and between 24 to 36 hour of life, cord TSH and subsequently TFT between 24 and 36 of life will be taken using non probability sampling (quota sampling- meaning that any newborn who fulfil the inclusion criteria will be recruited) for study group and non-probability convenient sampling in control group.

For the objective 2 which is to determine correlation between the severity of perinatal asphyxia to the level of thyroid hormone, a sheet will be used to record the severity of the asphyxia and a pearson's correlation will be used to with the respective level of thyroid hormone.

Study area Labour room, Operation Theatre and Neonate ICU (NICU) in Hospital USM.

Study population

All term newborn delivered in Hospital USM by non-thyroidal disease mother during the study period.

Subject criteria

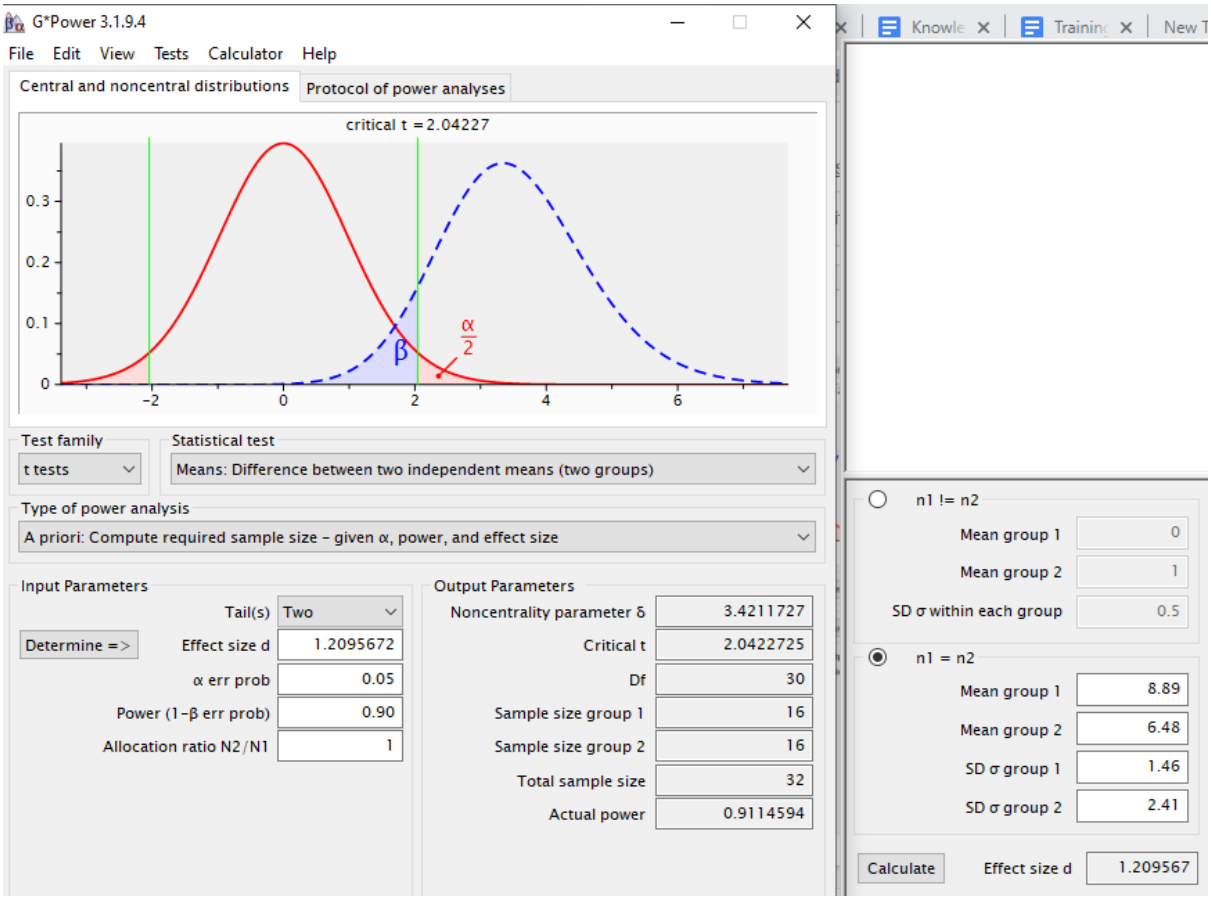
Criteria	Control Group	Study Group
Inclusion	1. All newborn from mothers with no underlying thyroid disease admitted to neonatal ward who are scheduled for blood taking between 24 to 36 hours of life 2. Newborn with Apgar score ≥8 and ≥9 at 1st and 5 minutes (no evidence of asphyxia) 3. Newborn delivered at ≥38 weeks gestation with birth weight appropriate to gestational age (AGA) ≥ 2.50kg 4. Delivered at Hospital USM	1. All term newborn delivered at ≥38 weeks gestation with birth weight appropriate to gestational age (AGA) ≥ 2.50kg 2. Delivered at Hospital USM 3. Evidence of birth asphyxia satisfying at least one of the criteria a. Apgar score ≤5 at 10 minutes b. Mechanical ventilation or resuscitation at 10 minutes c. Cord pH <7.1 OR arterial pH < 7.1 or Base deficit ≥12 within 60 minutes of birth 4. Evidence of encephalopathy according to Sarnat Staging (Finer1981, Sarnat 1976)
Exclusion	1. Refusal for study participation 2. Mothers who had thyroid disease during pregnancy which were on thyroid or antithyroid drug during pregnancy 3. Syndromic baby 4. All baby who during the period of study found out to fulfil criteria for sepsis- positive blood culture and sensitivity and positive CRP (for control group)	

Sample size estimation

- For objective 1: sample size estimation for objective 1 will be based on sample size calculation for difference between two independent means.

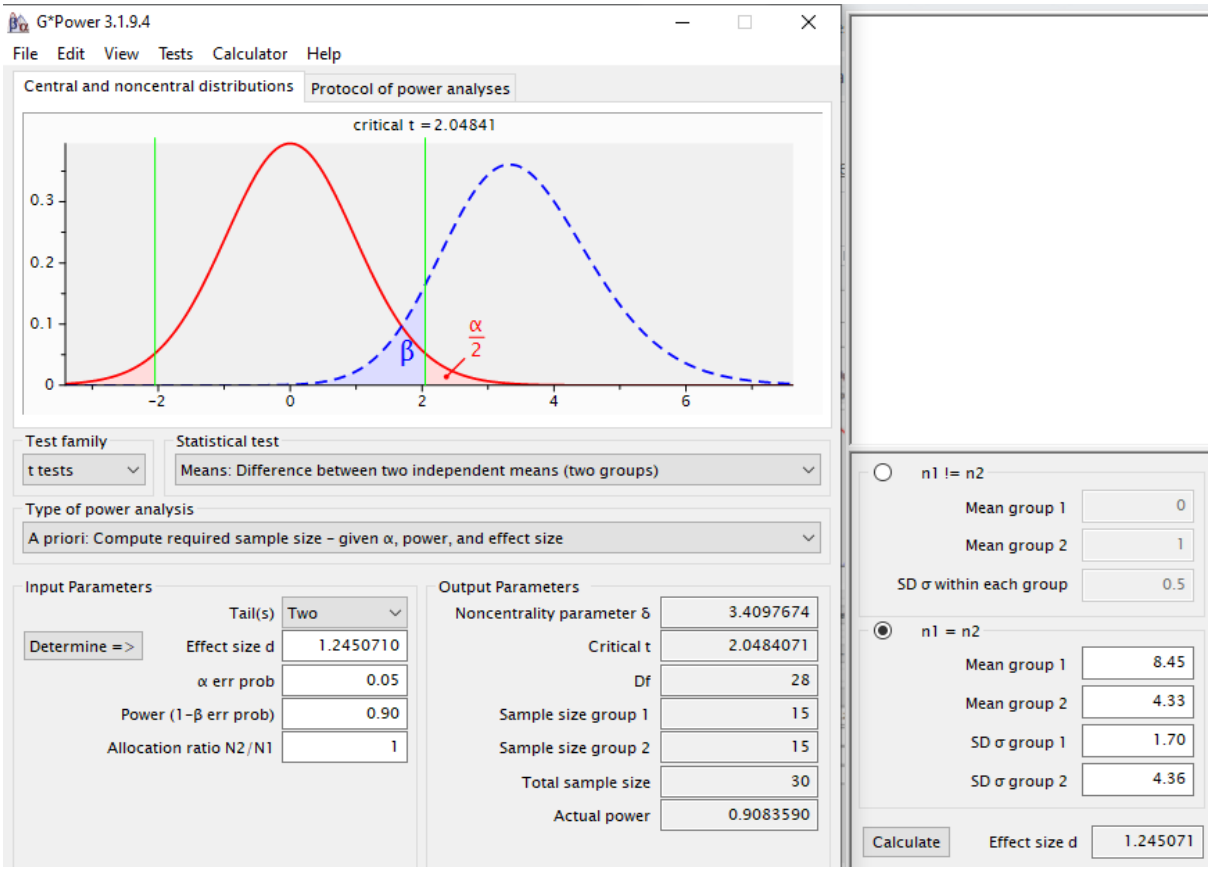
- To compare the level of Thyroxine (T4) in asphyxiated newborn with control group in cord blood and between 24 to 36 hours of life.

- From previous study Nishant Prabhakar [5], mean and SD of control group were 8.89 and 1.46, while mean and SD of the case were 6.48 and 2.41, with type 1-error of 0.05, and power of 0.90, and dropout rate of 20%, estimated sample size is 20 per group.



- To compare the level of TSH in asphyxiated newborn with control group in cord blood and between 24 to 36 hours of life.

- From previous study Nishant Prabhakar [5], mean and SD of control group were 8.45 and 1.70, while mean and SD of the case were 4.33 and 4.36, with type 1-error of 0.05, and power of 0.90, and dropout rate of 20%, estimated sample size is 19 per group.



- Objective 2: To determine association between severity of perinatal asphyxia (graded by cord blood gas pH, Apgar score at 10 minutes and highest Thompson scoring) with the level of thyroid hormone. No previous study about this correlation.

Based on estimated sample size for the 2 objectives, minimum required sample size to achieve all the objectives are 20 participant per group.

Sampling method and subject recruitment

We will use Convenient Sampling for of infant in the control group. Any infant admitted in NICU that fulfilled the eligibility criteria with a plan blood taking at between 24-36 hours of life will be selected as control.

We will use non probability (quota) sampling of infant into the study group. All infant fulfilled the eligibility criteria of the study group will be included in the study.

Research tool

1. Cord TSH: taken immediately at birth from cord blood into standard sampling tubes and will be sent to Endocrine Lab every next morning. Sample will be analysed using COBAS e411 analyser with electrochemiluminescence immunoassay (ECLIA) with sandwich principle. Measurement range 0.005 - 100µIU/L.

2. Arterial cord Blood Gas: sample taken from umbilical cord artery for delivery with evidence of fetal distress. Sample will be run immediately at the nearest ABG Machine ABL 90.
3. Thyroid Function Test (TFT)

    a. Free Thyroxine (T4) is analysed using COBAS e411 analyser with **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay (ECLIA) with competition principle. Measurement range is between 0.5- 100pmol/L. Values below the limit of detection will be reported as <0.5pmol/L and above the measuring range are reported as >100pmol/L.

    b. Thyroid stimulating hormone (TSH) is analysed using COBAS e411 analyser with **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay (ECLIA) with sandwich principle. Measurement range is between 0.005 -100µIU/L. Values below the limit of detection will be reported as <0.005 µIU/L and above the measuring range are reported as >100µIU/L.

4. Apgar score: A scoring system which is developed in year 1952 by Dr Virginia Apgar for rapid method of assessing clinical status of newborn at 1 minutes of age and the need for prompt intervention to establish breathing. The Apgar score comprise five component 1) Colour, 2) Heart rate 3) Reflex, 4) Muscle Tone, 5) Respiratory effort. Each of which is given a score of 0,1 or 2. A score of 10 indicated a baby in the best possible condition. (8)

Operational definition

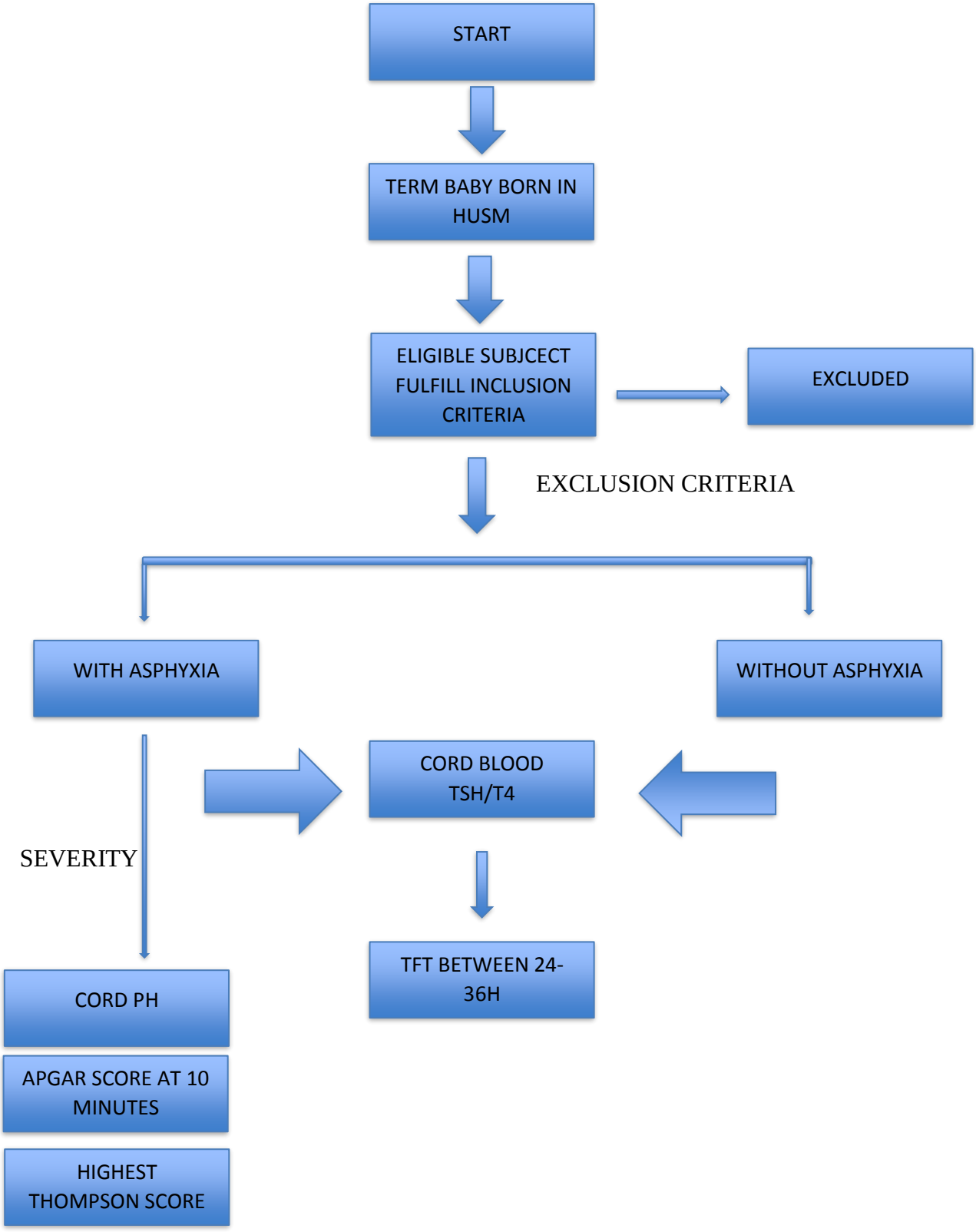
1. Birth asphyxia: baby delivered with one of the following criteria 1-Apgar Score <5 at 10 minutes of life (2) Require mechanical ventilation or resuscitation at 10 minutes of life (3) Cord blood gas with pH <7.1 or arterial pH < 7.1 or base deficit ≥ 12 within 60 minutes of life. Reference: Xenon as an adjuvant for therapeutic Hypothermia in near Term and Term newborn with HIE.
2. Cord blood thyroid hormone is the one of compulsory National birth blood screening taken from cord immediately after delivery.
3. Cord blood gas is blood gas analysis taken from arterial cord vessel with evidence of fetal distress**.** Lower pH (<7.35) indicating acidosis who is associated with worse severity of acidosis.
4. The Thompson HIE score is a neonatal scoring system used in the assessment of the severity of perinatal asphyxia. The score was found to have high sensitivity and specificity for hypoxic ischaemic encephalopathy adverse outcomes. It consists of 9 neurological assessment parameter which scoring 0 to 3 each. The sum of the individual points will be interpreted as HIE with mild, moderate and severe severity. (9)

Data collection method

After getting permission and approval from ethical committee, data collection activity will be conducted. Sheet where the information regarding the study will be disseminated to all Staff in Antenatal Ward, Labour Room, and Neonatal Ward in Hospital USM. The sheet will also contain the study information, informed consent. The sheet also will have the column to be filled with information of cord blood gas and TSH, Apgar score and Thompson score and value of TSH/T4 between 24 to 36 hours of life. The form will

include the participant's name and HUSM Registration Number but they will be identified as unique subject number to ensure confidentiality.

Study flowchart



Data analysis

Data will be entered and analysed using SPSS version *26*. Descriptive statistics will be used to summarise the socio-demographic characteristics of subjects. Numerical data will be presented as mean (SD) or median (IQR) based on their normality distribution. Categorical data will be presented as frequency (percentage).

Statistical Analysis

For objective number 1: Independent T-test to compare the level of thyroid stimulating hormone (TSH) and Thyroxine (T4) in asphyxiated newborn with control group in cord blood and between 24 to 36 hours of life.

For objective 2: Pearson's Correlation to determine the association between the severity of perinatal asphyxia (graded by cord blood gas pH, Apgar score at 10 minutes, and highest Thompson score) with the level of thyroid hormone (T4 and TSH).