PLACENTAL PATHOLOGY OF NEONATES DIAGNOSED WITH ENCEPHALOPATHY SOON AFTER BIRTH: A RETROSPECTIVE ANALYTIC STUDY

Candidate

Keaoleboga Lorraine Sebolai

MBChB, FC Paed, DCH

Student number: 2531002

Supervisors

Dr Omphile Mekgoe

MBChB, FC Paed, HIV DIP

Professor Sithembiso Velaphi

MBChB, FC Paed, MMed, PhD

Professor Colleen Wright

MBBCh (Wits) MMed, FC Path, FRCPath, FIACem, PhD

A dissertation submitted to the Faculty of Science, University of the Witwatersrand, in partial fulfilment of the requirements for the degree of Master of Medicine in Paediatrics and Child Health

Johannesburg, 2025

DECLARATION

I, Keaoleboga Lorraine Sebolai declare that this research report is my own work that I have accomplished through the assistance of my supervisors. I have ensured that all ideas and previously published intellectual property of others have been properly referenced. This report is being submitted for the Master of Medicine Degree in the Department of Paediatrics and Child Health at the University of the Witwatersrand, Johannesburg.

It has not been previously submitted for any degree or examination at any other university but some of the content has been presented at academic conferences by myself. This was done under the guidance of my supervisors.

Signature

Date: 30/07/2025

Place: Klerksdorp, North West Province, South Africa

lorrainelebopaul@gmail.com

076 190 1171

DEDICATION

First of all, Father God, You are above all things and that in you all things hold together. God, I thank you, for you have gone before me to see this research report to completion. I honor my loving husband and covering; Phillip Sebolai. You have been such a constant and amazing support throughout this whole journey. I have seen how you have diligently prayed and held me and how you graciously hid my absence from our children. I salute you.

To my dear children, Bonolo and Tlotlo Sebolai; you have taught me to be brave and I experienced the undeniable track record of God's faithfulness through you. To my mother, Jaqueline Paul and my mother in love, Lena Sebolai, I was able to hold my head high because I knew I had angels who were constantly interceding on my behalf. To Mamoliehi Tsoake, thank you for your gentle spirit and being a mother to our children while we were studying. To my siblings and friends, thank you for all the support and prayers.

Lastly, I dedicate this report to my father, Abram Paul. You have given me my identity, affirmed me and believed in me. You have fought the good fight and have ran your race. Continue to rest in eternal peace.

PRESENTATIONS

ABSTRACT PRESENTED AS AN ORAL PRESENTATION AT THE DEPARTMENTAL RESEARCH DAY

Placental Pathology of Neonates Diagnosed with Encephalopathy Soon After Birth: A Retrospective Analytic Study. Presented at the University of the Witwatersrand Paediatric Research day, 4 December 2023

ABSTRACT PRESENTED AS AN ORAL PRESENTATION AT A CONFERENCE

Placental Pathology of Neonates Diagnosed with Encephalopathy Soon After Birth: A Retrospective Analytic Study. Presented at the 42nd Conference on Priorities in Perinatal Care in Southern Africa, 12-15th March 2024, ATKV Goudini, South Africa

ABSTRACT PRESENTED AS AN ORAL PRESENTATION AT DR KENNETH KAUNDA DISTRICT RESEARCH DAY

Placental Pathology of Neonates Diagnosed with Encephalopathy Soon After Birth: A Retrospective Analytic Study. Presented at the Dr Kenneth Kaunda Research day, Potchefstroom, North West Province, 1 November 2024

MANUSCRIPT PLANNED FOR SUBMISSION

Placental Pathology of neonates Diagnosed with Encephalopathy soon after birth: A Retrospective Analytic Study (in preparation for submission to South African Medical Journal)

See attached author guidelines (Appendix G) and URL link:

https://scielo.org.za/revistas/samj/iinstruc.htm

ABSTRACT

Background: Placental pathology has been linked to neonatal encephalopathy (NE). Despite the high prevalence of NE in low- and middle-income countries (LMICs), there are limited published studies exploring this association from LMICs.

Objective: To describe and analyze the histopathology of placentas from neonates diagnosed with encephalopathy shortly after birth.

Methods: A retrospective analytic study was conducted on neonates diagnosed with encephalopathy at Klerksdorp/Tshepong (KT) hospital complex, South Africa from 1 December 2019 to 31 October 2022. Placentas from term and near-term neonates (birth weight ≥2000 grams) diagnosed with encephalopathy soon after birth were sent for histopathological examination. Neonates were grouped into those with encephalopathy with or without intrapartum hypoxia (IH) (base deficit ≥12 mmol/l). The severity of NE was assessed as mild, moderate and severe, according to Sarnat staging. The types and extent of placental lesions were compared between those with mild and moderate-to-severe encephalopathy and between cases with or without IH.

Results: Out of 16,336 live births, 271 neonates were diagnosed with NE, and of these, 193 (71.2%) had NE with IH. Placental histopathology results were available for 239 neonates (88.2%). The median placental weight was 428 grams, with 46.6% of placentas weighing <10th percentile. Cord abnormalities were observed in 27.2% of placentas. Nearly all placentas (98.2%) exhibited at least one histopathological lesion. The most common microscopic placental lesions identified were maternal vascular malperfusion (MVM) (55.6%), fetal vascular malperfusion (55.1%), chorioamnionitis (41.2%), and villitis of unknown etiology (28.9%). There was a significant association between NE with IH and the presence of MVM, with an adjusted odds ratio of 3.24 (95% CI 1.19–8.79). No significant association was found between the presence or number of different placental lesions and the severity of NE. Factors associated with severity of any NE (with and without IH) included an Apgar score of less than 7 at 10 minutes ($p<0.001$) and pH <7.00 ($p<0.001$).

Conclusion: A high proportion of neonates with encephalopathy showed evidence of intrapartum hypoxia. Almost all neonates with encephalopathy displayed at least one microscopic placental lesion. Furthermore, most placentas from neonates with NE exhibited abnormalities, indicating that placental pathology may play a significant role in the development of NE. A strong association was found between the presence of MVM and NE with IH.

ACKNOWLEDGEMENTS

To my supervisors, thank you all for the roles you have played that were so complementary from the beginning.

To Prof Velaphi, thank you for being readily available, despite being the Head of Department of one of the biggest hospitals in Southern Africa. Your passion for research, teaching, and patient care is unmatched.

To Dr. Mekgoe, I have learned from you as an excellent leader and clinician. Thank you for believing that I could complete this research report and for guiding me with such grace.

To Prof Wright, thank you for making this research project such a success with all the knowledge and experience you provided. Your encouragement is appreciated.

To Prof Thandrayen, thank you for doing the statistical analysis and explanation thereof for this report. Thank you for working tirelessly on the finer details and providing me with exceptional research advice.

Table of Contents

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ACA	Acute chorioamnionitis
ACOG	American College of Obstetricians and Gynecologists
CI	Confidence interval
CTG	Cardiotocograph
FVM	Fetal vascular malperfusion
HIC	High income countries
HIE	Hypoxic ischaemic encephalopathy
IH	Intrapartum hypoxia
IQR	Interquartile range
IUGR	Intrauterine growth restriction
KT	Klerksdorp/Tshepong
LMIC	Low- and middle-income countries
MVM	Maternal vascular malperfusion
NE	Neonatal encephalopathy
NICE	National Institute for Health and Care Excellence
OR	Odds ratio
SGA	Small for gestational age
VUE	Villitis of unknown aetiology

SUBMISSIBLE MANUSCRIPT FORMAT

Placental Pathology of Neonates Diagnosed with Encephalopathy Soon After Birth: A Retrospective Analytic Study

KL Sebolai[1,2] MBChB, FC Paed; OT Mekgoe[1] MBChB, FC Paed; CA Wright[3,4] MBBCh, FC Path, MMed, PhD; K Thandrayen[2] MBBCh, FC Paed, MMed, PhD, Cert of Endocrinology and Metabolism (Paed); SC Velaphi[2] MBChB, FC Paed, MMed, PhD

[1]Department of Paediatrics, Klerksdorp /Tshepong Hospital Complex, North West Province, [2]Department of Paediatrics and Child Health, School of Clinical Medicine, Faculty of Health Sciences, University of the Witwatersrand, Chris Hani Baragwanath Academic Hospital Johannesburg, South Africa; [3]Division of Anatomical Pathology, University of the Witwatersrand, Johannesburg, South Africa, [4]National Health Laboratory Services, Nelson Mandela Bay and SBD Eastern Cape, South Africa.

Corresponding Author

Lorraine Sebolai

123 Dr Yusuf Dadoo Avenue

Lahoff, Klerksdorp

2571

North West Province

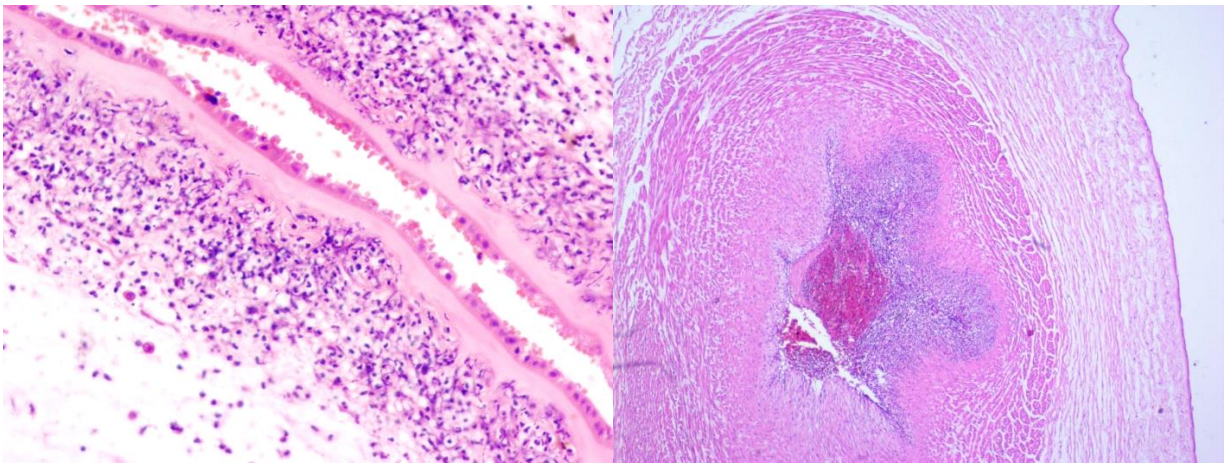
Telephone number: 076 190 1171

Email: lorrainelebopaul@gmail.com

INTRODUCTION

Neonatal encephalopathy (NE) is a clinically defined disorder characterized by impaired neurologic function in neonates born at term or near-term gestation. One of the causes of NE is hypoxic-ischaemic encephalopathy (HIE), which is due to impaired fetal cerebral blood flow and oxygen delivery. Globally, HIE is still considered to be a major contributor to perinatal mortality.[1] The incidence of HIE has been reported to be significantly higher in low- and middle-income countries (LMIC), as high as 14.9 per 1000 live births[2] compared to high-income countries (HIC) at 1.3-1.7 per 1000 live births.[3] In South Africa, studies from different provincial regions have also indicated a noticeable variability. Two studies conducted at two separate academic institutions in Gauteng reported an incidence of 3.6-15.2 per 1000 live births.[4,5]. There was a vast contrast in the findings of a similar population demographic to these Gauteng studies, with incidences reported as 2.3 to 4.3 per 1000 live births in the Western Cape.[6]

Placental pathology has been associated with an increased risk for the development of NE.[7] According to the 2016 Amsterdam Placental Workshop Group Consensus Statement, there are four significant microscopic lesions that have been associated with NE: acute chorioamnionitis (ACA) **(Figure 1)**, villitis of unknown aetiology (VUE) **(Figure 2)**, maternal vascular malperfusion (MVM) **(Figure 3)**, and fetal vascular malperfusion (FVM) **(Figure 4)**.



A. Maternal response (stage 2, grade 2) B. Fetal response (stage 3, grade 2)

Figure 1. Histopathological features of acute chorioamnionitis

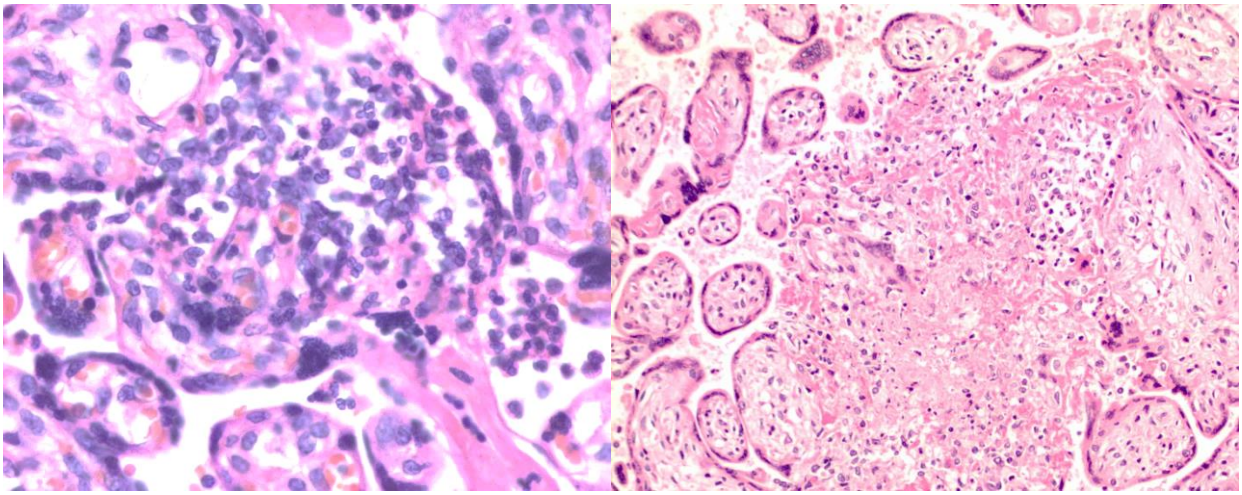
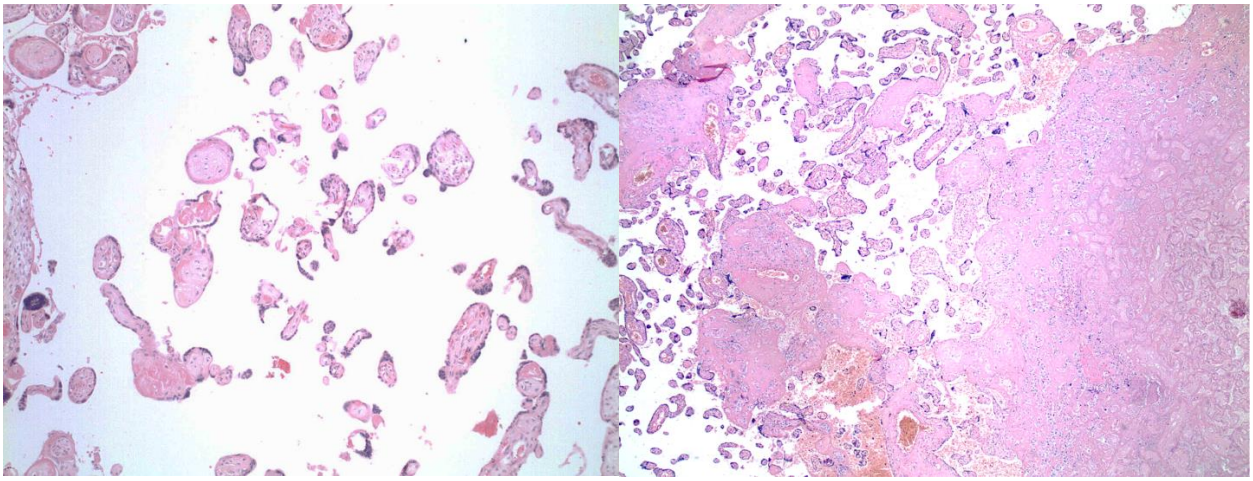
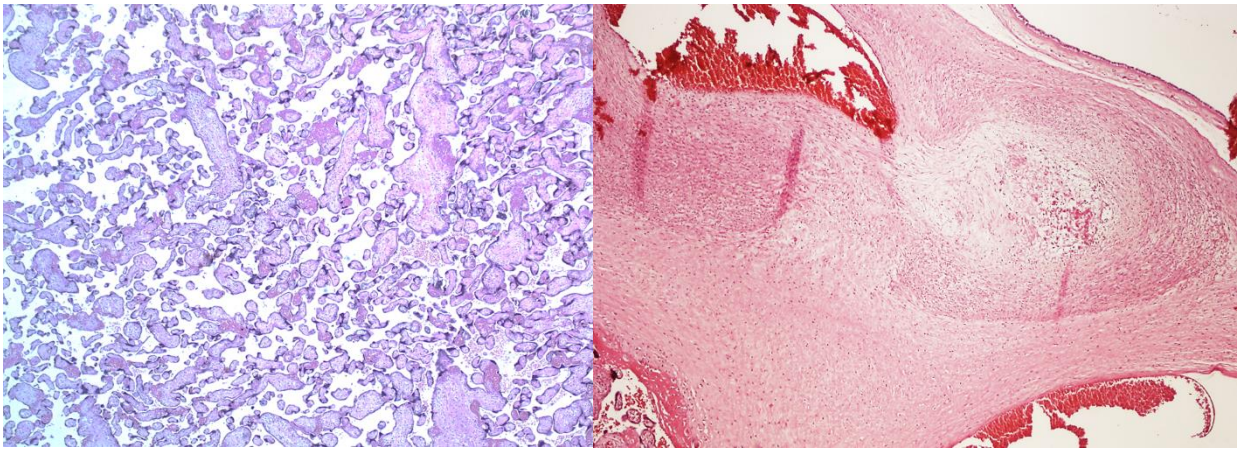


Figure 2. Histopathological features of chronic villitis (villitis of unknown origin)



A. Distal villous hypoplasia B. Accelerated maturation, increased syncytial knots and infarction

Figure 3. Histopathological features of maternal vascular malperfusion



A. Avascular villi, high grade B. Organizing thrombus with calcification

Figure 4. Histopathological features of fetal vascular malperfusion

These lesions have been described in previous studies as contributing to the poor neurological outcome of neonates. With regard to predictors of the outcome of NE, VUE has been shown to be the most significant predictor of abnormal long-term outcomes, while chorioamnionitis is associated with the severity of NE.[9] Neonates with NE have been shown to have a higher likelihood of placental inflammatory and flow restrictive lesions, which may predispose the neonatal brain to hypoxic-ischaemia.[9,10]

There is still a substantial lack of data on the risk profile of neonates with encephalopathy, and especially the contribution of placental histopathology. This is especially important in the LMIC setting where the incidence of NE is relatively high. As part of the supplement published from a South African Obstetric perspective, it is now recommended that placental histopathologic evaluation be part of the assessments when investigating causes of cerebral palsy, where NE was a suspected cause.[11] The aim of this study was to describe the histopathology of placentas from neonates born with encephalopathy and to determine whether there is an association between the type or extent of placental pathology and the severity of NE.

METHODS

Study Design

This was a retrospective, analytic study of neonates born at term/near term diagnosed with NE, who had placentas sent for histopathology.

Study Setting

This study was conducted in the Klerksdorp/Tshepong (KT) hospital complex, North West Province, South Africa. The KT hospital complex is a provincial and a satellite teaching hospital for the University of the Witwatersrand. It is a referral hospital for patients from Nic Bodenstein district hospital and 30 local clinics in the North West province. The hospital conducts about 6000 births per year. All neonates diagnosed with encephalopathy are admitted and managed in the neonatal unit, which has a capacity of 49 beds, including 12 neonatal intensive care unit (NICU) and 12 high care beds.

In December 2019, the hospital implemented a protocol that recommended that all placentas from neonates born with encephalopathy should be sent for histopathologic evaluation to assist in identifying factors that might contribute to NE. The following criteria were used to establish the

diagnosis of NE: requirement for resuscitation at birth, defined as the need for at least bag-mask ventilation, and signs suggestive of encephalopathy.

Study Population

Neonates born in KT hospital complex, weighing ≥ 2000g with a diagnosis of NE, with placentas sent for histopathologic assessment from 1st December 2019 to 31st October 2022, were enrolled in the study. Neonates who were born outside the hospital facility, had major congenital anomalies, or did not have a placenta sent for histopathology were excluded.

Study Procedures

Maternal and infant hospital records of the neonates diagnosed with NE were retrieved and reviewed for analysis. Maternal data included maternal age, gravidity, antenatal care, human immunodeficiency virus (HIV) status, mode of delivery, intrapartum monitoring, and presence of meconium-stained amniotic fluid. Bedside urine dipsticks were used to screen for urinary tract infection (UTI), which was evidenced by nitrites and/ or leucocytes. Suspicion of clinical chorioamnionitis was based on maternal tachycardia and fever. Intrapartum monitoring was performed by assessing the maternal clinical condition, plotting the progress of labour on a partogram, and using a cardiotocograph (CTG) to determine fetal heart rate. CTG tracings were reported as normal with reactive tracings or pathological according to the National Institute for Health and Care Excellence (NICE) guidelines.[12]

Neonatal data collected included anthropometry, sex, Apgar scores, resuscitation details, cord blood or arterial blood gas done within the first hour of birth, Thompson score, and Sarnat staging and outcome at hospital discharge. The Thompson score and/or Sarnat staging were used to assess the severity of NE.[13,14] The severity of NE was classified as mild, moderate, and severe NE. NE was also classified to be with or without intrapartum hypoxia (IH) defined as a blood gas with base deficit ≥12 mmol/l.

Placental histopathology was performed and reviewed by an experienced pathologist (CW). All placentas were reported on a standardized template and reported according to a globally accepted classification system.[8] The placental lesions were divided into macroscopic and microscopic characteristics. The macroscopic abnormalities included placental weight and cord abnormalities. The major significant microscopic lesions were identified and categorized into: acute chorioamnionitis (ACA), maternal vascular malperfusion (MVM), fetal vascular malperfusion

(FVM), and villitis of unknown aetiology (VUE). Further analysis was conducted, categorizing chorioamnionitis into maternal and/or fetal response as well as dividing FVM and MVM according to different grades. To assess the significance of these microscopic lesions, all placentas were further evaluated based on the total microscopic lesions that they each had and their correlation to the severity of NE and the type of NE (with or without evidence of IH). Permission to conduct the study was obtained from the hospital's Chief Executive Officer. Ethical approval was obtained from the University of the Witwatersrand Human Research Ethics Committee (Protocol reference number: M220119).

Data Analysis

Statistical analysis was conducted using STATA version 17.0 (Texas, USA). Neonates were divided into two groups based on the severity of NE: mild and moderate-to-severe NE. Additionally, they were classified based on the presence of intrapartum hypoxia (IH), with some having NE with IH and others without. Descriptive statistics were used to summarize data related to maternal and infant demographics, as well as placental characteristics. To compare infants based on the severity of encephalopathy, chi-square tests with Fisher's exact test were used for categorical variables, while Student's t-test or Mann-Whitney U test was applied for continuous variables. For multivariate analysis, all significant microscopic histopathological lesions and cardiotocographic findings were incorporated into the model, along with variables that had a p-value of less than 0.20 in the univariate analysis. Statistical results from both univariate and multivariate analyses were reported as crude and adjusted odds ratios (OR) with 95% confidence intervals (95% CI). Associations were deemed statistically significant if the 95% CI of the OR did not include value 1 and if p-values were less than 0.05.

RESULTS

Incidence

There were 16,336 live births during the study period, of which 271 were diagnosed with NE, with an incidence of 16.5 per 1000 live births **(Figure 5**). There were 270 singletons and one set of twins. Amongst the 216 who had arterial blood gases within an hour of delivery, 193 (89.4%) neonates were assessed as having NE due to intrapartum hypoxia (base deficit ≥12 mmols/l), giving an incidence of HIE at 11.8 per 1000 live births.

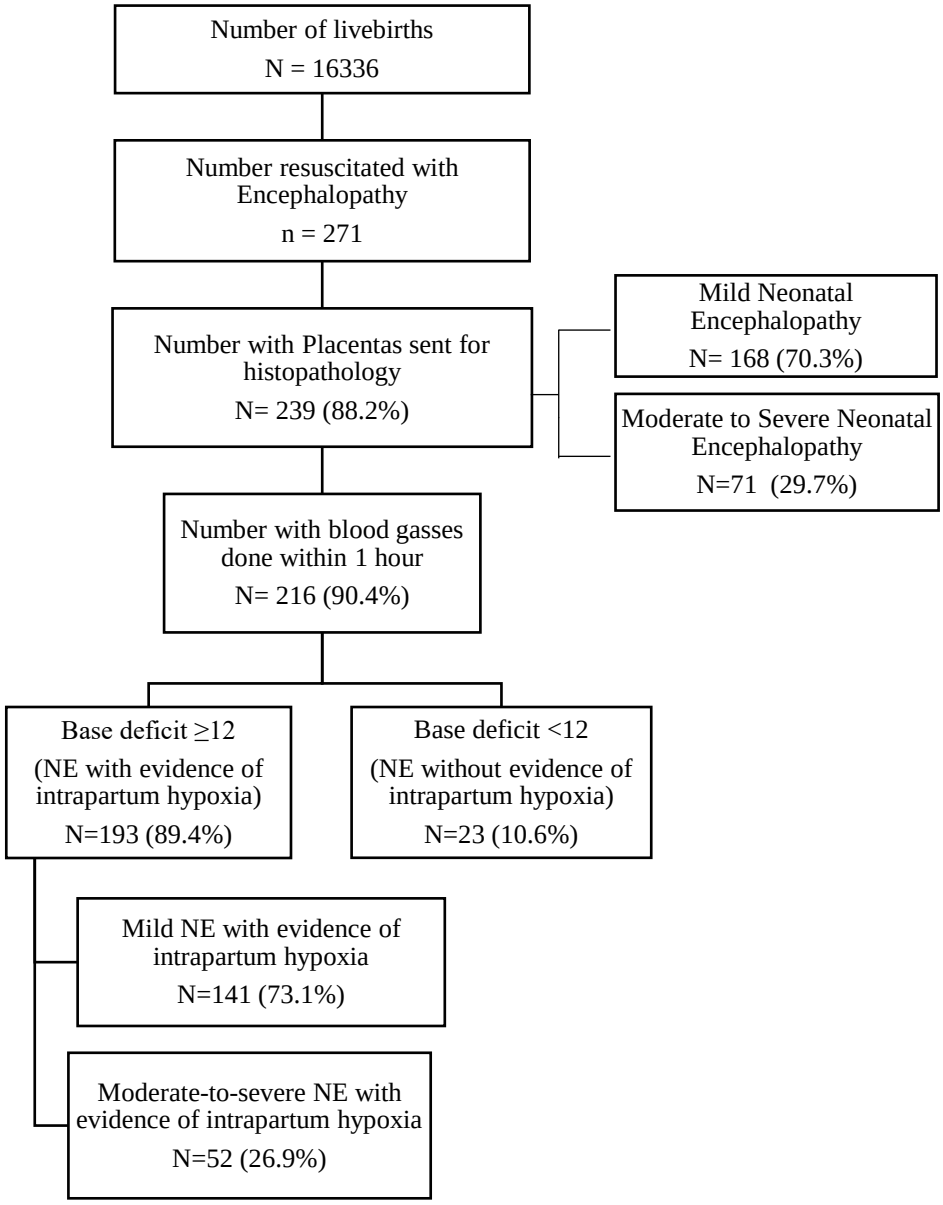


Figure 5. Flow chart of numbers of neonates with encephalopathy enrolled in the study

Maternal Characteristics

Table 1 shows the median maternal age was 23 years, with 56.7% of mothers being primigravidae. Most of the mothers (95.4%) had attended antenatal care, with 58.1% of them attending before 20 weeks of gestation. A quarter (24.8%) of mothers were positive for HIV. Among the mothers who had co-morbidities, the most common diagnosis was hypertensive disorders (32.2%), followed by urinary tract infections (26.9%). There were 103 (44.2%) pathological CTGs of those monitored. Meconium-stained amniotic fluid was observed in 40.3% of births and the caesarean section rate was 36.1%.

Table 1. Maternal age, clinical and delivery characteristics

Variable	**n / N (%)**
Maternal age, in years	23 (20-29)*
Primigravida	135/238 (56.7)
Attended antenatal care	227/238 (95.4)
Human Immunodeficiency virus, positive	59/238 (24.8)
Hypertensive disorders	77/238 (32.2)
Diabetes mellitus	13 (5.5)
Hypertensive and diabetes mellitus	10 (4.2)
Urinary tract infection	64/238 (26.9)
Pathological cardiotocograph	103/233(44.2)
Meconium-stained amniotic fluid	96/238 (40.3)
Mode of delivery, Caesarean section	86/238 (36.1)

*Median with interquartile ranges

Neonatal Characteristics

The median birth weight and gestational age were 3100g and 39 weeks, respectively. All neonates required a modality of resuscitation, with the majority (75.2%) responding to bag mask ventilation (BMV) only, 18.9% required chest compressions, and 5.9% required chest compressions and intravenous adrenaline (**Table 2**). Apgar score at 10 minutes was <7 in 31.5% and <5 in 8.8% of neonates. Among the 216 neonates who had arterial blood gas performed within the first hour of birth, the median pH and base deficit were 7.11 and 17.4 mmol/l, respectively, with 20.1% having a pH<7.00 and 89.4% with a base deficit of ≥12 mmol/l.

Therapeutic hypothermia was offered to 45.2% of the neonates. Overall, the mortality rate at hospital discharge was 2.9%.

Table 2. Neonatal characteristics, including management and outcome at hospital discharge

Variable	**n/N (%)**
Birth Weight, in grams	3100 (2800-3400)*
Gestational age, in weeks	39 (39-40)*
Sex, male	153/239 (64.0)
Apgar score at 10 minutes***	
<7	75/238 (31.5)
<5	21/238 (8.8)
Type of resuscitation required	
BMV only	179 (75.2)
BMV with chest compressions	45 (18.9)
BMV with chest compressions and adrenaline	14 (5.9)
Arterial blood gas within 1 hour of life**	
Blood gas pH*	7.11 (7.01-7.19)*
pH<7	48/216 (20.1)
Base deficit, in mmol /L	17.4 (13.9-21.0)*
Base deficit ≥12 mmol	193/216 (89.3)
All Neonates with Encephalopathy	239
Mild	168 (70.3)
Moderate to Severe	71 (29.7)
Encephalopathy without intrapartum hypoxia (base deficit <12 mmol/L)	23/216 (10.6)
Encephalopathy with intrapartum hypoxia (base deficit ≥12 mmol/L)	193/216 (89.4)
Mild	141/193 (73.1)
Moderate to Severe	52/193 (26.9)
Seizures	56/239 (23.4)
Thompson score >7	237/239 (99.2)
Managed with induced hypothermia (Cooling)	108/239 (45.2)
Number died	7/239 (2.9)

* - Median with interquartile ranges
**- 23 had blood gases missing
***- 1 neonates had no Apgar scores recorded
BMV – Bag mask ventilation
#There was one set of dichorionic twins, neonates n=239

Placental Histology

Reports for histopathology were available for 239 of the 271 (88.2%) neonates diagnosed with NE. The median placental weight was 428 grams, with 111 (46.6%) placentas being less than the 10th centile (**Table 3**). Cord abnormalities were present in 27.2% of placentas. These included hypercoiled umbilical cords, abnormal insertions such as marginal cord insertions and velamentous cord insertions. Amongst the neonates with NE with available placental pathology, 98.3% (235/239) had significant microscopic lesions on histopathology. The common microscopic placental lesions observed were maternal vascular malperfusion (MVM) (55.6%), fetal vascular malperfusion (FVM) (53.1%), chorioamnionitis (41.4%), villitis of unknown etiology (VUE) (28.9%), abruptio placentae (11.7%) and delayed villous maturation (11.7%) The remaining 4 placentas (2.8%) with no identified microscopic lesions had macroscopic pathology such as cord abnormalities, abnormally sized placentas (larger or smaller than gestational age) as well as non-specific features of intrauterine compromise.

Table 3. Placental characteristics from neonates with encephalopathy

Placental characteristics of all neonates	**N=239** **n (%)**
Placentas with microscopic histopathology	235 (98.3)
Placental weight, in grams	428 (360-490)*
Placental weight < 10th centile	111 (46.6)
Cord abnormalities	65 (27.2)
Chorioamnionitis present	**99 (41.4)**
Maternal & fetal response	61 (61.6)
Maternal response only	38 (38.4)
Fetal vascular malperfusion (FVM)	**127 (53.1)**
Low grade	63 (49.6)
High grade	64 (50.4)
Maternal vascular malperfusion (MVM)	**133 (55.6)**
Villitis of unknown etiology (VUE)	**69 (28.9)**
Low grade	39 (56.5)
High grade	30 (43.5)
Abruptio placenta	**28 (11.7)**
Delayed villous maturation	28 (11.7)
Meconium associated vascular necrosis	6 (2.5)
Intrauterine compromise	75 (31.4)

*- Median with interquartile ranges

Comparing the number of microscopic placental lesions according to the type and severity of neonatal encephalopathy

As shown in Table 4, there was a marginally significant difference in the proportion of placentas with ≥2 microscopic lesions in NE with IH compared to the group without IH (p=0.084). There were no significant differences in the proportion of placentas with ≥2 lesions with severity of NE, for all those with any NE (mild versus moderate-to-severe NE, p=0.248) and for those with NE with IH (mild versus moderate-to-severe NE, p=0.401).

Table 4. Number of placental lesions in comparison to the severity and type of encephalopathy

Number of placental lesions	**Severity of encephalopathy and/or presence of intrapartum hypoxia**		***p-value***
	Mild Encephalopathy N = 168 n (%)	***Moderate to Severe Encephalopathy N = 71 n (%)***	
One	51 (30.6)	27 (38.0)	0.248
Two or more	117 (69.4)	44 (62.0)	
	Encephalopathy with Intrapartum Hypoxia N= 193 n (%)	***Encephalopathy without Intrapartum Hypoxia N= 23 n (%)***	
One	58 (30.1)	11 (47.8)	0.084
Two or more	135 (69.9)	12 (52.2)	
	Mild Encephalopathy with Intrapartum Hypoxia N = 141 n (%)	***Moderate to Severe Encephalopathy with Intrapartum Hypoxia N = 52 n (%)***	
One	40 (28.4)	18 (34.6)	0.401
Two or more	101 (71.6)	34 (65.4)	

Comparison of maternal and neonatal characteristics, and placental histopathology between neonates with mild and moderate-to-severe encephalopathy

In both univariate and multivariate analysis, the factors that were associated with moderate to severe encephalopathy were Apgar score <7 at 10 minutes (p <0.001) and pH< 7.00 (p=0.001) **(Table 5).** There was no association with placental histopathology and severity thereof (p-values >0.05)

Table 5. Comparing maternal and neonatal characteristics, and placental pathology between mild and moderate-to-severe encephalopathy (all neonates)

Variable	Total n=239 n (%)	Mild Encephalopathy n=168 n (%)	Moderate to severe Encephalopathy n=71 n (%)	Univariate analysis OR (95% CI)	p-value	Multivariate analysis OR (95% CI)	p-value
Maternal age*	23 (20-29)	23 (20-29)	23 (20-29)	0.994 (0.95-1.03)	0.811		
Primigravida	135 (56.7)	92 (54.8)	43 (61.4)	0.788 (0.45-1.38)	0.409		
HIV positive	59 (24.8)	43 (25.5)	16 (22.9)	0.845 (0.44-1.63)	0.616		
Hypertension	77 (32.3)	49 (29.2)	28 (40.0)	0.632 (0.35-1.13)	0.122	0.581 (0.29-1.15)	0.121
Antenatal care attended	227 (95.4)	160 (95.2)	67 (95.7)	0.882 (0.23-3.43)	0.857		
Mode of delivery (vaginal)	107 (45.0)	75 (44.6)	32 (45.7)	1.013 (0.57-1.80)	0.964		
Pathological Cardiotocograph**	103/233 (44.0)	68/164 (41.5)	35/69 (50.7)	0.708 (0.40-1.24)	0.229	0.940 (0.48-1.82)	0.854
Birth weight*	3100 (2800-3400)	3052 (2785-3400)	3200 (2800-3400)	1.187 (0.50-2.81)	0.697		
Gestational age*	39 (39-40)	39 (39-40)	39 (38-40)	1.094 (0.88-1.36)	0.415		
Male	153 (64.0)	109 (64.9)	44 (62.9)	1.133 (0.64-2.01)	0.669		
Apgar score <7 at 10 min***	75/238 (31.5)	33/168 (19.6)	42/70 (60.0)	6.14 (3.3-11.3)	**<0.001**	4.65 (2.32-9.29)	**<0.001**
pH<7	48/228 (21.1)	19/161 (8.3)	29/67 (43.3)	5.70 (2.89-11.26)	**<0.001**	3.61 (1.70-7.66)	**0.001**
Base deficit*	17.4 (13.9-21.0)	16.7 (13.9-20.1)	19.3 (14.7-22.7)	0.957 (0.36-2.56)	0.931		
Chorioamnionitis	99 (41.2)	70 (41.7)	29 (40.8)	1.034 (0.59-1.82)	0.906	1.356 (0.68-2.69)	0.385
Maternal vascular malperfusion	133 (55.6)	92 (54.8)	41 (57.7)	0.886 (0.51-1.55)	0.671	0.657 (0.33-1.31)	0.234
Fetal vascular malperfusion	127 (53.1)	88 (52.4)	39 (54.9)	0.902 (0.52-1.56)	0.718	0.919 (0.46-1.82)	0.808
Villits of unknown aetiology	69 (28.9)	50 (29.8)	19 (26.8)	1.159 (0.62-2.16)	0.640	1.236 (0.59-2.61)	0.578
Placental lesions ≥ 2	161 (67.4)	117 (69.6)	44 (62.0)	0.710 (0.39-1.27)	0.249		

*- Median with interquartile range

**- 5 had no recorded CTG results

***-1 neonate had no Apgar score results

Comparison of maternal and neonatal characteristics, and placental pathology between neonatal encephalopathy with and without evidence of intrapartum hypoxia

On multivariate analysis the odds of neonates to have NE with intrapartum hypoxia were nearly thirteen-fold higher if the mother attended antenatal care (OR-12.91; 95% CI 2.08-80.0) and three-fold greater if the placental lesion was MVM (OR- 3.04 (1.04-8.88); p = 0.042) compared to those with NE without intrapartum hypoxia (**Table 6**).

Table 6. Comparing maternal and neonatal characteristics, placental pathology between encephalopathy with and without intrapartum hypoxia

Variable	Total N=216 n (%)	Encephalopathy with intrapartum hypoxia N=193 n (%)	Encephalopathy without intrapartum hypoxia N=23 n (%)	Univariate analysis OR (95% CI)	p-value	Multivariate analysis OR (95% CI)	p-value
Maternal age*	23 (20-29)	23 (20-29)	22 (20-29)	1.03 (0.96-1.09)	0.466		
Primigravida	121 (56.3)	108 (56.3)	13 (56.5)	0.97(0.41-2.34)	0.959		
HIV positive	55 (25.6)	49 (25.4)	6 (26.1)	1.03 (0.38-2.78)	0.942		
Hypertension	72 (33.5)	64 (33.3)	8 (34.8)	0.93 (0.37-2.31)	0.876		
Attended antenatal care	209 (95.4)	185 (96.4)	20 (87.0)	4.38 (1.04-18.42)	**0.044**	**12.91 (2.08-80.0)**	**0.006**
Mode of delivery (vaginal)	99 (45.8)	87 (45.1)	2 (52.2)	1.1 (0.46-2.74)	0.788		
Pathological cardiotocograph	91/210 (43.3)	83/193 (48.2)	8/2334.8)	1.48 (0.59-3.66)	0.394	1.48 (0.56-3.94)	0.430
Median Gestational age	39 (39-40)	39 (39-40)	39 (38-40)	1.11 (0.79-1.56)	0.535		
Birth weight	3100 (2765-3400)	3100 (2300-3400)	3000 (2360-3275)	0.99 (0.27-3.58)	0.990		
Male	139 (64.4)	121 (62.7)	18/23 (78.3)	0.47 (0.16-1.31)	**0.148**	0.53 (0.18-1.57)	0.254
Apgar score <7 at 10 min	64/215 (29.8)	58/192 (30.2)	6/23 (26.1)	1.23 (0.46-3.27)	0.683		
Thompson score	9 (8-11)	9 (8-11)	9 (8-11)	0.96 (0.36-2.56)	0.931		
Chorioamnionitis	92 (42.6)	84 (43.5)	8 (34.5)	1.44 (0.59-3.57)	0.425	1.62 (0.54-4.83)	0.387
Maternal vascular malperfusion	121 (56.0)	112 (58.0)	9 (39.1)	2.15 (0.89-5.21)	**0.090**	3.04 (1.04-8.88)	**0.042**
Fetal vascular malperfusion	113 (52.3)	104 (53.9)	9 (39.1)	1.81 (0.75-4.39)	**0.185**	1.85 (0.62-5.49)	0.268
Villitis of unknown aetiology	65 (30.1)	60 (31.1)	5 (21.7)	1.62 (0.57-4.58)	0.359	1.97 (0.56-6.92)	0.288
Placental lesions ≥ 2	147 (68.1)	135 (70.0)	12 (52.1)	2.13 (0.89-5.11)	**0.089**	1.00 (0.29-3.42)	0.996

*Median with interquartile ranges

Comparison of maternal and neonatal characteristics, and placental pathology between neonates with mild and moderate-to-severe neonatal encephalopathy with intrapartum hypoxia.

On univariate analysis, factors associated with moderate to severe NE with IH were maternal hypertension (p=0.021), Apgar score <7 at 10 minutes (p<0.001), and pH <7.00 (p<0.001). On multivariate analysis, the risk of having moderate to severe NE with IH was almost 4-fold greater if the Apgar score was <7 at 10 minutes, and 3-fold greater if the pH was <7.00 and 2-fold higher if the mother had hypertension (**Table 7**).

Table 7. Comparing maternal and neonatal characteristics, and placental pathology between mild and moderate-to-severe encephalopathy with intrapartum hypoxia

Variable	Total N=193 n (%)	Mild NE with intrapartum hypoxia n=141 n (%)	Moderate to severe NE with intrapartum hypoxia n= 52 n (%)	Univariate analysis OR (95% CI)	p-value	Multivariate analysis OR (95% CI)	p-value
Median Maternal age	23 (20-29)	23 (20-29)	23 (20-29)	1.00 (0.95-1.05)	0.977		
Primigravida	108 (56.3)	77 (55.0)	31 (59.6)	0.82 (0.43-1.55)	0.535		
HIV positive	49 (25.5)	37 (26.2)	12 (23.5)	0.84 (0.399-1.78)	0.654		
Hypertension	64 (33.3)	40 (28.4)	24 (47.1)	0.46 (024-0.89)	**0.021**	2.09 (1.01-4.32)	**0.047**
Attended antenatal care	185 (96.4)	135 (95.7)	50 (98.0)	0.44 (0.05-3.75)	0.454		
Mode of delivery (vaginal)	87 (45.3)	61 (43.6)	26 (50.0)	1.01 (0.52-1.97)	0.962		
Pathological cardiotocograph	83/193 (43.0)	58 (41.1)	25 (48.1)	0.76 (0.40-1.45)	0.412	1.04 (0.51-2.14)	0.908
Median Birth weight*	3100 (2800-3400)	3100 (2800-3100)	3110 (2750-3400)	0.94 (0.37-2.40)	0.898		
Median Gestational age*	39 (39-40)	39 (39-40)	39 (39-40)	1.07 (0.83-1.39)	0.596		
Male	121 (63)	87 (61.7)	34 (65.4)	0.85 (0.44-1.66)	0.639		
Apgar score <7 at 10 min	58 (30.1)	30 (21.3)	28 (53.8)	4.50 (2.27-8.92)	**<0.01**	3.81 (1.77-8.20)	**0.001**
pH <7.00	38 (19.7)	18 (12.8)	20 (38.5)	4.27 (2.02-9.00)	**<0.01**	2.92 (1.28-6.66)	**0.011**
Chorioamnionitis	84 (43.5)	60 (42.6)	24 (46.2)	0.86 (0.46-1.64)	0.655	1.09 (0.53-2.29)	0.802
Maternal vascular malperfusion	112 (58.0)	81 (57.4)	31 (59.6)	0.91 (0.48-1.75)	0.787	0.59 (0.27-1.25)	0.167
Fetal vascular perfusion	104 (53.9)	76 (53.9)	28 (53.8)	1.00 (0.53-1.89)	0.995	1.07 (0.51-2.25)	0.854
Villitis of unknown aetiology	60 (31.1)	43 (30.5)	17 (34.0)	0.90 (0.46-1.79)	0.771	0.93 (0.42-2.06)	0.863
Placental lesions ≥ 2	135 (70.0)	101 (71.6)	34 (65.4)	0.75 (0.38-1.47)	0.402		

*Median interquartile ranges

DISCUSSION

Placental histopathology is important in identifying the causes of various neonatal adverse outcomes. Its inclusion and analysis in supporting diagnosis is now recommended as part of the investigation of neonates with neonatal encephalopathy.[11] In this study, we assessed for macroscopic and microscopic abnormalities in placentas of neonates with NE in a LMIC setting. The main findings indicated that, on a macroscopic level, approximately half of the placentas weighed below the tenth percentile, and around a quarter exhibited abnormal cord abnormalities. Microscopically, nearly all placentas showed abnormalities during histopathological assessment. The most commonly identified placental lesions were maternal vascular malperfusion (MVM), fetal vascular malperfusion (FVM), chorioamnionitis, and villitis of unknown etiology (VUE). The types, severity, and quantity of these placental lesions did not change based on the severity of encephalopathy. Additionally, there was an independent association between the presence of MVM and neonatal encephalopathy (NE) with intrapartum hypoxia (IH). The proportion of neonates with NE with IH and two or more histopathologic lesions was marginally greater than those without IH (69.9% vs 52.2%, p=0.084) This "placental lesion multiplicity" finding has been associated as a risk factor in other neonatal conditions such as intrauterine growth restriction (IUGR) and abnormal cranial ultrasound findings.[15]

The proportion of placentas below the 10^{th} percentile was much higher than the neonates who were small for gestational age. The majority (87%) of the neonates weighed ≥2500g, with 30 neonates (12.6%) being small for gestational age (SGA). The type of SGA (symmetrical, or asymmetrical) was not documented. Of these SGA neonates, 23 (76.7%) had two or more significant microscopic lesions. This finding does correlate with previous studies that implicated multiple placental lesions with IUGR.[15] Most cases of neonatal encephalopathy (NE) occurred among mothers aged 21 to 35 years, which likely reflects the typical age distribution for women in childbearing years. However, no specific age group was associated with a higher incidence of NE.

Lack of adequate antenatal care has been shown to be a risk factor for the development of HIE.[16,17] A study conducted in Nigeria found mothers who had no antenatal care to have 3 times the risk of HIE compared those who received adequate antenatal care. [16] A case-control study also noted that lack of antenatal care appeared to be a significant risk factor for HIE (0R 2.1 95% CI 1.3-3, 3.2; p=0.002). [17] However, in our study, we found that mothers who received antenatal

care were nearly thirteen times more likely to have neonates with encephalopathy associated with intrapartum hypoxia (IH) compared to those who did not receive antenatal care. This finding may seem counterintuitive; it is possible that these mothers had high-risk pregnancies and an underlying predisposition to encephalopathy. It would have been helpful to know the total number of antenatal visits to better understand the potential underlying conditions.

The two chronic maternal conditions recognized to increase the risk of placental insufficiency or maternal vascular malperfusion (MVM), along with potential complications at the time of delivery, are hypertension and diabetes mellitus. A previous descriptive analysis conducted in South Africa found that the prevalence of hypertensive disorders among first-time (primigravid) women was 12.5%.[18] Another study by Yang et al. concluded that maternal hypertensive disorders carried a 2.4-fold risk of NE with hypoxia.[19] In our study population, hypertensive conditions were noted in 32.2% of the overall group. MVM was the most prevalent microscopic lesions occurring in more than 50% of the placentas. We also found that neonates born to mothers with hypertensive disorders had an increased risk of moderate to severe NE with IH. This may imply that hypertensive disorders predispose the placenta to vascular injury, even in the absence of perinatal hypoxia. An international consensus published in 1999 concluded that intrapartum hypoxia was unlikely to be the cause of cerebral palsy in more than 90% of affected children.[20]

Factors associated with moderate to severe NE were Apgar score <7 at 10 minutes and pH <7.00. There were no significant differences in the placental histopathology in the different groups and severity of encephalopathy. However, it was clear that certain microscopic lesions were more common in moderate to severe NE, as seen in previous studies.[10, 19] The most significant microscopic lesions noted in all groups were MVM and FVM. Additionally, MVM was associated with a 3 times greater risk of developing encephalopathy with IH, on the multivariate model after adjusting for other confounding factors such as FVM, VUE, ≥ 2 placental lesions, and chorioamnionitis. MVM is diagnosed with a constellation of findings, and a minimum of three macroscopic and/or microscopic items are required to confirm the diagnosis.

MVM has been implicated in studies in neonates with HIE/NE, either alone or more frequently in combination with other pathologies such as FVM or VUE. [22] All three of these pathological entities may contribute to a placenta that is small, further compromising the ability of fetus in its

ability to withstand the hypoxic stress of labour. This chronic intrauterine injury primes the fetal brain for additional injury in the immediate perinatal period, potentially leading to poor neurological outcomes, and this may be identified using early brain imaging.[23,24] Although our cohort did not show any association between the severity of NE and cord abnormalities, just over a quarter of placentas had cord abnormalities. These included hypercoiled umbilical cords, as well as abnormalities of cord insertion such as marginal and velamentous cord insertions. These may cause intermittent or partial umbilical cord compression and obstruction of fetal blood flow. Although this may be associated with FVM, the microscopic lesion may not be identified due to sampling. FVM has been seen in 20-30 % of cases of presumed HIE.[21] In the study of Mir et al. FVM was the only individual predictor of adverse neurological outcomes.[10] This study also suggested that when present in the placenta, FVM may predict resistance to head cooling. Our cohort did not show any association of FVM with the severity of NE or the presence of NE with intrapartum hypoxia. Microscopically, FVM is seen as extensive avascular villi, vascular obstructive lesions, and vascular thrombi, and can often be accompanied by umbilical cord abnormalities. Acute chorioamnionitis or involvement of the chorionic membranes is a maternal response to ascending infection or cell death, while funisitis or chorionic vasculitis indicates a fetal inflammatory response (FIR), causing release of cytokines and chemokines into the fetal blood. It is an acute lesion in most cases, present for 12-48 hours prior to delivery. Chorioamnionitis is a common pathology in the placenta, and may be present in 21-24% of placentas delivered between 21 to 24 weeks.[25] Chorioamnionitis was the third most common lesion (41.2%) in our study, with maternal and fetal responses being more prevalent. There was no association drawn between the occurrence of chorioamnionitis and the type or severity of NE.

Villitis of unknown aetiology (VUE) is a diagnosis made after infectious causes have reasonably been ruled out, occurs predominantly in the third trimester, and is dependent on placental sampling. It is thought to be a graft-vs-host immune rejection phenomenon and is associated, amongst others, with NE and neonatal seizures.[26] Of the microscopic lesions in this study, VUE accounted for 28.9%. Though it was not statistically significant, it carries important clinical implications. It is a chronic lesion, present for at least one week prior to delivery of the placenta.[27] An increased frequency of basal ganglia and thalamic injury has been found with VUE compared to other placental lesions.[21] Additionally, some studies have made an association to resistance to therapeutic hypothermia to VUE.[10]

Neonatal encephalopathy (NE) is linked to both morbidity and mortality, with more severe cases predicting worse outcomes. [4] This condition is a significant and prevalent issue in middle- and low-income countries. Parameters that were significantly associated with the severity of NE were intuitive: lower Apgar scores at 5 and 10 minutes, level of resuscitation required, lower blood pH, occurrence of seizures, and the Thompson score, all predicted the severity of NE. As these are well-described criteria for, and associations with NE, and are expected findings. [28] Deaths occurred only in those categorized as moderate-severe NE. While the factors associated with NE are known and can be targeted for intervention, many cases occur at birth without any warning. Limited resources are a major barrier to addressing these modifiable factors. This study shows that sending placentas for histopathological examination in all newborns with encephalopathy is feasible. Additionally, examining placental pathology can play a crucial role in identifying the factors that contribute to the development of NE.

The strength of this study is that we reviewed a large number of placentas from neonates with NE. The diagnosis of encephalopathy was based on the need for resuscitation and presence of signs of encephalopathy, and differentiated those with encephalopathy with or without evidence of intrapartum hypoxia based on an arterial blood gas taken within an hour of birth. The placentas were reviewed, and standard evaluation was used to determine the pathology.

The main limitation of this study was that there were no controls or placentas from neonates who did not have NE and were healthy, therefore unable to assess if the prevalence of histopathological lesions observed in this study was out of proportion to those who are healthy. The lack of association between placental pathology and severity of NE could be due to a relatively small sample size to confidently draw a comparison between the mild and moderate-to- severe NE as well as the IH group in this study. Logistic regression model could possibly have different outcomes of the placental lesions if the sample was of the same size for both mild and moderate- to-severe NE and severity of IH. Additionally, it was also a single-center study; therefore, unable to generalize findings from this study.

Previous studies have looked at the frequency and type of histopathologic lesions in the placentas delivered by women with no adverse pregnancy outcomes. Most placentas from these seemingly normal pregnancies had lesions consistent with inflammatory or vascular lesions.[29] This finding

warrants a follow-up case-control study to our current research. The inclusion of a control group would assist in determining whether it is indeed worthwhile to continue evaluating placental histopathology in our setting and may lead to new implementations of managing at risk pregnancies to prevent adverse outcomes.

CONCLUSION

Placental histopathology with features of MVM is associated with NE and intrapartum hypoxia. Furthermore, most placentas from neonates with NE exhibited abnormalities, indicating that placental pathology may play a significant role in the development of NE. This study underscores the significance of placental histopathological evaluation for all births that required neonatal resuscitation, as this may assist in determining whether neonatal encephalopathy (NE) is attributed to inadequate perinatal care or to underlying placental pathology. We recommend that all neonates should have placentas sent for histopathologic evaluation at birth. Due to the potential financial burden this may impose, this may not be possible, it would be most beneficial to at least send the placentas for neonates who required resuscitation at birth. Future research on the association between placental histopathology and NE should consider conducting case-control studies, as this could help clarify the role of placental histopathology in the development of NE.

REFERENCES

1. Lawn JE, Cousens S, Zupan J. 4 million neonatal deaths: when? Where? Why? Lancet 2005;365(9462):891–900. https://doi.org/10.1016/S0140-6736(05)71048-5

2. Lee ACC, Kozuki N, Blencowe H, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res 2013;74 Suppl 1(Suppl 1):50–72. https://doi.org/10.1038/pr.2013.206

3. Kurinczuk JJ, White-Koning M, Badawi N. Epidemiology of neonatal encephalopathy and hypoxic-ischaemic encephalopathy. Early Hum Dev 2010;86(6):329–338. https://doi.org/10.1016/j.earlhumdev.2010.05.010

4. Bruckmann EK, Velaphi S. Intrapartum asphyxia and hypoxic ischaemic encephalopathy in a public hospital: Incidence and predictors of poor outcome. S Afr Med J 2015;105(4):298. https://doi.org/10.7196/SAMJ.9140

5. Ballot, D.E., Padayachee, N. Outcomes of neonates with perinatal asphyxia at a tertiary hospital in South africa, Johannesburg. South African Journal of Child Health 2013;7(3):87–94. https://doi.org/doi.org/10.7196/sajch.574

6. Horn AR, Swingler GH, Myer L, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. J Perinat Med 2013;41(2):211–217. https://doi.org/10.1515/jpm-2012-0107

7. Vik T, Redline R, Nelson KB, et al. The Placenta in Neonatal Encephalopathy: A Case-Control Study. J Pediatr 2018;202:77-85.e3. https://doi.org/10.1016/j.jpeds.2018.06.005

8. Khong TY, Mooney EE, Ariel I, et al. Sampling and Definitions of Placental Lesions: Amsterdam Placental Workshop Group Consensus Statement. Archives of Pathology & Laboratory Medicine 2016;140(7):698–713. https://doi.org/10.5858/arpa.2015-0225-CC

9. Nelson KB, Penn AA. Is infection a factor in neonatal encephalopathy? Arch Dis Child Fetal Neonatal Ed 2015;100(1):F8–F10. https://doi.org/10.1136/archdischild-2014-306192

10. Mir IN, Johnson-Welch SF, Nelson DB, Brown LS, Rosenfeld CR, Chalak LF. Placental pathology is associated with severity of neonatal encephalopathy and adverse developmental outcomes following hypothermia. Am J Obstet Gynecol 2015;213(6):849.e1–7. https://doi.org/10.1016/j.ajog.2015.09.072

11. Bhorat I, Buchmann E, Soma-Pillay P, Nicolaou E, Pistorius L, Smuts I. Cerebral Palsy and Criteria Implicating Acute Intrapartum Hypoxia in Neonatal Encephalopathy - An Obstetric Perspective for the South African Setting. S Afr Med J 2021;111(3b):277–279. https://doi.org/10.7196/SAMJ.2021.v111i3b.14923

12. O'Heney J, McAllister S, Maresh M, Blott M. Fetal monitoring in labour: summary and update of NICE guidance. BMJ 2022;379:o2854. https://doi.org/10.1136/bmj.o2854

13. Thompson CM, Puterman AS, Linley LL, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr 1997;86(7):757–761. https://doi.org/10.1111/j.1651-2227.1997.tb08581.x

14. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol 1976;33(10):696–705. https://doi.org/10.1001/archneur.1976.00500100030012

15. Viscardi RM, Sun CC. Placental lesion multiplicity: risk factor for IUGR and neonatal cranial ultrasound abnormalities. Early Hum Dev 2001;62(1):1–10. https://doi.org/10.1016/s0378-3782(01)00114-1

16. Owolabi AT, Fatusi AO, Kuti O, Adeyemi A, Faturoti SO, Obiajuwa PO. Maternal complications and perinatal outcomes in booked and unbooked Nigerian mothers. Singapore Med J 2008;49(7):526–531.

17. Butt TK, Farooqui R, Khan MAU. Risk factors for hypoxic ischemic encephalopathy in children. J Coll Physicians Surg Pak 2008;18(7):428–432.

18. Moodley J, Onyangunga OA, Maharaj NR. Hypertensive disorders in primigravid black South African women: A one-year descriptive analysis. Hypertens Pregnancy 2016;35(4):529–535. https://doi.org/10.1080/10641955.2016.1193190

19. Yang W, Wang L, Tian T, et al. Maternal hypertensive disorders in pregnancy and risk of hypoxic-ischemia encephalopathy. J Matern Fetal Neonatal Med 2021;34(11):1754–1762. https://doi.org/10.1080/14767058.2019.1647529

20. MacLennan A. A template for defining a causal relation between acute intrapartum events and cerebral palsy: international consensus statement. BMJ 1999;319(7216):1054–1059. https://doi.org/10.1136/bmj.319.7216.1054

21. Harteman JC, Nikkels PGJ, Benders MJNL, Kwee A, Groenendaal F, de Vries LS. Placental pathology in full-term infants with hypoxic-ischemic neonatal encephalopathy and association with magnetic resonance imaging pattern of brain injury. J Pediatr 2013;163(4):968-995.e2. https://doi.org/10.1016/j.jpeds.2013.06.010

22. Bingham A, Gundogan F, Rand K, Laptook AR. Placental findings among newborns with hypoxic ischemic encephalopathy. J Perinatol 2019;39(4):563–570. https://doi.org/10.1038/s41372-019-0334-9

23. Hagberg B, Hagberg G, Beckung E, Uvebrant P. Changing panorama of cerebral palsy in Sweden. VIII. Prevalence and origin in the birth year period 1991-94. Acta Paediatr 2001;90(3):271–277.

24. Cowan F, Rutherford M, Groenendaal F, et al. Origin and timing of brain lesions in term infants with neonatal encephalopathy. Lancet 2003;361(9359):736–742. https://doi.org/10.1016/S0140-6736(03)12658-X

25. Redline RW, O'Riordan MA. Placental lesions associated with cerebral palsy and neurologic impairment following term birth. Arch Pathol Lab Med 2000;124(12):1785–1791. https://doi.org/10.5858/2000-124-1785-PLAWCP

26. Nowak C, Joubert M, Jossic F, et al. Perinatal prognosis of pregnancies complicated by placental chronic villitis or intervillositis of unknown etiology and combined lesions: About a series of 178 cases. Placenta 2016;44:104–108. https://doi.org/10.1016/j.placenta.2016.04.017

27. Redline RW. Placental pathology: Pathways leading to or associated with perinatal brain injury in experimental neurology, special issue: Placental mediated mechanisms of perinatal brain injury. Exp Neurol 2022;347:113917. https://doi.org/10.1016/j.expneurol.2021.113917

28. Executive summary: Neonatal encephalopathy and neurologic outcome, second edition. Report of the American College of Obstetricians and Gynecologists' Task Force on Neonatal Encephalopathy. Obstet Gynecol 2014;123(4):896–901. https://doi.org/10.1097/01.AOG.0000445580.65983.d2

29. Romero R, Kim YM, Pacora P, et al. The frequency and type of placental histologic lesions in term pregnancies with normal outcome. J Perinat Med 2018;46(6):613–630. https://doi.org/10.1515/jpm-2018-0055

APPENDICES

APPENDIX A: STUDY PROTOCOL

PLACENTAL PATHOLOGY IN NEONATES DIAGNOSED WITH ENCEPHALOPATHY SOON AFTER BIRTH: A RETROSPECTIVE ANALYTIC STUDY

Researcher:

Keaoleboga Lorraine Sebolai

Student number: 2531002

MBChB (Stell), DCH (SA)

Supervisors:

Dr Omphile Mekgoe

Head of Paediatrics: Klerksdorp/Tshepong hospital complex

MBChB (UKZN), FC Paed (SA), HIV DIP (SA)

Professor Sithembiso Velaphi

Head of Paediatrics: Chris Hani Baragwanath Academic Paediatrics

MBChB (UKZN), FC Paed (SA), MMed (Wits), PhD (Wits)

Professor Colleen Wright

Principal Pathologist, Anatomical Pathology, NHLS, MBBCh (Wits) MMed, FC Path, FRCPath, FIACem, PhD

INTRODUCTION

The United Nations Children's Fund estimated the neonatal mortality rate at 18 per 1000 live births in 2019, with approximately 6,700 neonatal deaths occurring every day.[1] Perinatal asphyxia was identified to be a significant cause of neonatal mortality, ranging between 34% and 40% in Sub-Saharan Africa and South Asia, respectively.[2] It is reported that up to three-quarters of neonates with neonatal encephalopathy develop long-term neurodevelopmental disabilities.[3] Efforts in reducing neonatal deaths and the number of children with disabilities should include determining factors associated with the development of neonatal encephalopathy.

Neonatal encephalopathy is a clinically defined disorder characterized by impaired neurologic function in neonates born at or near term. It is characterized by the altered level of consciousness, seizures, inability to initiate and maintain adequate respiration, and may be associated with multi-organ dysfunction. There are various risk factors associated with neonatal encephalopathy. These factors may be maternal, fetal or placental related. Intrapartum factors play a much more significant role in contributing to encephalopathy than antepartum factors [4]. Metabolic and genetic factors have also been identified, even though there is limited data linking the latter to predisposition to neonatal encephalopathy.

Causes and epidemiology of neonatal encephalopathy

Causes of neonatal encephalopathy are classified as hypoxic and non-hypoxic. Further investigations are often deemed necessary to identify or exclude non-hypoxic ischemic causes of neonatal encephalopathy. Non-hypoxic causes of neonatal encephalopathy include, but are not limited to, infections, trauma, inborn errors of metabolism, and genetic and chromosomal disorders. Hypoxic causes often have perinatal antecedents, symptomatology, and clinical course that are consistent with intrapartum hypoxia. Hypoxic ischemic injury is a major cause of neonatal encephalopathy and is responsible for twenty-five percent of the neonatal mortality rate globally and is among the top five leading causes of under-5-year mortality rate worldwide, [5, 6]

Low- and middle-income countries have a higher prevalence of neonatal hypoxic encephalopathy due to resource constraints. The incidence of neonatal hypoxic encephalopathy is reported as 1.5 per 1000 live births in high-income countries as compared to an alarming 14.9 per 1000 live births in Sub-Saharan Africa.[7] In the South African context, the incidence rates have been reported to range from 2.3 to 13.3 per 1000 live births.[8-10]. The outcomes of infants who had suffered

intrapartum asphyxia have remained relatively unchanged over the years despite the introduction of therapeutic hypothermia. Previously, Sarnat and Sarnat noted that 25% of newborns with moderate encephalopathy and 100% of newborns with severe encephalopathy had severe neurodevelopmental outcomes or were dead at 1 year of age.[11] Thompson score, which was described in 1997, reports that a score ≤10 assures a normal neurological outcome at 1 year of age and that 65% of infants with a score of >10, and 92% of those with a score >15, will have abnormal neurological outcomes at 1 year of age.[12]. Ballot et al concluded that perinatal asphyxia may be a significant cause of cerebral palsy in sub-Saharan Africa, with estimates of 15.5% of cases of cerebral palsy being due to neonatal encephalopathy.[13]

Risk factors associated with neonatal encephalopathy

Maternal factors

Maternal factors associated with encephalopathy include chronic diseases, infections and teratogenic drugs. A recently published review identified that maternal hypertension was an important antenatal risk factor for neonatal encephalopathy.[14] Other risk factors include advanced maternal age, lack of antenatal care, increased body mass index, antepartum haemorrhage, medication and history of herbal use in pregnancy, smoking and pre-eclampsia.[15] Clinical chorioamnionitis has been reported to account for 40% of cases with neonatal encephalopathy.[14, 16]

Intrapartum factors

Inadequate fetal monitoring can lead to adverse outcomes. Sentinel events such as placental abruption, shoulder dystocia, and cord prolapse predispose to the development of hypoxic-ischaemic encephalopathy. A case–control study conducted by Burrois et al. has shown that induction of labour and emergency caesarean sections are more likely to be associated with hypoxic-ischaemic encephalopathy.[15] In low-income countries, where there is poor intrapartum care, the proportion of neonates with encephalopathy-related complications is significantly higher than in well-resourced settings.

Placental factors

The placenta plays an important role and is critical for the healthy development of the fetus. It forms a barrier to toxins and infective organisms. Information on placental lesions may be

beneficial in neonatal care and can assist in explaining an abnormal neonatal outcome.[17] Placental pathology has been associated with increased risk for the development of neonatal encephalopathy.[18] It has been shown that patients with features of neonatal encephalopathy often have excessively lighter or heavier than those of the same gestational age who do not have neonatal encephalopathy.[19] A prospective cohort study performed in 2010, looking at placentas of term newborns with hypoxic-ischemic encephalopathy, reported that 87% had abnormal placental findings.[20] Another study reported incidences of 75 to 83% in neonates with encephalopathy having abnormal placental changes.[21] Placental pathologies are often identified in neonatal encephalopathy as a combination of acute and chronic placental lesions.[22] It is important that placental lesions are well defined and classified. According to the 2016 Amsterdam consensus document, placental lesions associated with neonatal encephalopathy can be classified into the four major lesion categories: fetal vascular malperfusion (FVM) maternal vascular malperfusion (MVM), acute chorioamnionitis (ACA), and chronic villitis.[23]

Fetal vascular malperfusion

Perinatal asphyxia has been most associated with placental lesions affecting fetal vascular supply. These lesions are umbilical cord complications (cord tear, hypercoiled cord and cord hematoma), chorioamnionitis with fetal vasculitis, and fetal thrombotic vasculopathy. One of the most common placental lesions recorded is the fetal thrombotic vasculopathy which signifies a chronic thrombotic process within the fetal vasculature.[14, 20, 24] Fetal vascular malperfusion could prime the infant's brain for acute peripartum hypoxic-ischaemic injury.

Maternal vascular malperfusion

Maternal vascular malperfusion is the presence of both macroscopic and microscopic findings indicative of chronically compromised maternal blood flow through abnormal spiral arteries. [23] This leads to ischemic damage and reduced placental growth. Most lesions in the maternal vascular side can originate from early abnormalities in placental development caused by underlying abnormalities in the uterus and its vascular supply.[25] Maternal factors associated with abnormal vascular remodeling are linked to underlying vascular disease such as essential hypertension and diabetes.[26] Loss of integrity in the maternal circulation can lead to marked retroplacental hemorrhages such as abruptio placenta.

Inflammatory mechanisms

Acute chorioamnionitis is the most common inflammatory lesion caused by microorganisms gaining access to the placental membranes and amniotic fluid.[27]. Inflammatory cytokines and other circulating inflammatory mediators have been associated with an increased risk of neurological complications, including cerebral palsy.[28] Infection has been associated with perinatal hypoxia as a risk factor as well as a cause for poor prognosis and worsened brain injury.[29]

Pathogenesis of hypoxic-ischaemic encephalopathy

Hypoxic ischemic encephalopathy (HIE) is an encephalopathy seen after intrapartum asphyxia which is defined as a biochemical condition of impaired respiratory gaseous exchange leading to hypoxemia, hypercapnia and metabolic acidosis. The underlying pathology is due to impaired cerebral blood flow and oxygen delivery to the brain. The pathological events of hypoxic ischemic encephalopathy occur in two phases, namely primary and secondary energy failure. Primary energy failure is because of the initial reduction in cerebral blood flow. The impaired cerebral blood flow leads to decreases in oxygen and glucose supply, which leads to significantly less energy in the form of adenosine triphosphate (ATP) and increased lactate production. The low ATP levels cause failure of mechanisms that maintain cell integrity, particularly the sodium/potassium pumps and mechanisms to maintain low intracellular calcium. This leads to excessive influx of the positively charged sodium ions that precipitate massive depolarization of neurons, leading to the release of glutamate. The glutamate binds to glutamate receptors allowing additional influx of intracellular calcium and sodium. Increased intracellular calcium lead to cerebral edema, ischemia and microvascular damage that result in necrosis and cell death.[30] The extent of primary energy failure contributes to further injury in the secondary energy failure phase.

The secondary energy failure occurs 6 to 48 hours after initial insult with a mechanism appearing to be related to oxidative stress, excitotoxicity and inflammation.[30]. Excitotoxicity occurs when excessive levels of extracellular neurotransmitters, especially glutamate, over stimulate excitatory receptors. The overstimulation leads to an additional influx of sodium and calcium into the neural cells. The overproduction of free radicals leads to necrosis and apoptosis which cause oxidative stress. Oxidative stress is harmful to the neonatal brain due to low concentrations of antioxidants and a high consumption of oxygen when transitioning from fetal to neonatal life. The hypoxic-ischemic state causes the iron that was bound to proteins, be released. Free iron (Fe2+) is then

available to react with peroxides and form free radicals. The neonatal brain has a decreased ability to eliminate free radicals and the increased susceptibility to the free radicals leading to damage of neuronal tissue.

Diagnosis of hypoxic-ischemic encephalopathy

There are various determinants that must be kept in mind when making a diagnosis of HIE. Often, but not always there is an identifiable sentinel event leading to hypoxic-ischemic event. In confirmed cases of neonatal encephalopathy, it is important to determine the likelihood of peripartum or intrapartum event as a contributory factor. Neonatal encephalopathy that is due to hypoxic-ischemic event will be accompanied by abnormal neurological signs, fetal acidemia, evidence of hypoxic-related injuries to other organs and sentinel events related to labor and delivery.[31] The abnormal neurological signs that are in keeping with an acute hypoxic event include failure to initiate or maintain spontaneous breathing needing resuscitation, depressed level of consciousness and muscle tone/ activity as evidenced by low Apgar scores <5 at 5 and 10 minutes of life. On a biochemical point of view, intrapartum hypoxia is supported by presence of fetal acidemia defined by blood pH less than 7.0 or base deficit greater than or equal to 12 mmol/L, or both. [32, 33] The presence of multi-organ dysfunction supports the evidence for hypoxic–ischemic encephalopathy. Multisystem organ failure can include particularly neurologic dysfunction, renal and hepatic injury, hematologic abnormalities, cardiac dysfunction, metabolic derangements, and gastrointestinal injury. An objective assessment of severity of HIE can be made based on Thompson score (see Table 1).[12] Thompson score is derived from nine components of the neurological examination in Sarnat and Sarnat staging of HIE (see Table 2).[11] It has been demonstrated that a Thompson score of more than 10 during the first 7 days of life is indicative of an abnormal outcome.

Table 1. Thompson score

	0	1	2	3
Level of consciousness	Normal	Hyperalert or stare	Lethargic	Comatose
Fontanelle	Normal	Full, not tense	Tense	
Posture	Normal	Fisting, cycling	Strong, distal flexion	Decerebrate
Tone	Normal	Hypertonia	Hypotonia	Flaccid
Moro	Normal	Partial	Absent	
Grasp	Normal	Poor	Absent	
Suck	Normal	Poor	Absent+- Bites	
Respiration	Normal	Hyperventilate	Brief apnea	IPPV
Seizures	None	Infrequent (<3 per day)	Frequent (>2 per day)	

Table 2. The Sarnat Staging for Neonatal Encephalopathy

SEVERITY	**STAGE 1 (Mild)**	**STAGE 2 (Moderate)**	**STAGE 3 (Severe)**
Level of consciousness	Hyperalert	Lethargic or obtunded	Stupor or coma
Activity	Normal	Decreased	Absent
Neuromuscular control			
Muscle tone	Normal	Mild hypotonia	Flaccid
Posture	Mild distal flexion	Strong distal flexion	Intermittent decerebration
Stretch reflexes	Overactive	Overactive	Decreased or absent
Complex/ primitive reflexes			
Suck	Weak	Weak or absent	Absent
Moro (startle)	Strong	Weak	Absent
Tonic neck	Slight	Strong	Absent
Autonomic function			
Pupils	Mydriasis	Miosis	Variable
Heart rate	Tachycardia	Bradycardia	Variable
Seizures	None	Common	Uncommon

Justification for the study

Klerksdorp hospital has encountered an increase in the number of neonates with encephalopathy in the past three years. Nationally, there has also been an increase in the number of medico-legal cases related to encephalopathy. Data from the Klerksdorp hospital Perinatal Problem Identification Programme (PPIP) from August to 0ctober 2019 reported that poor monitoring during labor, delay in performing caesarean section due to unavailable theater facilities, failure to promptly respond to fetal distress, and no therapeutic hypothermia being offered were identified as modifiable factors in intrapartum hypoxia-related deaths (unpublished data). Thus, it is often assumed that all cases of neonatal encephalopathy are probably related to the above-mentioned modifiable factors. It is not clear if there are antecedent factors like placental pathology place fetuses at risk of developing intrapartum asphyxia even during normal labour. To identify factors that might be contributing to the increase in incidence of neonatal encephalopathy at Klerksdorp hospital, a protocol was established where all placentas from neonates who had encephalopathy are sent for histopathological investigations. This protocol was established in 2019. Knowledge of placental abnormalities in neonates with encephalopathy will assist in better understanding the causes of neonatal encephalopathy in our local setting. There is limited data from South Africa on placental pathological abnormalities in neonates with encephalopathy. Systematically describing the histopathological features of placentas from neonates with encephalopathy will assist in better understanding the role of placental pathology in development of encephalopathy and its associated long term sequelae.

AIM

To determine histopathologic features of placentas from neonates with encephalopathy born and admitted at Klerksdorp hospital over a period of 24 months.

OBJECTIVES

1. To describe the histopathological features of placentas from neonates with encephalopathy
2. To determine clinical characteristics of neonates with the diagnosis of encephalopathy
3. To determine the proportion of neonates with encephalopathy due to intrapartum hypoxia
4. To compare placental histopathology between neonates with encephalopathy with or without evidence of intrapartum hypoxia
5. To compare placental histopathology between neonates with mild HIE and those with moderate to severe HIE

METHODS

Study design

This will be a retrospective analytic study.

Study setting

The study will be conducted in Klerksdorp hospital in the North West province. Klerksdorp hospital is a provincial hospital and a satellite teaching institution for the University of the Witwatersrand. It receives patients from Nic Bodenstein district hospital, 30 local clinics and the surrounding private hospitals for patients who do not have medical aid. The total deliveries conducted per month range from 400 to 500 births. The obstetric unit has labor and delivery ward which is a seven bedded unit with two neonatal resuscitation areas. It is staffed with three professional nurses and two enrolled nurses per shift. The doctors who manage the labor and delivery room are interns and medical officers under supervision of registrars and specialists. All neonates who are born with features suggestive of encephalopathy undergo a thorough examination and placenta is sent for histopathology. The neonatal unit has a total bed capacity of 49 beds. The neonatal intensive care unit has 12 beds, with six of these beds allocated for isolation. High care unit is nine bedded, and the general neonatal ward and kangaroo mother care unit has 28 beds. All neonates are managed by general pediatricians as there are no neonatologists. The medical personnel in the neonatal unit consist of one full time and one part-time general pediatrician, one registrar, three medical officers and three medical interns. There is one unit manager, six registered nurses and four enrolled nurses to cover the entire unit per shift. The diagnosis of encephalopathy is based on the Thompson score or Sarnat and Sarnat staging. All newborn babies who require resuscitation soon after birth have blood taken from the umbilical cord or from the neonate within an hour of birth for blood gas analysis. As from January 2019, placentas from deliveries of neonates who required resuscitation and had any features of encephalopathy have been sent for histopathology. The placental specimens are taken to Lancet laboratories, accompanied by a brief history of patient details where a group of pathologists examine and report on the findings. It is therefore important to have a standardized method of what would be the criteria for pathologic findings in neonatal encephalopathy and be able to differentiate hypoxic from non- hypoxic ischemia. In view of the nature of the study, a pathologist, as a co-investigator would be beneficial. The role would be to look at the placental histology reports that have been selected to be part of the study to ensure standardized methods of preparing specimens for examination, consistent

manner of recording all the placental histology reports and ensuring that the objectives for examining the placenta are achieved. A pathologist from Lancet laboratories will be part of the study.

The Lancet laboratory has a standardized criteria for reporting the histology of neonates that have clinical evidence of encephalopathy:

- Macroscopy of placenta (weight, dimensions, membrane insertion, cord length, presence of obstructive lesions)
- Appearance of fetal surface of the placenta
- Appearance of maternal surface of the placenta
- Microscopy of placenta (presence of chorioamnionitis, villitis and looking at the maturity of placenta and to determine whether there is fetal vascular malperfusion)

A conclusion is reached based on the examination and a possible cause of encephalopathy determined.

Study population

Patients diagnosed with neonatal encephalopathy in Klerksdorp hospital from 30 November 2019 to 30 November 2021 and had placentas sent for histopathology. Thorough examination will include ascertaining whether neonate needed resuscitation at birth, the APGAR scores, and the neurological status in the form of Thompson score as well as obtaining a cord gas or arterial gas within an hour of birth.

Inclusion criteria

- All neonates with birth weight of 2000g or more, with a diagnosis of neonatal encephalopathy:
- Born in Klerksdorp hospital
- Available placental histopathology results

Exclusion criteria

- An underlying chromosomal or genetic condition
- Any major congenital abnormality

Study procedures and data collection

Patients' hospital clinical and laboratory records will be reviewed. Data will be collected from admission sheets, PPIP data sheets and patients' files and will be captured in the proposed sheet and then entered into Microsoft excel. Data to be collected will include maternal demographics, antenatal history including maternal co-morbidities and social habits, information on monitoring and management of labour and delivery details. Data to be collected on neonatal characteristics will include birth weight, head circumference, length, gestational age, sex, cord or arterial blood gas results, need for resuscitation, Apgar score, diagnosis, severity of encephalopathy, placental histopathology and outcome at hospital discharge.

Sample size

Assuming that the prevalence of abnormalities in placental findings in neonates with encephalopathy is 80%[21] with precision or confidence level of 5% and confidence interval of 95%, the number of neonates to be enrolled is 245 based on the following statistical equation:

$$N = \frac{Z^2_{\alpha/2} \times p\,(1-p)}{d^2}$$

Where

N = Sample size

$Z^2_{\alpha/2}$ = 1.96 (95% confidence interval)

d = level of precision or confidence level (5%)

p = proportion of neonates with encephalopathy have abnormal placenta (85%).

Data analysis

Data will be described using standard statistical methods. Categorical variables will be described using frequencies and percentages. Continuous variables will be described using means with standard deviations for data with a normal distribution and medians with interquartile ranges for skewed data. The sample will be divided into two groups, namely, moderate to severe encephalopathy and mild encephalopathy. These groups will be compared using Chi-square tests for categorical variables and Student independent t-test for continuous variables with a normal distribution. Non-parametric tests will be used for skewed data. Statistical significance will be defined as p-value of less than 0. 05.

Ethical considerations

Ethics approval will be obtained from University of the Witwatersrand. The records will be obtained upon approval from the clinical manager of Klerksdorp hospital. To maintain confidentiality, no patient information will be disclosed during the data collection period. No names and contact details will be revealed during the entire study. Identity of patients will be protected by allocating numbers to the files. There will be no risk imposed on patients participating in the study. Patients will also not receive any direct benefit from being in the study. No informed consent will be acquired from patients as the study will be retrospective and patients have already been discharged and it will be impossible to re-establish contact with their parents to get informed consent.

Funding/ costs

The anticipated costs for internet and printing of material will be covered by the researcher. Analysis of data and costs pertaining to professional advice received from statisticians will be the responsibility of researcher. The anticipated costs for internet and printing of material, buying statistical software from the University will be covered by the researcher. The cost of these is estimated not to be more than R3 000. Analysis of data will be conducted by the investigator, supported by the supervisors and therefore there are no expected costs towards a statistician.

Study management and timelines

	Nov 2021	Dec 2021	Jan 2021	Feb 2022	Mar 2022	Apr 2022	May 2022	Jun 2022	Jul 2022	Aug 2022	Sep 2022
Literature review											
Protocol preparation											
Protocol assessment											
Ethics application											
Data collection											
Data analysis											
Writing up the report											

REFERENCES

1. Estimates developed by the UN Inter-agency Group for Child Mortality Estimation. Levels & Trends in Child Mortality.; 2019.

2. The Alliance for Maternal and Newborn Health Improvement (AMANHI) mortality study group. Population-based rates, timing, and causes of maternal deaths, stillbirths, and neonatal deaths in south Asia and sub-Saharan Africa: a multi-country prospective cohort study. Lancet Glob Health. 2018;6:e1297-308.

3. Tann CJ, Webb EL, Lassman R, Ssekyewa J, Sewegaba M, Musoke M, et al. Early Childhood Outcomes After Neonatal Encephalopathy in Uganda: A Cohort Study. EClinicalMedicine. 2018;6:26-35.

4. Martinez-Biarge M, Diez-Sebastian J, Wusthoff CJ, Mercuri E, Cowan FM. Antepartum and intrapartum factors preceding neonatal hypoxic-ischemic encephalopathy. Pediatrics. 2013;132(4):e952-9.

5. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015;385(9966):430-40.

6. Lawn JE, Blencowe H, Oza S, You D, Lee AC, Waiswa P, et al. Every Newborn: progress, priorities, and potential beyond survival. Lancet. 2014;384(9938):189-205.

7. Lee AC, Kozuki N, Blencowe H, Vos T, Bahalim A, Darmstadt GL, et al. Intrapartum-related neonatal encephalopathy incidence and impairment at regional and global levels for 2010 with trends from 1990. Pediatr Res. 2013;74 Suppl 1:50-72.

8. Bruckmann EK, Velaphi S. Intrapartum asphyxia and hypoxic ischaemic encephalopathy in a public hospital: Incidence and predictors of poor outcome. S Afr Med J. 2015;105(4):298-303.

9. Padayachee N, Ballot D. Outcomes of neonates with perinatal asphyxia at a tertiary academic hospital in Johannesburg, South Africa. S Afr J CH. 2013;7(3):89-94.

10. Horn AR, Swingler GH, Myer L, Harrison MC, Linley LL, Nelson C, et al. Defining hypoxic ischemic encephalopathy in newborn infants: benchmarking in a South African population. Journal of perinatal medicine. 2013;41(2):211-7.

11. Sarnat HB, Sarnat MS. Neonatal encephalopathy following fetal distress. A clinical and electroencephalographic study. Arch Neurol. 1976;33(10):696-705.

12. Thompson CM, Puterman AS, Linley LL, Hann FM, van der Elst CW, Molteno CD, et al. The value of a scoring system for hypoxic ischaemic encephalopathy in predicting neurodevelopmental outcome. Acta Paediatr. 1997;86(7):757-61.

13. Ballot DE, Rakotsoane D, Cooper PA, Ramdin TD, Chirwa T, Pepper MS. A prospective observational study of developmental outcomes in survivors of neonatal hypoxic ischaemic encephalopathy in South Africa. S Afr Med J. 2020;110(4):308-12.

14. Aslam S, Strickland T, Molloy EJ. Neonatal Encephalopathy: Need for Recognition of Multiple Etiologies for Optimal Management. Front Pediatr. 2019;7:142.

15. Barrois M, Patkai J, Delorme P, Chollat C, Goffinet F, Le Ray C. Factors associated with neonatal hypoxic ischemic encephalopathy in infants with an umbilical artery pH less than 7.00. Eur J Obstet Gynecol Reprod Biol. 2019;236:69-74.

16. Stanek J. Hypoxic patterns of placental injury: a review. Arch Pathol Lab Med. 2013;137(5):706-20.

17. Roescher AM, Timmer A, Erwich JJ, Bos AF. Placental pathology, perinatal death, neonatal outcome, and neurological development: a systematic review. PLoS One. 2014;9(2):e89419.

18. Zhang P, Benirschke K. Placental pathology casebook. Serpentine aneurysms of the placenta with fetal consequences. J Perinatol. 2000;20(1):63-5.

19. Vik T, Redline R, Nelson KB, Bjellmo S, Vogt C, Ng P, et al. The Placenta in Neonatal Encephalopathy: A Case-Control Study. J Pediatr. 2018;202:77-85 e3.

20. Wintermark P, Boyd T, Gregas MC, Labrecque M, Hansen A. Placental pathology in asphyxiated newborns meeting the criteria for therapeutic hypothermia. Am J Obstet Gynecol. 2010;203(6):579 e1-9.

21. Chang KT, Keating S, Costa S, Machin G, Kingdom J, Shannon P. Third-trimester stillbirths: correlative neuropathology and placental pathology. Pediatr Dev Pathol. 2011;14(5):345-52.

22. Penn AA, Wintermark P, Chalak LF, Armstrong J, Redline R, Scher MS, et al. Placental contribution to neonatal encephalopathy. Semin Fetal Neonatal Med. 2021;26(4):101276.

23. Redline RW, Ravishankar S, Bagby CM, Saab ST, Zarei S. Four major patterns of placental injury: a stepwise guide for understanding and implementing the 2016 Amsterdam consensus. Mod Pathol. 2021;34(6):1074-92.

24. Volpe JJ. Placental assessment provides insight into mechanisms and timing of neonatal hypoxic-ischemic encephalopathy. J Neonatal Perinatal Med. 2019;12(2):113-6.

25. Gruenwald P. Fetal deprivation and placental pathology: concepts and relationships. Perspect Pediatr Pathol. 1975;2:101-49.

26. Redline RW. Placental pathology: a systematic approach with clinical correlations. Placenta. 2008;29 Suppl A:S86-91.

27. Redline RW, Faye-Petersen O, Heller D, Qureshi F, Savell V, Vogler C, et al. Amniotic infection syndrome: nosology and reproducibility of placental reaction patterns. Pediatr Dev Pathol. 2003;6(5):435-48.

28. Pierrat V, Haouari N, Liska A, Thomas D, Subtil D, Truffert P, et al. Prevalence, causes, and outcome at 2 years of age of newborn encephalopathy: population based study. Arch Dis Child Fetal Neonatal Ed. 2005;90(3):F257-61.

29. Jenster M, Bonifacio SL, Ruel T, Rogers EE, Tam EW, Partridge JC, et al. Maternal or neonatal infection: association with neonatal encephalopathy outcomes. Pediatric research. 2014;76(1):93-9.

30. Allen KA, Brandon DH. Hypoxic Ischemic Encephalopathy: Pathophysiology and Experimental Treatments. Newborn Infant Nurs Rev. 2011;11(3):125-33.

31. American College of Obstetricians and Gynecologists. Executive summary: neonatal encephalopathy and neurologic outcome. . Obstetrics and gynecology. 2014;123:896-901.

32. Low JA. Intrapartum fetal asphyxia: definition, diagnosis, and classification. American journal of obstetrics and gynecology. 1997;176(5):957-9.

33. Low JA, Lindsay BG, Derrick EJ. Threshold of metabolic acidosis associated with newborn complications. American journal of obstetrics and gynecology. 1997;177(6):1391-4.

APPENDIX B: DATA COLLECTION SHEET

Maternal History			
Patient Number:		Folder No:	
Age at Delivery:	____ years		
G:___ P:___ M:___		Gestational Age:	___ /40 weeks By: □ Early Sonar □ Late Sonar □ Dates □ SFH
Pregnancy			
Booked: □ Yes □ No If Yes: Booked at gestational age: _____weeks			Antenatal Clinic: _____
Rh: □ Positive □ Negative □ Not tested			
RPR: □ Positive □ Negative □ Not tested Titre if positive: ____ Date: _____ Treatment completed: □ Yes □ No			
RVD: Rapid at delivery: □ Positive □ Negative □ Not Tested RVD neg : Late last tested: _____ RVD pos : CD4 count: _____ Date done: _____ VL: _____ Date done: _____ On ART since: _____			
Co-Morbidities: □ Hypertensive Disease (PET/Hypertension) □ Diabetes □ Epilepsy □ Cardiac □ UTI/Chorioamnionitis			
Smoker: □ Yes □ No		Alcohol use: □ Yes □ No	
Labour and Delivery			
Place of Birth:_____ Time: □ Day □ Night			
Monitoring during labour: □ Monitored □ Not Monitored □ Arrived HOP PV: _____ CTG: Total: _____ □ Reassuring □ Suspicious □ Pathological ACTION: □ IPR □ Delivery Partogram: □ Plotted □ Not Plotted Duration of labour: _____ hours □ Normal □ Prolonged □ Precipitous If Prolonged: □ LPL □ Second Stage □ Third Stage Crossed Action Line: □ Yes □ No Analgesia Given: □ Yes □ No If Yes, hours before delivery: _____ hours Other:_____ Medication:_____ Drugs:_____			
Baby			
Birth date: _____ Weight:_____g HC: _____cm Length: _____cm			
APGAR: ___/1min ___/5min ___/10min ___/20min			
Gender: □ Male □ Female			
Ventilator Breaths:	□ Yes □ No		Duration: _____ min
Chest Compressions:	□ Yes □ No		Duration: _____ min
Adrenaline:	□ Yes □ No		Duration: _____ min
Cord Blood Gas:	pH:____ PCO2:____mmHg PO2:____mmHg BE:___ Lactate:____		
Baby Blood Gas (< 1hour)	pH:____ PCO2:____mmHg PO2:____mmHg BE:___ Lactate:____		
Thompson Score:	D1:___ D2:___ D3:___ D4:___ D5:___ D6:___ D7:___		
Sarnat Score:	D1:___ D2:___ D3:___ D4:___ D5:___ D6:___ D7:___		
Classification	□ Mild □ Moderate □ Severe		
Therapeutic Hypothermia:	□ Yes □ No		

		If No, reason: □ Machine in use □ Machine broken □ Passed 6hrs			
Blood Culture					
CSF		□ Meningitis □ No meningitis			
CUS		□ Normal □ Abnormal			
Placental Findings:					
Macroscopic:	Weight	____ g	**Microscopic:**	Chorioamnionitis/villitis	□ Yes □ No
	Cord length	____ cm		Maturation microscopic infarction	□ Yes □ No
	Dimensions			Fetal vascular malperfusion	□ Yes □ No
	Membrane			Maternal vascular malperfusion	□ Yes □ No
	Insertions			Retroplacental haematoma	□ Yes □ No
	Meconium	□ Yes □ No			
	Obstructive lesions	□ Yes □ No			
	No. of coils				
Fetal Surface:	Congested/ thrombosed	□ Yes □ No	**Maternal Surface:**	□ Complete □ Incomplete	
	Chorionic vessels	□ Yes □ No		Indentations	□ Yes □ No
	Sub amniotic hemorrhage	□ Yes □ No		Retroplacental clot	□ Yes □ No
				Infarction/ Intervillous hemorrhage	□ Yes □ No
			Conclusion of placental histology:		

Amended placental histopathology collection tool

<table>
<tr><th colspan="6">Placental Findings:</th></tr>
<tr><td rowspan="9">Macroscopic:</td><td>Weight</td><td>____ g</td><td rowspan="9">Microscopic:</td><td>Acute chorioamnionitis
Villitis</td><td>□ Yes □ No
□ Yes □ No</td></tr>
<tr><td>Cord length</td><td>____ cm</td><td>Accelerated Maturation
Microscopic infarction</td><td>□ Yes □ No
□ Yes □ No</td></tr>
<tr><td>Dimensions</td><td></td><td>Fetal vascular malperfusion</td><td>□ Yes □ No</td></tr>
<tr><td>Membrane</td><td></td><td>Maternal vascular malperfusion</td><td>□ Yes □ No</td></tr>
<tr><td>Insertion</td><td></td><td rowspan="3">Retroplacental haematoma</td><td rowspan="3">□ Yes □ No</td></tr>
<tr><td>Meconium</td><td>□ Yes □ No</td></tr>
<tr><td>Obstructive lesions</td><td>□ Yes □ No</td></tr>
<tr><td>No. of coils</td><td></td></tr>
<tr><td rowspan="3">Fetal Surface:</td><td>Congested/ thrombosed vessels</td><td>□ Yes □ No</td><td rowspan="3">Maternal Surface:

Conclusion of placental histology:</td><td colspan="2">□ Complete □ Incomplete</td></tr>
<tr><td rowspan="2">Sub amniotic hemorrhage</td><td rowspan="2">□ Yes □ No</td><td>Retroplacental clot</td><td>□ Yes □ No</td></tr>
<tr><td>Infarction
Intravillous hemorrhage</td><td>□ Yes □ No
□ Yes □ No</td></tr>
</table>

APPENDIX C: ETHICS CLEARANCE CERTIFICATE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Dr KL Sebolai and Ms K Barnard

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M220119

NAME: **(Principal Investigator)**	Dr KL Sebolai and Ms K Barnard
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	*Placental pathology in neonates diagnosed with encephalopathy soon after birth: a retrospective analytic study*
DATE CONSIDERED:	2022/01/28
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Dr O Mekgoe and Professor S Velaphi
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/09/19

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

APPENDIX D: APPROVAL FROM NATIONAL HEALTH LABORATORY SERVICES

NATIONAL HEALTH LABORATORY SERVICE

Academic Affairs and Research
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 555 0367/0406
Email: babatyi.kgokong@nhls.ac.za
academic.research@nhls.ac.za
Web: www.nhls.ac.za

07 September 2022

Applicant: Keaoleboga Sebolai
Institution: University of the Witwatersrand
E-mail Address: lorrainelebopaul@gmail.com
Cell: 076 190 1171

CC: Colleen Wright

Project Title: Placental pathology in neonates diagnosed with encephalopathy soon after birth: a retrospective analytic study
Reference Number: PR2225936

Research Application Type(s):
1. Request for Data

RE: APPROVAL LETTER: REQUEST TO ACCESS NHLS RESOURCES FOR RESEARCH PURPOSES

This letter serves to advise that the application requesting permission to conduct the above-mentioned research using the listed NHLS resources has been reviewed and **"Approved"**. Please note that the approval is granted on the condition that you comply with the NHLS Research Material and Data Access Policy and requirements stated below.

1. All material and data requested shall be used as per the research protocol submitted to the NHLS and as approved by the relevant Health Research Ethics Committee (HREC) in South Africa.
2. Access to the NHLS material and/or data shall be limited to the minimum required for successful completion of the approved study and shall be made available ***without patient names***.
3. Confidentiality shall be maintained at the participant and institutional level and there shall be no disclosure of personal information or confidential information.
4. The material and/or data obtained from the NHLS shall be anonymised and not, for any reason, be used to track or recruit patients as no pre-approval/consent is obtained from patients.
5. Processes shall be discussed with the relevant NHLS departments (i.e. Corporate Data Warehouse (CDW), NHLS Laboratory Management, Operations Office, etc.) and agreed upon.
6. Any amendments to the study requirements, including the use of the material and/or data for purposes not initially disclosed to the NHLS) shall be cleared by an approved HREC and submitted to the NHLS for approval via the AARMS system – https://aarms.nhls.ac.za.
7. The NHLS shall be acknowledged as a source of material and/or data in any output, such as abstracts and journal articles, emanating from the project.
8. A final report of the research study and any published output resulting from this study shall be submitted to the NHLS via AARMS

Please note that this letter constitutes approval by the NHLS Academic Affairs and Research Office. The NHLS entities tasked with providing the material and/data may have additional requirements for access. Data related queries may be directed to NHLS CDW, email: zarina.sabat@nhls.ac.za; contact number: 011 386 6074 and sample related queries (if applicable) shall be directed to the relevant business manager.

P.P

Dr Babatyi Malope-Kgokong
National Manager: Academic Affairs and Research

APPENDIX E: SIGNED DECLARATION ON PLAGIARISM

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I, **Keaoleboga Lorraine Sebolai** (Student number: **2531002**) am a student registered for the degree of **Master of Medicine in Paediatrics and Child Health** in the academic year 2025.

I hereby declare the following:
- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date**: 01/05/2025**

APPENDIX F: TURNITIN REPORT

final document for turnitin 30.04.25-1.docx

by Keaoleboga Sebolai

Submission date: 01-May-2025 09:10PM (UTC+0200)
Submission ID: 2663473030
File name: final_document_for_turnitin_30.04.25.docx (102.65K)
Word count: 5255
Character count: 30276

final document for turnitin 30.04.25-1.docx

ORIGINALITY REPORT

11%	8%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.frontiersin.org Internet Source	2%
2	wiredspace.wits.ac.za Internet Source	1%
3	assets.researchsquare.com Internet Source	1%
4	Imran N. Mir, Sarah F. Johnson-Welch, David B. Nelson, Larry S. Brown, Charles R. Rosenfeld, Lina F. Chalak. "Placental pathology is associated with severity ofneonatal encephalopathy and adverse developmental outcomes following hypothermia", American Journal of Obstetrics and Gynecology, 2015 Publication	1%
5	hdl.handle.net Internet Source	1%
6	www.science.gov Internet Source	1%
7	Torstein Vik, Raymond Redline, Karin B. Nelson, Solveig Bjellmo et al. "The Placenta in Neonatal Encephalopathy: A Case–Control Study", The Journal of Pediatrics, 2018 Publication	<1%
8	www.omicsdi.org Internet Source	<1%
9	Lauren L. Jantzie, Raymond W. Redline. "Placental pathology: Pathways leading to or associated with perinatal brain injury in experimental neurology, special issue: Placental mediated mechanisms of perinatal brain injury", Experimental Neurology, 2021 Publication	<1%

APPENDIX G: GUIDELINE FOR AUTHORS FROM SOUTH AFRICAN MEDICAL JOURNAL

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are to fill a gap in literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarize the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text.

Structured abstract

- This should be 250-400 words, with the following recommended headings:
    - **Background:** why the study is being done and how it relates to other published work.
    - **Objectives:** what the study intends to find out
    - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
    - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
    - **Conclusion:** it must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

APPENDIX H: DECLARATION BY CO-AUTHORS

Declaration: Student's contribution to article(s) and agreement of co-author(s)

I, Keaoleboga Lorraine Sebolai, student number 2531002, declare that this Thesis/Dissertation/Research Report is my own work and that I contributed adequately towards research findings published in the article(s) stated below which are included in my Thesis/Dissertation/Research Report.

Signature of Student 

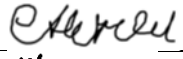
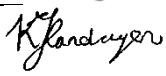
Date: 01/05/2025

Name of Primary Supervisor: Professor S C Velaphi

Signature of Primary Supervisor:  **Date:** 2 May 2025

Agreement by co-authors: By signing this declaration, the co-authors listed below agree to the use of the article(s) by the student as part of his/her Thesis/Dissertation/Research Report. In cases where the student is not the 1st author of a published article, the primary supervisor must explain (under comments) why the student is entitled to use the paper for his/her degree purposes.

Article: Title: Placental pathology of neonates diagnosed with encephalopathy soon after birth: A retrospective analytic study
Journal name, year, volume and page numbers: South African Medical Journal (in preparation for submission)

Authors	Name	Signature	Date
1st author	KL Sebolai		01/05/2025
2nd author	OT Mekgoe		02/05/2025
3rd author	CA Wright		1/05/2025
4th author	K Thandrayen		2/05/2025
5th author	SC Velaphi		02/05/2025

Comments by primary supervisor: Dr. Sebolai is the primary author of this paper. She conceptualized the study, developed the research protocol, collected the data independently, and wrote the initial draft. While she analyzed and interpreted the data with some assistance, she conducted the research with minimal support, aside from the statistical analysis. Thus, I fully support Dr. Sebolai in submitting this research paper as part of her research report.