ADDIS ABABA UNIVERSITY
COLLEGE OF HEALTH SCIENCES
CENTER FOR INNOVATIVE DRUG DEVELOPMENT AND THERAPEUTIC TRIALS FOR AFRICA

TIME TO RESPONSE AND PREDICTORS OF SEIZURE RESPONSE TO PHENOBARBITAL THERAPY AMONG NEONATES ADMITTED WITH HYPOXIC-ISCHEMIC ENCEPHALOPATHY AT NEKEMTE COMPREHENSIVE SPECIALIZED HOSPITAL, ETHIOPIA

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Time to Response and Predictors of Seizure Response to Phenobarbital Therapy among Neonates Admitted With Hypoxic-Ischemic Encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia

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June, 2024

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university, and that all sources of material used for this thesis have been properly recognized.

Wase Benti Hailu Signature June, 2024

APPROVAL

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This is to certify that thesis prepared by Wase Benti, titled "*Time to Response and Predictors of Seizure Response to Phenobarbital Therapy among Neonates Admitted With Hypoxic-Ischemic Encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia*, has been submitted in partial fulfillment of the requirements for the degree of Masters of Science in Clinical Trials, complies with the regulation of the university and meets the accepted standards with respect to originality and quality.

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Above all, I praise God for His gracious care that that enabled me quell sorrow and endure turbulences for glory. I am always indebted for the support of CDT -team throughout hardships.

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ACRONYMS

AHR	Adjusted hazard Ratio
APGAR	Appearance, Pulse, Grimace, Activity, Respiratory
CI	Confidence Interval
DHS	Demographic Health Survey
EEG	Electroencephalography
GABAA	Gamma γ-Aminobutyric acid type A
HIE	Hypoxic-Ischemic Encephalopathy
ILAE	International league against epilepsy
NICU	Neonatal Intensive Care Unit
NICU	Neonatal Intensive Care Unit
NMR	Neonatal Mortality Rate
NSE	Neonatal Status Epilepticus
PNA	Prenatal Asphyxia
PvEEG	Polygraphic video–electroencephalography
SD	Standard Deviation
WHO	World Health Organization

ABSTRACT

Background: Hypoxic-ischemic encephalopathy is a brain injury that occurs in newborns when there is not enough blood flow to the brain. Recent studies have raised concerns about how well phenobarbital works for treating seizures in newborns, as it may not effectively control seizures with the initial loading and repeated doses.

Objective: This study evaluated the time to response and predictors of seizure response to phenobarbital therapy among neonates admitted with hypoxic-ischemic encephalopathy.

Methods and Materials: A retrospective cohort study was conducted at Nekemte Comprehensive Specialized Hospital, using randomly selected medical records of 284 neonates who were treated between January 2020 and December 31, 2023. The study included neonates diagnosed with perinatal asphyxia stage II and III hypoxic-ischemic encephalopathy, who were treated with naso-gastric phenobarbital. The treatment included an initial loading dose of 20mg/kg and two repeated doses of 10mg/kg. Survival analysis was conducted. Predictor variables with a p-value ≤ 0.25 in bivariate Cox regression were included in the multivariable Cox regression analysis. Adjusted Hazard Ratios with 95% confidence intervals were computed, and a p-value < 0.05 was considered statistically significant.

Results: Out of the 284 neonates, 210 (73.9%) responded to the phenobarbitone treatment. The incidence rate of response was 27.73 per 1000 person-hours of observation, with a median time to response of 29 hours (IQR 26.5-32 hours). Low birth weight (AHR=0.59; 95%CI 0.58, 0.98), subtle seizure type (AHR: 2.35; 95% CI 1.09, 5.08), severe hypothermia (AHR=0.23; 95% CI 0.052, 0.26), and seizure frequency of twice or more (AHR=0.436, 95% CI 0.31, 0.61) were identified as predictors of seizure response.

Conclusion and recommendation: Overall incidence rate of response was low. Having history of twice/more frequency of seizure insult, severe hypothermia and low birth weight (LBW) decreased the response rate while subtle type of seizure increased likelihood of response to phenobarbitone therapy. Electroencephalogram-confirmed seizures treatment and combined management with therapeutic hypothermia for high-risk newborns needs to be started for better response and reduced response time. Further controlled studies utilizing both clinical and neuroimaging for definitive outcome measurement are recommended.

Keywords: neonate, time to recovery, NICU, censored, incidence, predictors.

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CHAPTER ONE: INTRODUCTION

1.1. Background

Hypoxic-ischemic encephalopathy (HIE) is a brain dysfunction resulting from inadequate blood flow (ischemia) or oxygen flow (hypoxia) period. It can happen due to various etiologies during pregnancy, labor, or post-natal period. Commonest prenatal causes include preeclampsia, maternal diabetes/vascular disorder, alcohol or drug abuse, severe fetal anemia, congenital fetal infection, and lung malformations (1,2). While prolonged late stage of labor, abruption of placenta, breech fetal presentation low blood pressure of the mother, excess placental bleeding are among causes during delivery, it can also happen due to extreme preterm (less than 28weeks of gestation),)severe cardiac disorders, trauma to brain or skull, serious infections and cardiac arrest or respiratory failure during post-natal period (2,3).

The widely accepted grading of perinatal HIE is Sarnat and Sanat scoring system of three clinical stages to determine neurological insults of asphyxiated newborns (4). Stage 1 is characterized by stretch reflexes, hyper-alertness, uninhibited moro and stretch reflexes, sympathetic effects, and a normal electroencephalogram lasting less than 24 hours, whereas stage 2 has obtundation, hypotonia, strong distal flexion, multifocal seizures and periodic patters on EEG. Stupor, flaccidity, suppression of brain stem and autonomic activities, is potential or had irregular cyclical discharges on EEG characterize the severe, stage-III (4,5). Incidence of moderate (stage 2) to severe HIE (stage 3) in developed countries ranges from 1-8 per 1000 live births, while it varies from 2.3 to 26.5 per 1000 live births in developing countries, and 3 to 22 in sub-Saharan Africa (6–9).

Seizures are common in neonates with in all three grades of hypoxic-ischemic encephalopathy in as much as 62% (10). Despite the varying types of seizure in moderate and severe HIE, it is pertinent in both grades as it is among the grading criteria. Without prompt management, seizures are highly associated with adverse neurodevelopmental outcomes. International league against epilepsy (ILAE) classifies neonatal seizure as ‘epilepsies and syndromes with subheading of generalized and focal seizures. In 2017, ILAE established neonatal classification framework with addition of electroencephalogram to aid categorization as automatism (voluntary movement), impaired cognition, tonic, clonic, epileptic spasm, myoclonic, behavioral arrest, electro-graphic only seizure and unclassified seizure. However, this algorithm might not be functional in absence of EEG in the setting and also for critically ill

neonates(11). In the absence of EEG categorization of Volpe (subtle, tonic, clonic, myoclonic and non-paroxysmal repetitive behaviors) is commonly used as mentioned in American Academy of Pediatrics (Nelson Essentials of Pediatrics) and other literatures (11–13).

Ethiopian national neonatal treatment guideline and Nelson essentials of pediatric care recommend initial intravenous or oral phenobarbital dose of 20mg/kg which aims to reach blood level of 20 µg/mL to deliver visible anticonvulsant effect in the neonate(14). If seizure persists, repeated dose of 5-10mg/kg until seizure is controlled, with the maximum total dose of 40mg/kg is administered or patient developed dose-limiting adverse event (15). Despite neonates less than 30 weeks gestational age need lesser dose, gestational age and weight of the neonate doesn't appear to affect dose-blood level relationships(16).

There is a dearth of evidence on to what extent neonatal seizures are controlled using current treatment modalities. Currently, WHO recommends the use of phenobarbital and phenytoin as first-line treatment options. However, there is a variability of response over time and neurological conditions. To our understanding, this study is the first of its kind in Ethiopia to assess the response rate, time to response and predictors of seizure response to phenobarbital therapy among neonates admitted with Hypoxic-Ischemic Encephalopathy.

1.2. Problem Statement

Seizures are the most common neurological emergency in newborns. Neonatal seizures has been reported in 30% to 62% of newborns with all grades of hypoxic-ischemic encephalopathy that emanates from perinatal asphyxia (10,17). Various studies reported 22-80% of mortality, 11.3-38.9% of epilepsy ,12-84.6% of cerebral palsy , and 20-42.7% of intellectual disability or developmental delay rate among preterm neonates and 30% mortality ,13% neurological abnormalities for term neonates (17,18). Birth asphyxia is the third cause(23%) of newborn deaths and 29% of early neonatal mortality worldwide(19). Africa shoulders 25% of global neonatal mortality from which 24% were due to perinatal asphyxia (20). Incidence of HIE in high-income countries was 0.5 compared to 1.8 in low and middle-income countries while it ranges from 1.6 to 7.9 per 1000 livebirths in Sub-Saharan Africa (21,22). A recent systematic review and meta-analysis reported pooled prevalence of birth asphyxia in Ethiopia as 19.3% and incidence rate of HIE ranging from 10.7 and 30.9 per 1000 live birth (23–25). A study from North West Ethiopia reported that neonatal seizure was present in 74.4% of newborns with HIE, and were responsible for 73.9% deaths (26). Furthermore, seizures in HIE are associated with poor neurodevelopmental outcome, independent of HIE severity(27), more severe hippocampal cell death(28), and elevated serum concentrations of interleukin-8, an inflammatory cytokine that increases seizure susceptibility and induces organ damage (29).

The neonatal brain is dynamic and vulnerable to acute seizures and subsequent epileptogenesis. In HIE, neonatal seizures may exacerbate the injury caused from hypoxic ischemia. Experimental models suggest that the combination of hypoxic-ischemia and seizures increase hippocampal brain damage compared to those with hypoxic ischemic injury alone (30,31). Clinical studies have shown HIE to result in progressive decline in mitochondrial aerobic metabolism and subsequent loss of high-energy phosphate compounds (27,32).

Neonates with HIE represent an especially difficult population to treat. Perinatal systemic ischemia often causes significant damage outside the nervous system, resulting in multi-organ dysfunction which can affect metabolism of common antiepileptic drugs, altering their effect of controlling seizure use in this population (33).

There is a paucity of research examining pharmacological management of seizures exclusively in neonates with HIE. Few studies had reported varying and inadequate level of seizure cessation using the recommended initial and repeated doses of phenobarbital in neonatal seizure. According to Gilman and colleagues, approximately 70% control of clinical seizure

were reported (34). Whereas other controlled study of 59 neonates attained complete control of seizure in 43% of neonates(35). Another open-label RCT from India found seizure control (seizure-free period of 24hours) in 72% of neonates that received initial 20mg/kg dose of phenobarbital (36). A retrospective study of 91 neonates with neonatal seizure treated showed 63% complete cessation of seizure ,17% partial or reduction of seizure episodes and 21% non-response to initial 20mg/kg phenobarbital dose (37).

There is a mix of evidence on factors determining seizure response in HIE. Neonatal seizure response to phenobarbital treatment in HIE seemed to determine by neonatal, clinical or seizure related factors. Some studies showed that combined administration with benzodiazepines, initial lower seizure frequency and fewer seizure burden, >7 APGAR score at 5^{th} minute of life, and term gestational stages were predictive of good response rate. In contrary, other studies reported that response rate didn't vary by gestational age, sex, birth weight or APGAR score at 5^{th} minutes after birth rather hypothermia management, underlying HIE were are determinants of good response

Despite recommendations of phenobarbitone for neonatal seizure, recent studies have raised concerns about the effects of phenobarbital treatment on neonatal brain structures affecting seizure response and admission outcomes(30,38). There is a mix of evidence on factors determining seizure response in HIE. Neonatal seizure response to phenobarbital treatment in HIE is determined by neonatal, clinical or seizure related factors. Few studies showed that combined administration with benzodiazepines, initial lower seizure frequency and fewer seizure burden, 4-7 APGAR score at 5^{th} minute of life, and term gestational stages were predictive of good response rate (31,39–43). In contrary, another studies reported that response rate didn't vary by gestational age, sex, birth weight or APGAR score at 5^{th} minutes after birth rather hypothermia management , underlying HIE were are determinants of good response (44–48)

There is limited data on the efficacy of phenobarbital in treating neonatal seizures in Africa, particularly in Ethiopia. Examining the seizure response and determination of its predictors among neonates having HIE has huge contribution to the literature, and evidence-based clinical practice, developing possible guidelines and thus reduction of neonatal mortality and neurological sequel of the condition.

1.3. Significance of the Study

Hypoxic-ischemic encephalopathy is leading morbidity in asphyxiated neonates. Majority of neonates having HIE experience seizure that has detrimental effect on the neurological outcome and discharge outcome. Besides the mortality rate, the long-term complication to the neonates is alarming. Significant amounts of epilepsy during childhood could have been prevented through effective management of neonatal seizures while poorly controlled seizures can also pose the risk of seizure in their later childhood ages. This has a multi-faceted socio-economic impact on the wellbeing of the babies, families, and health system repercussions.

World Health Organization's guideline for neonatal seizure management, American Pediatric Association (APA) and Nelson's Textbook of Pediatrics (2021 Edition) recommends combined treatment of neonatal seizure with therapeutic hypothermia (also called cooling) and phenobarbital therapy as first-line therapy followed by third generation anti-epileptic drugs. However, hypothermia management is only available in few tertiary care settings and phenobarbitone is used in greater majority of the clinical setting of Ethiopia. Newborns with neonatal seizure attributing to HIE are kept at room temperature and receive pharmacotherapy. The growing concern of phenobarbitone lower efficacy, use of phenytoin as second-line than latest products like levetiracetam compounded by long-term effect in developing brain of newborns like preterm necessitates evidence for clinical decisions and policy considerations. Further RCTs studies will utilize this finding as a baseline to establish the exact therapeutic effect of phenobarbitone across various setups to contribute to evidence-based care and policymaking.

1.4. Study Objectives

1.4.1. General Objective

- To assess response rate, time to response and predictors of seizure response to Phenobarbital therapy among neonates admitted with hypoxic-ischemic encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia.

1.4.2. Specific Objectives

- To estimate the incidence of seizure response to phenobarbital therapy in neonates admitted with hypoxic-ischemic encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia, from January 4 to March 6,2024.
- To estimate median time of seizure response to phenobarbital therapy among neonates admitted with hypoxic-ischemic encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia, from January 4 to March 6,2024.
- To identify predictors of seizure responses to phenobarbitone Therapy among neonates admitted with hypoxic-ischemic encephalopathy at Nekemte Comprehensive Specialized Hospital, Ethiopia from January 4 to March 6,2024.

CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

During the neonatal period, neurologic diseases manifest themselves mostly with seizures that are often related to an unfavorable outcome, such as death, cerebral palsy, intellectual disability, and epilepsy. Incidence of seizure are estimated both in population-based or clinical diagnosis. The more advanced approaches including Polygraphic video–electroencephalography (v-EEG) monitoring could be employed to understand if the paroxysmal movement is epileptic in origin, and it is the only way to identify electrical-only seizures in high-risk infants.

Acute symptomatic neonatal seizure is the most prevalent in common neurological diseases necessitating NICU admissions. Hypoxic ischemic encephalopathy (HIE),ischemic stroke and intracranial hemorrhage are the leading acute neurologic disorders of neonates attributing to 38%,18% and 12% respectively (49,50). Other causes of neonatal seizure includes transient metabolic derangements (4%), central nervous system infections such as meningitis (4%), congenital causes like brain malformations (4%),inborn infant metabolic disorders (3%), and other genetic etiologies of benign (3%) or severe onset neonatal epilepsies (6%) (51,52). Hospital based study from Nepal reported incidence of HIE among asphyxiated neonates as 14 per 1000 livebirths and 44% of asphyxiated newborns had seizure(53). From this study, 44% had stage II HIE whereas 24% had stage III HIE. Retrospective cohort study of 476 neonates(273 term and 203 preterm) from Parma university hospital, Italy reported incidence of 2.29/1000 live births (95% CI: 1.87-2.72) (54).

Phenobarbital remains the standard first-line pharmacotherapy for neonatal seizures resulting from HIE, even though data indicate it is only is effective in 50-60% (55–57). It is also the only drug strongly recommended in the WHO guideline, notably with very low evidence. The standard loading dose of phenobarbital is 20 mg/kg and may be repeated if seizure persists with maintenance dose of 10mg/kg to the maximum of 40mg/kg. In clinical practice, even though choice of second-line anti-epileptic drug varies, Ethiopian national guideline recommends addition of phenytoin thereafter. This could be because it has good safety and efficacy, availability and cost-effectiveness profile compared to other second line AEDs, though precautions are required for newborns requiring inotropic support which it may cause myocardial depression. Midazolam has a shorter half-life while clonazepam is good in EEG

control. A 2023 recommendation of the Neonatal Task Force of the International League Against Epilepsy (ILAE) also supports this notion. It recommend the use of Phenobarbitone as first line, regardless of etiology (exception of channelopathy from family history which opts use of phenytoin/carbamazepine), and second line AEDs (if not responding to first line) phenytoin, levetiracetam, midazolam, or lidocaine may be used as a second-line ASM(58).

2.2. Phenobarbitone

Phenobarbital is a long-acting barbiturate that has long been considered the standard first-line agent for neonatal seizures, due to its success in suppressing seizures in children and adults. Since therapeutic hypothermia (cooling) has become standard of care for HIE, many studies have examined the use of phenobarbital in combination with hypothermia for seizure control and neuroprotection (56,59,60).

2.2.1. Mode of action

γ-Aminobutyric acid type A (GABAA) inhibitory activity is enhanced by phenobarbital, which may restrict glutamate excitement (61). GABA activity is hypothesized to have an excitatory effect in the developing brain(62). Animal investigations have demonstrated that different brain areas have variable quantities of Cl- transporters, resulting in different intracellular Cl- concentrations (63). In particular, thalamic neurons have low Cl- concentrations and are inhibited by GABA receptor activation, whereas cortical neurons have relatively high amounts. As a result, GABA action excites these cells. This study demonstrates that other brain regions, despite their immaturity, may maintain low Cl- concentrations, and that phenobarbital may suppress seizures depending on the place of seizure genesis in the neonatal brain(51).

2.2.2. Pharmacokinetics

Because phenobarbital is metabolized by the liver and eliminated by the kidneys, these processes may be compromised in neonates with hepatic or renal impairment following HIE. The medication binds to plasma proteins 40-60% of the time (64). Comparisons of phenobarbital administration in neonates with and without birth asphyxia show variations in drug processing, including lower clearance and greater minimum blood concentrations (59). Asphyxiated newborns have clearance values of 4.1 +/- 1.0 mL/kg/h [36], 0.0034 L/h/kg [38], and 0.08 +/- 0.03 mL/min/kg, implying that these patients require only about half the maintenance dose of non-asphyxiated newborns to achieve comparable blood concentrations.

2.2.3. Dosing

Ethiopian NICU guideline (2021), WHO guideline for neonatal seizure and Nelson essentials of pediatric care recommend initial intravenous or oral phenobarbital dose of 20mg/kg which aims to reach blood level of 20 µg/mL to deliver visible anticonvulsant effect in the neonate(14). If seizure persists, repeated dose of 5-10mg/kg until seizure is controlled, with the maximum total dose of 40mg/kg is administered or patient developed dose-limiting adverse event (15) It is available for oral use in tablet and elixir form as well as in vials of sterile solution for parenteral use. The 2021 Ethiopian NICU guideline also recommend use of the same IV dose but due to longer time of absorption from oral doses (oro-gastric or naso-gastric administration), reload every four to six hours.

2.2.4. Adverse events

World Health Organization recommended use of phenobarbitone for neonatal seizure acknowledging inadequate efficacy level, established use in older children and several RCTs are being explored to investigate safety profile(46,65). Some high income countries have shifted to use more safer third generation AED like levetiracetam and others than phenobarbitone(39) Phenobarbital use in asphyxiated neonates has been a controversy arising from the thrive to control the seizure perplexed by its adverse effect on developing brain. Despite studies reported 40mg/kg over one hour and plasma concentration of 25mg/L had no effect on pulse rate, respiratory , blood pressure or blood gas values (15), adverse events like sedation, irritability, hypotension, hepatotoxicity hypotension were equally concerning (35). Furthermore, one animal study has shown evidence of neuronal apoptosis at blood levels (41) and other study also reported worse neurodevelopmental outcomes at two years of age (40).

Based on the available literature, phenobarbital remains the standard first-line agent for neonatal seizures, despite its potentially harmful effects and limited efficacy. Possible secondary medications that may be considered include phenytoin, bumetanide, topiramate, levetiracetam, lidocaine, or benzodiazepines. However, it remains unclear which of these options would be the most effective (66,67). Ongoing trials of these drugs in neonates with or without HIE should provide more direction for clinicians.

2.3. Response Rate of neonatal seizure to Phenobarbital treatment in HIE

Seizures are common in neonates with moderate and severe hypoxic ischemic encephalopathy (HIE) and independently of HIE severity associated with worse outcome The impact of seizure activity on the developing brain and the most effective way to manage these seizures remain surprisingly poorly understood. There is a dearth of evidence for whether and to what extent neonatal seizures exacerbate brain damage after HIE and how best to manage them with the current treatment modalities. Currently, WHO recommends use of phenobarbital and Phenytoin as first-line treatment options. However, there is a varying level of its efficacy, response over time and neurological outcomes; particularly among neonates with seizures related to Hypoxic-Ischemic encephalopathy (28,55,60).

Studies reported varying level of seizure cessation using the recommended initial and repeated doses of phenobarbital in neonatal seizure. According to Gilman and colleagues, approximately 70% control of clinical seizure were reported (34). Whereas other controlled study of 59 neonates attained complete control of seizure in 43% of neonates(35).

Another open-label RCT from India found seizure control (seizure-free period of 24hours) in 72% of neonates that received initial 20mg/kg dose of phenobarbital (36). A retrospective study of 91 neonates with neonatal seizure treated showed 63% complete cessation of seizure ,17% partial or reduction of seizure episodes and 21% non-response to initial 20mg/kg phenobarbital dose (68).

According to retrospective study conducted by Thibault *et al*, seizure cessation was attained in 18/31 (58%) of neonatal seizure treatment with initial loading dose of phenobarbital following cardiac surgery (37). This study also reported 8(14%) of neonates developed adverse event; seven hypotension and one respiratory depression. A randomized, prospective study that administered Phenobarbital 40 mg/kg IV over one hour in term neonates with severe perinatal asphyxia depicted good outcome(seizure cessation) in 48.4%(15/31) neonates and no adverse event (15).

A retrospective, single-center cohort study comparing seizure resolution to either phenobarbitone, levetiracetam alone or combined with benzodiazepines showed 61.6 %(45/73 neonates) for phenobarbitone alone and 94.1% when combined with benzodiazepines (47).

Deepak and colleagues' retrospective chart review of 50 neonates for examining reasons for phenobarbital response failure in neonates with HIE and seizure showed significant difference among non-responders higher mean seizure score(frequency) and more injury of white matter, parenchymal cortex on MRI scan (69).

A recent retrospective cohort study of 108 neonates with electroencephalography (EEG)-confirmed seizures treated with either of Phenobarbitone or levetiracetam gained an equal of 36% complete seizure response (70). According to etiology response rate for seizure treatment with Phenobarbitone in neonates with HIE (n=32), total seizure cessation were 41%(9 of 22) for phenobarbitone and 50%(5 of 10). In contrary, other multi-center RCT reported 80% response rate to phenobarbital treatment which could be attributed to continuous video-EEG implementation that enables early identification of at-risk neonates (55,71). Varying levels of complete seizure cessation has been reported. Connell et al reported 6%, ye and Flanagan's 25%(8/32) within 120 minutes of administration while Boylan and colleagues had 29%(4/14)neonates responding to initial loading dose (20mg/kg) of Phenobarbitone(72–74). There has been reported cases of seizure recurrences within few days and majority of them had low seizure burden and normal/mildly abnormal ECG findings (75).

Current advances in clinical practices including continuous monitoring and remote specialist review of EEG video and development of automated neonatal seizure detection algorithms remain impractical on low-resource setting but can bring differing response rate to the treatment (55,76,77)

2.4. Predictors of Seizure Response to Phenobarbitone in HIE

The predictors of response to phenobarbital treatment in neonatal seizures presenting in hypoxic-ischemic encephalopathy are multi-factorial including neonatal characteristics, clinical profiles, and seizure related and treatment related factors.

Seizure resolution after treatment with phenobarbitone among neonates with hypoxic-ischemic encephalopathy has shown to be influenced by co-administration of benzodiazepines. According to study done by Wagner and colleagues, combined administration of benzodiazepines with phenobarbitone had increased neonatal seizure response from 61.6% to 94.1% (16/17 neonates) (47).

The average loading dose of phenobarbitone was 19.67 ± 3.96mg/kg in line with the recommended 20mg/kg and average maximum maintenance dose was 4.48 mg/kg/dose ± 2.18 for phenobarbital (47) while other study used initial maximum loading dose of 20mg/kg (15-20mg/kg) (78). Controlled seizure with initial dose of phenobarbitone was 63.9%, 77.4% including second dose, and average hospital stay of 1.88 days before response or outcome (MD 1.88 days: 95% CI 0.84–2.91) (79). According to study conducted by Rao *et al*., examining phenobarbital for the treatment of neonatal seizures associated with hypoxic–ischemic encephalopathy seizure frequency which is highest during the first 24 hours and seizure burden were predictive of time and response to treatment (78).

There is a mix of evidence on impact of neonatal seizure and treatment on neurodevelopmental outcome in hypoxic–ischemic encephalopathy. Secondary data analysis of neonatal research network's trial on effect of hypothermia in treatment of HIE, seizure burden were related with underlying severity of HIE rather than serious adverse neoro-developmental outcomes including death and disability (80). Similarly, another study reported that initial seizure frequencies were not determinant of the time to response or seizure free(78). However, there is a broad body of literature opposing this notion. For instance, animal study from laboratory showed poor neuro-developmental outcomes were derived from seizure burden and longer response time (44).

Furthermore, treatment of acute symptomatic seizures like in HIE with phenobarbitone had less risk of adverse long-term neuro-developmental outcomes unlike refractory seizures including neonatal status epilepticus (42,81,82). Study from India showed that neonatal seizures resulting from HIE with moderate to severe EEG background responded poorly to phenobarbital treatment of loading or maintenance doses(69).

Seizure frequency was one of the predictors of favorable or unfavorable treatment outcome in neonatal seizure management in Hypoxic-ischemic encephalopathy. Study by Linda et al identified that the risk of incomplete response to phenobarbitone treatment were 2.22, 3.26 and 3.53 times higher occasional seizures, frequent seizures, status epilepticus respectively, while gestational age, etiology, seizure frequency, EEG findings didn't yield significant effect on the response of treatment (70)

Various studies report that response to phenobarbitone treatment didn't vary by gestational age, sex, birth weight , APGAR score at fifth minute after birth, etiology or initial EEG findings (28,35,37). However, another study by Carolis et al reported therapeutic serum concentration is achieved at initial similar loading dose of 20mk/kg for preterm neonates and should be followed by 5mg/kg due to toxicity warning(83). Study done by Bath et al showed that efficacy of phenobarbital in treating neonatal seizure in hypoxic–ischemic encephalopathy were not significantly increased with an increase in dose administered (70). Additionally single bind RCT showed treatment response of 57% which was inversely associated with severity of seizure (35).

Hypoxic-ischemic encephalopathy in full term neonates accounts for over 80% of all seizures in the first two days of life and increased risk of clinical seizure in extremely low birth weight infants with or without other comorbidities (2,3). Neonatal seizure has detrimental effect on preterm newborns ranging from long-term neurodevelopmental impairment to death, cerebral palsy and epilepsy(84). Despite this effect, the incidence of its response to treatment is highly dependent on the method used to diagnose it. The most common adverse events following phenobarbitone treatment included hypotension, respiratory suppression, and sedation (70). Consistently, hypotension due to Phenobarbitone treatment of seizure has been reported in one half of neonates with hypoxic-ischemic encephalopathy and one-sixth of neonatal seizure treated after cardiac surgery requiring prompt improvement in management approaches for these newborns having hemodynamic instability (37,85)

2.5. Conceptual Framework

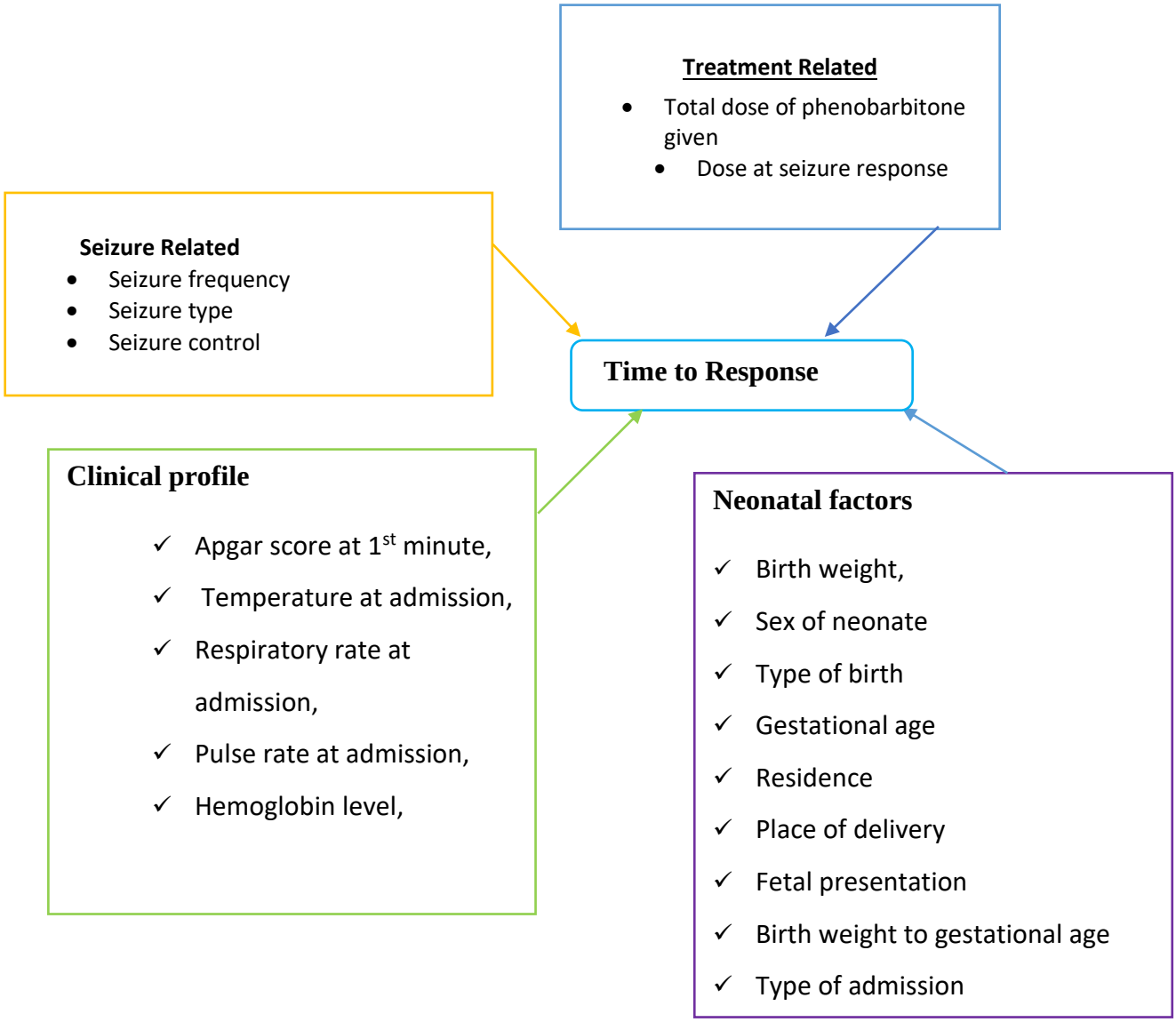


Figure 1: Conceptual framework relating response rate and time to response with predictors of seizure response (clinical profile, neonatal factors and comorbidities) to Phenobarbital therapy among neonates admitted with HIE at NCSH **(10,39,48,62,86–89)**

CHAPTER THREE: METHODS AND MATERIALS

3.1. Study Setting and Period

The study was conducted in Nekemte comprehensive specialized hospital which is located in Nekemte town; 333Kms West of the capital. East Wollega zone is climatically categorized as "Sub-tropical". This zone is bordered in the East by West Shoa, in the North by Horo Guduru Wollaga zone and Benishangul Gumuz region, in the South by Bunno Bedele, and in the West by West Wallaga zone. East Wallaga is divided into 17 woredas and one town. Its population is estimated to 1.6 million people. There are five government hospitals, and 67 health centers in this zone. Nekemte comprehensive specialized hospital was founded by Swedish missionaries in 1932. The town is home of 75,219 total population; 38,385 men and 36,834 were women(90). The hospital provides various comprehensive and specialist services including pediatrics and child health, surgery, gynecological and Obstetric care, medical care, oncology unit and other services. Its pediatric department has neonatal intensive care unit with 40 total bed, 42 beds for pediatric ward (with NRU, critical care and other service units). One pediatrician, one general practitioner and six NICU nurses were working in the unit for 2020 and 2021. Since 2022, two pediatricians (one daytime the other night shift), one GP and 11 NICU nurses are overseeing the pediatric department. Over the preceding four years (from January 1st ,2020 to December 31st , 2023), a total of 1044 neonates were treated according to national guideline for the neonatal seizure due to perinatal asphyxia, from which 588,75 are PNA Stage II& III HIE according to Sarnat and Sanat scoring system. The data was retrieved from January 4 to March 6,2024 from the record of neonates treated with neonatal seizure in stage II& III HIE during four year period, from January 1st ,2020 to December 31st , 2023.

3.2. Study Design

Hospital-based retrospective cohort study was conducted.

3.3. Population

3.3.1. Source Population

All neonates admitted to neonatal intensive care unit of Nekemte Comprehensive Specialized hospital with the diagnosis of PNA stage II and III hypoxic-ischemic encephalopathy and treated with phenobarbital (initial and repeated two doses) from January 1st ,2020 to December 31st , 2023.

3.3.2. Study Population

Randomly selected neonates admitted to NICU of Nekemte Comprehensive Specialized hospital with the diagnosis of PNA stage II and III Hypoxic-Ischemic Encephalopathy and treated with initial (20mg/kg) or repeated (10mg/kg if seizure persists) naso or oro-gastric phenobarbitone from January 1st , 2020-December 31st 2023, fulfilling eligibility criteria.

3.4. Eligibility Criteria

3.4.1. Inclusion Criteria

The study included preterm and term neonates admitted and treated at NICU:

- ✓ All clinically diagnosed PNA stage II and III Hypoxic-Ischemic Encephalopathy and treated with naso-gastric phenobarbital (any of tablet or syrup preparation of 30,50,100mg) initial loading dose of 20mg/kg, repeated 10mg/kg (twice if seizure persists till total dose of 40mg/kg) from January 1st , 2020- December 31st , 2023.

3.4.2. Exclusion Criteria

- If other causes of seizure including congenital brain anomalies, and other congenital anomalies incompatible with life, neonatal meningitis, hypoglycemia, electrolyte imbalance
- Those diagnosed with PNA stage I Hypoxic-Ischemic Encephalopathy
- Those diagnosed with *neonatal status epilepticus* was excluded, due to its known refractoriness to phenobarbital therapy. NSE is previously defined as continuous seizure activity for at least 30 minutes or recurrent seizures lasting a total of 30 minutes without definite return to the baseline neurologic condition between seizures, in any 1-hour period , currently International league Against Epilepsy (ILAE) defined as an occurrence of a seizure with five minutes or more of continuous clinical and/or electrographic seizures activity, or recurrent seizure activity without recovery between seizures (91–93)

3.5. Sample Size Determination

To calculate sample size, the general formula to calculate sample size for time to event data in survival analysis was computed as:-

Sample size (n) = Number of event (E)
Probability of event (PE)……(94)

To calculate sample size and number of event, STATA version 14 was used using command ***"stpower logrank 0.5, hratio ( ) power (0.8) wdprob (0.15)",*** taking HR for different variables (Table 1), Log rank 0.5 and withdrawal probability of 15% for incomplete medical chart. Hazard ratio taken from previous study conducted at UCLA Mattel Children's Hospital neonatal intensive care unit, USA yielded maximum sample size of 284 (39). After sample size and number of event obtained from STATA, probability of event estimated based on number of events and sample size.

- To calculate sample size for survival data, log rank method was used, which depends on power and hazard ratio of the study. Log rank method used to calculate sample size for survival data by assuming proportional hazard assumption, and adjustment for withdrawal. The value of log rank is equal with**=0.5**.

Hypothesis

- H0: $S_1(t) = S_2(t)$

$S_1(t)$ =Survival function at time t1,

$S_2(t)$ =Survival function at time t2,

Table 1: Sample size calculation for assessing response rate, time to response and predictors of seizure response to Phenobarbital therapy among neonates admitted with PNA-HIE at Nekemte Comprehensive Specialized Hospital, 2024

Variables	**HR**	**CI (95%)**	**Event**	**Probability of event**	**Sample size**
Higher Severity score of seizure (<1HR- non/partial response)	0.56	0.39-0.79	100	0.35	**284** (39)
Frequent seizure (higher IRR indicate incomplete/non-response)	4.95	1.44-27.1	18	0.6	30 (70)
Less severe HIE stage (>1HR-complete response)	2.57	1.26–5.27	42	0.58	72 (39)
Abnormal EEG finding (<1HR-non/partial response)	0.25	0.1-0.59	22	0.28	78 (39)

3.6. Sampling Procedure

The study considered all neonates admitted to intensive care unit those fulfilling the inclusion criteria. The hospital's report for PNA and HIE admissions are shown below (Table 2)

Table 2: Total report of neonates admitted to NICU of Nekemte comprehensive specialized hospital with the diagnosis of PNA and HIE, 2024

Year	PNA (total n=1044) Categorized using Sarnat and Sanat Score		
	HIE stage I	HIE stage II	HIE stage III
2020	53	174	32
2021	68	222	26
2022	79	192	17
2023	52	91	38
Total	252	679	113
Monthly Average	29		

Systematic random sampling technique was used to select individual patient record. Sampling interval was calculated as K=N/n where N is source population, n is calculated sample size and K is sampling frame. K=1044/284~4, meaning every 4^{th} medical record of neonate treated for neonatal seizure in HIE stage II& III were used. After the first four medical record numbers are listed, the first case was selected using lottery method.

3.7. Study Variables

3.7.1. Dependent Variable

Time to response to phenobarbitone therapy

3.7.2. Independent Variables

- ***Neonatal characteristics:*** birth weight, post-natal age, sex of neonate, type of birth, , gestational age, residence, place of delivery, fetal presentation , type of admission ,
- ***Clinical Profile:*** Apgar score at 1st minute, Apgar score at 10th minute, temperature at admission, respiratory rate at admission, pulse rate at admission, oxygen saturation at admission, hemoglobin, blood glucose,
- ***Seizure related :*** Seizure frequency before medication, seizure type, seizure response, seizure time of onset after birth
- ***Medication related*** :
    - Total dose of phenobarbitone given
    - Dose at complete seizure response

3.8. Variable and Operational Definitions

Response: Is complete cessation of clinical seizure following an administration of initial loading (20mg/kg) or two repeated doses(10mg/kg each) of phenobarbitone; then kept on maintenance dose (5mg/Kg in two divided dose until discharge) and no administration of further antiepileptic drug.

Survival time: the time from admission to the neonatal intensive care unit to the occurrence of an event or censoring

Event: Referred to those neonates who discharged by pediatrician in charge as controlled clinical seizure (complete seizure cessation after full course of phenpoobarbitone therapy)

Censored status: Neonates that didn't develop an event (death, shifted to further anti-epileptic drug combination treatment)

Low birth weight baby: a baby who was born with a weight of less than 2,500 gram(95).

Premature baby: a baby who was born before 37 completed weeks of pregnancy(95).

APGAR score classification: A score of 7-10 is considered normal, while < 7 is low(can be further classified as 3-6 and 4-7) and need expanded scoring and management(96).

Perinatal asphyxia: defined based on the presence of an Apgar score < 5 at 5 minutes, need for resuscitation with positive pressure ventilation and oxygen for>1minute immediately after birth, an arterial pH of< 7.10, and a base deficit of greater than -12 mmol/L^{-1} within the first hour after birth(29).

3.9. Data Collection tool and Procedures

Data collection tool is adopted from studies conducted by Battig *et al* and Rao *et al.,* (39,70). The data source for this study was secondary data from the hospital NICU registration logbook and patient folder. The tool consists of five parts, which comprises neonatal characteristics, clinical profile of the baby, seizure related, medication related, and comorbidity conditions. The starting point for retrospective follow-up was the time from first date/hour of admission and the endpoint of follow-up will be the date/time of experiences of response and censored. Data was collected by using chart review with data extraction tool by five, trained BSc pediatric Nurses. Data extraction checklist was prepared in English but supported by translated narration in Afan Oromo language.

3.10. Data Quality Assurance

One day training was given for data collectors regarding the tool and objective of the study. The data extraction tool was pre -tested on 5% (14) of the sample at Wollega University Referral Hospital. Based on the findings and feedback obtained from the pre-testing process, minimal modification was done on the tool in accordance with objective of the study. A supervisor was assigned during data collection for ensuring completeness, accuracy and consistency of data.

3.11. Data management and Analysis

Data were checked for their completeness and consistency. Data was analyzed using STATA MP version 17 for statistical analysis. Before analysis, data was cleaned by using simple frequencies and cross tabulation. Re-categorization of categorical variables and categorization of continuous variables was done to make suitable for analysis. Finally, the outcome of each participant was dichotomized into censored or recovered. Incidence rates were calculated for the entire study period, the number of responses within the follow up divided by the total person time at risk on follow up and reported per 1000PHs. Hour was used as a time scale to calculate median survival time. The life table was used to estimate the cumulative survival probability of neonates. Kaplan Meier survival curve was used for the estimation of survival probability and Log rank test for statistical difference of survival probability among the groups.

Testing the proportional hazard assumption is vital for interpretation and use of fitted proportional hazard models. Schoenfeld residuals test for the individual covariates, log-log plot and global tests were used to assess proportional hazard assumption. Backward stepwise procedures were employed (procedures deleting one variable with large p-value at a time as the regression model progresses). The Log-likelihood (LL) value was considered to select the best fit model. Finally, Cox Snell residual graph was used to assess overall model adequacy of proportional hazard model. A Cox proportional hazards regression model was used to identify predictors of response to phenobarbital treatment. Independent variables with P-value ≤ 0.25 in bi-variable Cox-regression were candidate variables for multivariable Cox regression analysis. Adjusted Hazard Ratios (AHR) with 95% confidence intervals was computed and statistical significance set at (p-value < 0.05). Finally, data is presented using tables, narratives, figures and graphs.

3.12. Ethical Clearance

Ethical clearance was obtained from the IRB of Addis Ababa University College of Health Sciences, CDT-Africa. Additionally, permission letter was secured from hospital management to guide and ease access of medical records and necessary cooperation at department level. Information sheets were presented to hospital management about the study objective and data collection, privacy and anonymity during extraction and publication. Based on the utilization of secondary data sources (patient medical record) Addis Ababa University, college of Health Sciences' Scientific and Ethics Review Committee waived the need for informed consent. All data were collected ensuring confidentiality and anonymity of patient record by using codes to de-identify patient profile.

3.13. Result Dissemination Plan

The study report is submitted and presented to Addis Ababa University College of Health Sciences, CDT-Africa. It will be published in peer-reviewed international journal and presented in scientific conferences.

CHAPTER FOUR: RESULTS

4.1. Sociodemographic characteristics of neonates

This study analyzed the medical records of 284 neonates who were admitted to the neonatal intensive care unit of Nekemte Comprehensive Specialized Hospital with a diagnosis of hypoxic-ischemic encephalopathy. The study period was from January 1, 2020, to December 31, 2023. Male neonates accounted for slightly over half (52.47%) of the admissions. The majority of participants were born at term (80.64%) and 70.77% had a cephalic presentation during delivery. Nearly three-fourths (73.2%) had a normal birth weight and 77.11% were delivered via spontaneous vertex delivery. Similarly, the majority of them (93.66%) were delivered in a health institution, and over half (58.45%) were admitted after being delivered in the same hospital (Table 3).

Table 3: Socio-demographic characteristics of neonates of neonates admitted with hypoxic ischemic encephalopathy in Nekemte comprehensive specialized hospital, 2024 (N=284?)

Variable	**Response Category**	**Status**		**Total**	
		Responded	**Censored**	**No**	**Percent**
Sex of neonate	Male	109	40	149	52.47%
	Female	101	34	135	47.53%
Gestational age	Post term (>42wks)	14	6	20	7.04%
	Term [37-42wks]	170	59	229	80.64%
	Preterm [28-37)	26	9	35	12.32%
Fetal presentation	Cephalic	146	55	201	70.77%
	Brow	13	3	16	5.63%
	Breech	51	16	67	23.59%
Birth weight	Normal [2.5-4kg]	152	56	208	73.24%
	High (>4kg)	31	10	41	14.44%
	Low [1-2.5kg)	27	8	35	12.32%)
Type of birth	SVD	111	58	219	77.11%
	C/Section	17	4	21	7.39%
	Assisted vaginal delivery	32	12	44	15.49%
Place of delivery	Home	10	8	18	6.34%
	Health institution	200	66	266	93.66%
Residence	Urban	32	90	122	42.96%
	Rural	42	120	162	57.04%
Type of admission	Inborn	129	37	166	58.45%
	Referred from other	75	34	109	38.38%
	From Home	6	3	9(3.17%)	

4.2. Clinical profile of neonates admitted with HIE

A majority (69.72%) of newborns delivered at a health institution had an APGAR score between 4 and 7 at one minute after birth. Additionally, 72.89% were moderately hypothermic, 77.11% were appropriate for gestational age (AGA), and 63.73% had a normal range of breathing pattern. In terms of their hemoglobin level, 86.97% had a normal level. It is worth noting that for the type of seizure, most cases (68.66%) were either not recorded or unknown due to home delivery (Table 4).

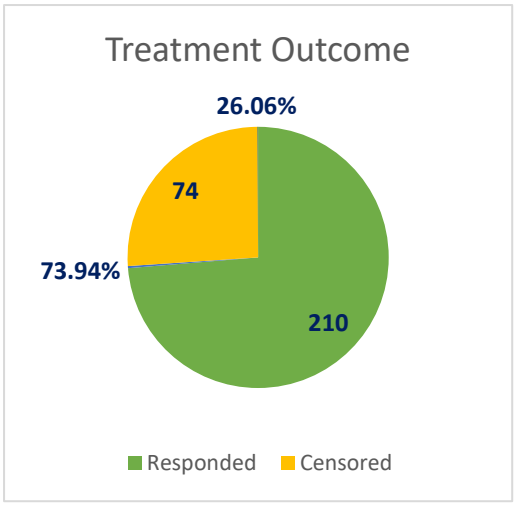
Table 4: Clinical characteristics of neonates admitted with hypoxic-ischemic-encephalopathy in Nekemte comprehensive specialized hospital, 2024 (N=284)

. **Variable**	**Response category**	**Status**		**Total**	
		Responded	**Censored**	**No**	**Percent**
APGAR score at 1st min of birth	1-3	29	39	68	23.94%
	4-7	171	27	198	69.72%
	Unknown/Not recorded	10	8	18	6.34%
Body Temperature	Normal (36.5^{0}C -37.5^{0}C)	17	9	26	9.15%
	Febrile (>37.5^{0}C)	2	3	5	1.76%
	Mild Hypothermia (36.0-36.4^{0}C)	23	15	48	16.90%
	Moderate Hypothermia (32.0-35.9^{0}C)	166	41	207	72.89%
	Severe Hypothermia (<32.0^{0}C)	2	6	8	2.82%
Birth weight to GA	AGA	182	37	219	77.11%
	LGA	14	27	41	14.44%
	SGA	14	10	24	8.45%
Respiratory rate(breathes/min)	30-60brpm	156	25	181	63.73%
	<30brpm	31	29	70	24.65%
	>60brpm	23	20	43	15.14%
Pulse rate((beats/min))	120-160bpm	137	16	153	53.87%
	<120bpm	41	32	73	25.70%
	>160bpm	32	26	58	20.42%
Hemoglobin(mg/dL)	13.5-20mg/dl	188	59	247	86.97%
	<13.5mg/dl	19	9	28	9.86%
	>20mg/dl	3	6	9	3.17%
Seizure type	Subtle	61	1	62	21.83%
	Clonic	2	3	5	1.76%
	Myoclonic	1	1	2	0.70%
	Not mentioned	137	58	195	68.66%
Seizure number	Once	57	2	59	20.77%
	Twice	146	25	171	60.21%
	≥ 3	7	47	54	19.01%

AGA/LGA/SGA-Appropriate, large and small for gestational age

4.3. Treatment outcome of neonates admitted with HIE at Nekemte comprehensive specialized hospital ,2023

Of the neonates admitted with neonatal seizures in hypoxic-ischemic encephalopathy, 210 responded to phenobarbital treatment, while 26.06% did not respond (censored) during the study period (Fig 2 A). Among those who responded, the majority (61%) had their seizures controlled after the second dose (first reloading dose of phenobarbital) (Fig 2B).



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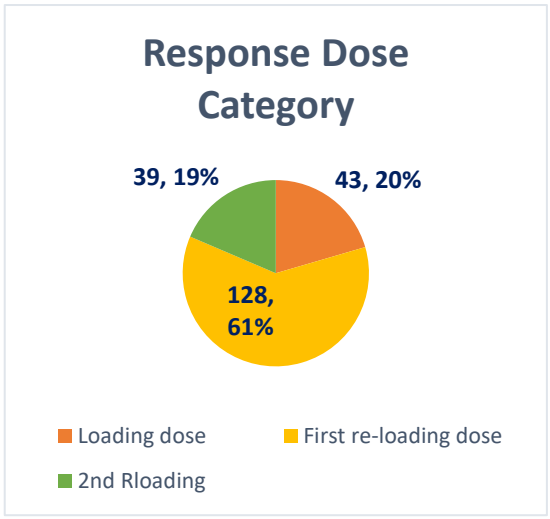

B

Figure 2: Treatment outcome and dose seizure control among neonates admitted with HIE in Nekemte comprehensive specialized hospital, 2024

4.4. Overall Kaplan- Meier survival estimate

Overall, Kaplan-Meier was used to estimate survival (in this case, response to treatment). Out of the 284 neonates included in the study and admitted to the NICU, 119 (41.9%) responded to phenobarbital therapy by the end of the 30th hour. The median response time was 29 hours with an interquartile range (IQR) of 26.5-32 hours and a mean of 26.66 hours (95% CI 25.94, 27.39). During the follow-up period, a total of 7,572.7 person-hours were observed, with a minimum follow-up time of 7 hours and a maximum of 38 hours. The overall incidence rate of response was 27.73 (95% CI 24.22, 31.74) per 1000 neonates with HIE person-hours. Figure 3 displays the overall Kaplan-Meier survivor function, which shows a rapid decrease in response rates after the 25th hour of treatment, indicating that most neonates responded to phenobarbital during this time.

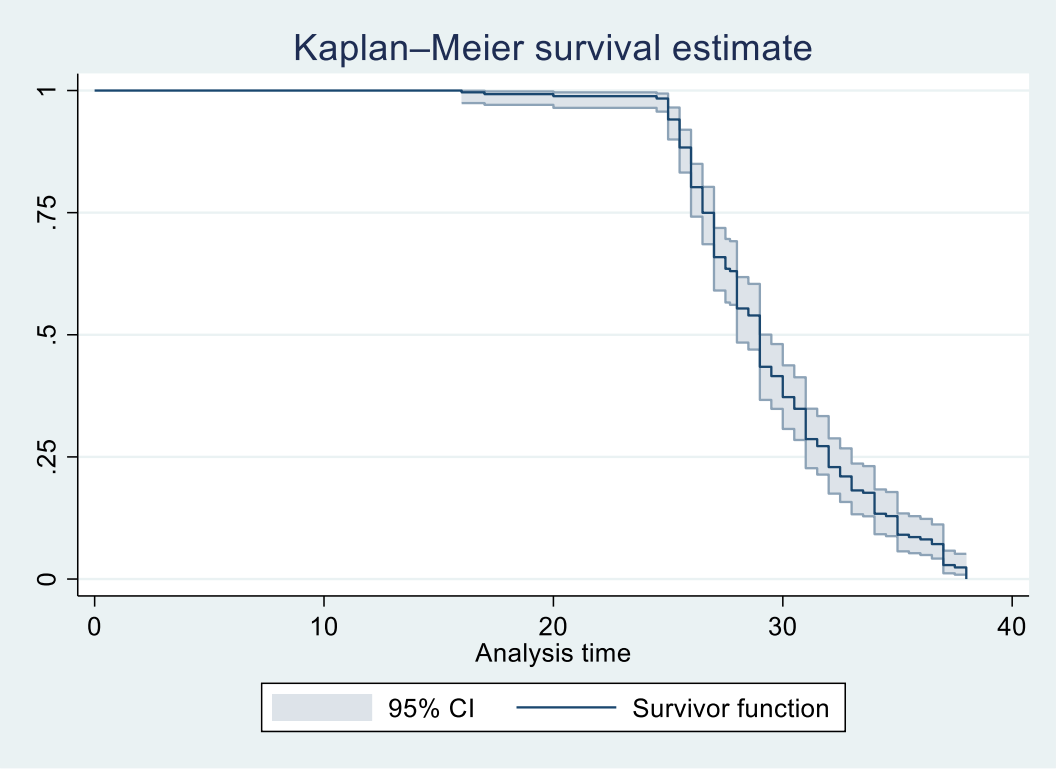

Figure 3: Overall Kaplan-Meier Survival probability curve among neonates admitted to NICU with HIE in Nekemte comprehensive specialized Hospital, Ethiopia ,2024.

The cumulative survival probability at the end of 20th, 25th ,30th, 35th hours was 2.34, 9.21,171.92 and 240.82 per 1000person-hour observations respectively (Table 5).

Table 5: Life table of cumulative survival analysis of time to response and its predictors among neonate admitted with HIE in NICU of Nekemte comprehensive specialized Hospital, Ethiopia ,2024.

Estimated person-hour and incidence rates

Cohort	Person-time	Failures	Rate	[95% conf. interval]	
(0- 5]	1420.00	0	0.0000	-	-
(5-10]	1414.00	0	0.0000	-	-
(10-15]	1392.00	0	0.0000	-	-
(15-20]	1284.00	3	0.00234	0.00075	0.00724
(20-25]	1085.50	10	0.00921	0.00496	0.01712
(25-30]	692.20	119	0.17192	0.14364	0.20575
(30-35]	245.00	59	0.24082	0.18658	0.31082
>35	40.00	19	0.47500	0.30298	0.74469
Total	7572.70	210	0.02773	0.02422	0.03175

4.4.1. Comparison of Kaplan-Meier curve functions for different categorical variable

The Kaplan-Meier estimator survival curve provides an estimate of survival among different groups of covariates, allowing for comparisons. Separate graphs were constructed to show the estimates of the Kaplan-Meier survivor function for different covariates, as described below. In general, if one survival function lies below another, it means that the group defined by the lower curve has a better recovery time or a higher recovery rate than the group defined by the upper curve.

Additionally, a log-rank test was performed to determine whether the observed differences seen on the plot are significant or not. Separate Kaplan-Meier graphs and log-rank tests were performed to estimate survival probability and test for statistical differences in survival probability among the groups, respectively. .

There was a difference in median response time for neonates with normal birth weight, high birth weight, and low birth weight. The median response time for neonates with normal birth weight was 29 hours, while it was 27 hours for the rest (P value = 0.029). The median response time for neonates who had an APGAR score between 4 to 6 was 29 hours, compared to 30 hours for those with an unknown status (P value = 0.041).

The median response time for neonates who had severe hypothermia was 26.5 hours, while it was 30.5 hours and 29 hours for neonates with a normal body temperature and febrile neonates, respectively (P value = 0.035). The median response time for neonates born large for gestational age was shorter at 27 hours, while it was 29 hours for both small for gestational age (SGA) and appropriate for gestational age (AGA) neonates (P value = 0.044). Neonates who had a history of one seizure had a median response time of 27.5 hours, compared to 29 hours and 19 hours for those who had a seizure frequency of twice and thrice, respectively (P value = 0.00) (APPENDIX III).

4.5. Response rate and median response time among admitted neonate to NICU

Out of a total of 284 admitted neonates included, 210 observations (73.94%) experienced an event (responded). The median response time was 29 hours, with an interquartile range (IQR) of 26.5-32 hours, and a mean of 26.66 hours (95% CI 25.94, 27.39). During the follow-up period, a total of 7,572.7 person-hours were observed, with a minimum of 7 hours and a maximum of 38 hours of follow-up time. The overall incidence rate of response was 27.73 (95% CI 24.22, 31.74) per 1000 neonates with HIE person-hours. The highest incidence of response was observed at the end of the 30th and 35th hours of enrollment, with rates of 171.92 and 240.82 per 1000 person-hour observations, respectively (Table 5).

4.6. Test of proportional hazard assumption

For each covariate, the Cox proportional hazard assumption was performed individually. The Schoenfeld residuals proportional hazard assumption test and log-log plot test were used, along with simultaneous global tests. As a result, it was determined that each covariate (p-value > 0.05) and all covariates simultaneously (Global test for Cox proportional hazard: p-value = 0.6638> 0.05) met the proportional hazard assumption. Additionally, the assumption of a constant hazard ratio over time was satisfied. Therefore, the Cox PH model was deemed adequate for this data (APPENDIX IV).

4.7. Multi-collinearity Test

Measure of multi-collinearity between predictor variable was measured by using variance inflation factor (VIF), which was based on the binary cox-regression outcome. VIF is an estimate of how much the variance of a regression coefficient is inflated due to multi-collinearity, which will not affect the explanatory power of the variable but its statistical significance. This study's mean VIF value was 1.43, with the greatest value of 3.15 (less than 10 was conventionally tolerable) (APPENDIX V).

4.8. Predictors of response to phenobarbitone therapy

To identify predictors of response, we conducted bi-variable and multivariable Cox regression analyses. In the multivariable Cox analysis, we selected variables based on a p-value ≤ 0.25 from the bi-variable Cox regression. We then performed a full multivariable Cox analysis using a backward stepwise selection process, including all potential risk factors that had a p-value ≤ 0.25 in the bi-variable Cox proportional hazard analysis .

As a result, we selected 7 variables for the stepwise variable selection process. These variables were seizure frequency, birth weight, sex, APGAR score at 1st minute, respiratory rate, body temperature at admission, and seizure type. We chose the optimal model based on the Log Likelihood Ratio (LLH). After conducting a backward stepwise variable selection process, we identified the first model as the best model according to LLH (Table 6).

Table 6 : Model selection based on Log likelihood ratio (LLH)

Model	Observation	DF	LLH
1	284	1	-1324.0524
2	284	2	-1292.5387
3	284	3	-1255.3871
4	284	4	-1244.6165
5	284	6	-1244.6015
6	284	7	-1244.6015

In general, for model building, all variables that met at least two of the assumptions were selected. Additionally, all variables satisfied the assumption of proportional hazard assumption. Finally, it was discovered that four variables had a statistically significant association with the incidence of seizure response to phenobarbital therapy. These variables are birth weight, body temperature at admission, seizure frequency, and seizure type (Table 8).

Multivariable Cox proportional regression analysis revealed that the frequency of seizures was significantly associated with the response to phenobarbital therapy. Neonates who had two or more seizures during admission with HIE had a 56.4% lower response rate compared to those who had only one seizure (AHR=0.436, 95% CI 0.31, 0.61).

Birth weight was one of the factors that predicted the recovery of neonates. Neonates with low birth weight had a 41% lower response rate to phenobarbital therapy compared to newborns with normal birth weight (AHR=0.59; 95% CI 0.58, 0.98).
The body temperature of neonates during admission showed a statistically significant association with seizure response in this study. The risk of seizure response among neonates with severe hypothermia was 77% lower compared to babies with normal body temperature (AHR=0.23; 95% CI 0.052, 0.26).

The type of seizure also showed a statistically significant association with seizure response. Neonates who had subtle seizures were 2.35 times more likely to respond to phenobarbital treatment compared to those with tonic seizures (AHR: 2.35; 95% CI 1.09, 5.08).

Table 7 :Multivariable cox proportional analysis of time to response and its predictors among neonate admitted with HIE in NICU of Nekemte comprehensive specialized Hospital, Ethiopia, 2024

Variable	Category	Survival status		CHR (95%CI)	AHR (95%CI)	P- value
		Responded	Censored			
Seizure frequency	Once	57	2	1	1	1
	Twice	146	25	.49(0.34,0.65)	0.44(0.32,0.61)	**0.00***
	≥ 3	7	47	-	-	-
Birth weight	Normal	190	18	1	1	1
	High (>4kg)	14	27	.79(.54, 1.18)	0.51(0.87,1.07)	0.833
	Low (1-2.5Kg)	6	29	0.84(0.75,0.97)	0.59(0.058,0.98)	**0.026***
Sex	Male	109	40	1	1	1
	Female	101	34	0.78(0.83,0.96)	1.24(0.92,1.65)	0.165
APGAR Score at 1st min of birth	1-3	29	39	1	1	1
	4-6	171	27	0.8(0.54,0.87)	0.74(0.54,1.02)	0.069
	Unknown	10	8	0.47(0.23,0.98)	0.58(0.32,1.06)	0.076
Respiration rate	Normal (30-60brpm)	156	25	1	1	1
	Bradypnea (<30brpm)	31	29	0.78(0.21,0.53)	0.76(0.50,1.15)	0.189
	Tachypnea (>60brpm)	23	20	0.72(0.50,0.71)	0.81(0.52, 1.31)	0.387
Body temp at admission	(36.5^{0}C -37.5^{0}C)	17	9	1	1	1
	>37.5^{0}C	2	3	0.91(0.43,0.68)	1.73(0.38,7.81)	0.476
	36.0-36.4^{0}C	23	15	1.93(1.02,3.66)	1.85(0.95,3.59)	0.069
	(32.0-35.9^{0}C	166	41	1.51(0.91,2.51)	0.52(0.88,1.61)	0.132
	<32.0^{0}C	2	6	0.43(035,0.55)	0.23(0.05,0.26)	**0.019***
Seizure type	Tonic	8	11	1	1	1
	Subtle	61	1	2.16(1.025,4.5)	2.35(1.09,5.09)	**0.030***
	Clonic	2	3	2.01(0.42,9.56)	1.49(0.35,6.26)	0.587
	Myoclonic	1	1	0.56(07,4.52)	0.28(0.38,2.08)	0.215
	Not mentioned	137	58	1.72(0.84,3.53)	0.89(0.65,1.23)	0.480

4.9. **Model goodness of fit**

Following multivariable Cox proportional hazards model, the adequacy of the fitted model was observed using Cox-Snell residuals. The Nelson-Aalen cumulative hazard function graph was compared to the Cox-Snell residuals variable against the diagonal line. A hazard function closely followed 45-degree line suggesting data best fitted the model (Figure 5).

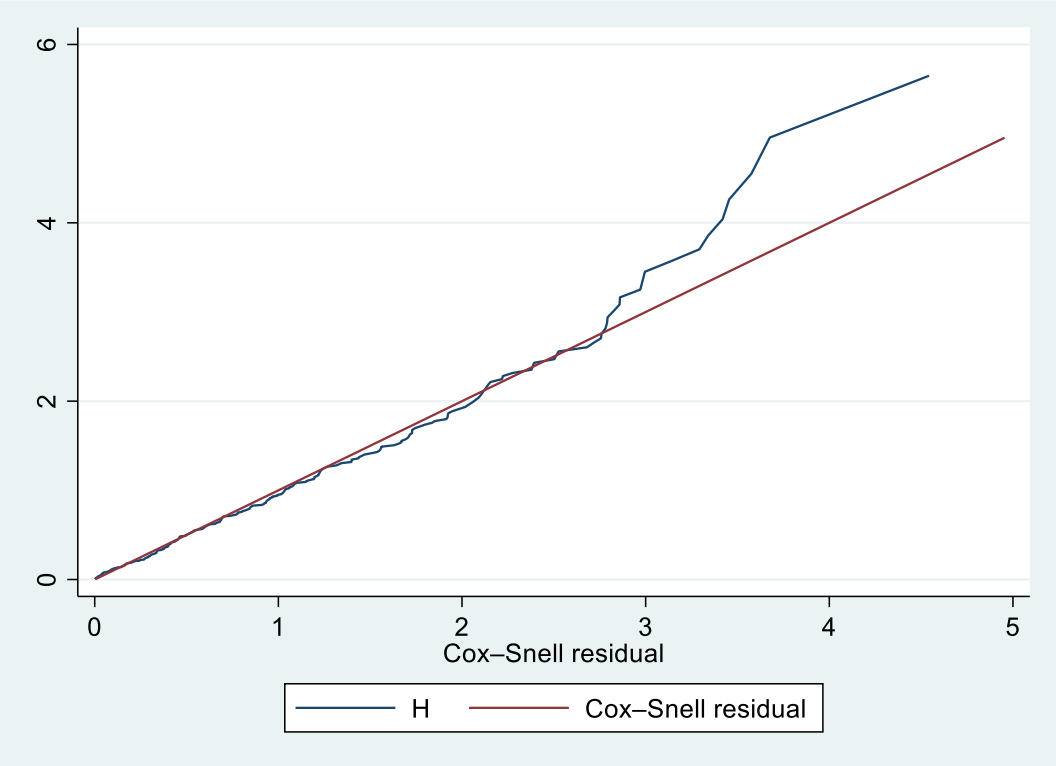

Figure 4:Cox Snell residual test for overall adequacy of the model

CHAPTER FIVE: DISCUSSION

This retrospective study assessed the time to response and its predictors among neonates admitted with HIE in the NICU of Nekemte Comprehensive Specialized Hospital. This study shows that the overall response rate of neonatal seizure in HIE was 73.9%. This is somehow similar to a study done by the National Institute of Child Health and Human Development trial study in the United States (77.4%), but higher compared to other reports (14,24,33,69,83).

Furthermore, 63.9% of neonatal seizures in this study responded to the initial loading dose, while 77.4% of seizure freedom was attained after the first re-loading dose of phenobarbitone. Thibault and other studies (71, 73, 85) reported a higher response rate compared to this study finding with the initial loading dose. This gap might be due to differences in the care provided, equipped material and quality of care, in those study settings. In addition, absence of electrographic seizure diagnosis and management in current study setting would contribute underdiagnosis of neonatal seizures.

Neonates that had two sessions of seizures during admission with HIE had a lower response rate by 56.4% compared to those who had a seizure history of once. This is in line with the study conducted by Linda and colleagues which identified that the risk of incomplete response to phenobarbitone treatment was 3.26 times higher in more frequent seizures compared to a single or second episode (69). Another study also reported poor response and neurodevelopmental outcome derived from seizure burden and longer response time. This could be explained by the fact that seizure burden is related to the underlying severity of HIE, and continued seizure attacks have a biochemical effect on the neonate's vital organs.

Neonates with low birth weight had a 41% lower response rate to phenobarbitone therapy compared to normal birth weight newborns. This is consistent with another study which reported that nearly half of the preterm neonates responded to the phenobarbital therapy (69,83). This could be because of hypoxic-ischemic encephalopathy on the developing neonatal brain, the detrimental effect of seizures on preterm newborns ranging from long-term neurodevelopmental impairment, and hemodynamic instability that needs prompt improvement in management approaches for these newborns.

The body temperature of neonates during admission showed a statistically significant association with seizure response in this study. The risk of seizure response among neonates

with severe hypothermia was lower by 77% compared to babies with normal body temperature. This finding contradicts a study conducted by Pressler, which reported a consensus that mild hypothermia reduces the severity and burden of seizure in HIE (95). However, this was when therapeutic hypothermia (32-36°C) was used as a management approach. Other studies support this finding, with severe hypothermia (<32°C) indicating a lower response to phenobarbital therapy (77, 91). Severe hypothermia worsens HIE symptoms by exacerbating hypoxia, causing pulmonary vasoconstriction and pulmonary hypertension, and impairing surfactant production (48, 96).

In this study, subtle seizures comprised 89.8% of the overall seizure types mentioned in the medical records. This is consistent with a study from Gondar, which reported rates of 60.6%, 87.3%, and 60.7% in preterm, post-term, and all neonates, respectively (25). Similarly, other studies from various settings have also reported consistent findings (97–99). In contrast, there were studies reporting clonic seizures as the most common type in HIE (96, 97), while others reported tonic seizures as the predominant type (100). .

The subtle type of seizure also showed a statistically significant association with seizure response. Neonates who had subtle seizures were 2.35 times more likely to respond to phenobarbital treatment compared to those with tonic seizures. This finding agrees with some findings and contradicts others (95, 101). The discrepancy can be attributed to a greater number of seizures in the current study (195, or 68.66%) not being mentioned (categorized by the health worker in charge), and also to the fact that the other studies used EEG to diagnose and classify, while this study used clinical parameters (like symptoms algorithm). The clinical signs of subtle seizures are usually unnoticed because they mimic normal behaiviors and reactions. They are typically less severe than tonic or other variants, clinically modest, inconspicuous, and lack a discernible post-ictal state Furthermore, the prognosis for tonic seizures is poor since they often occur with intraventricular hemorrhage. Because myoclonic seizures often occur as part of the early myoclonic encephalopathy syndrome, which is the earliest presenting form of epileptic encephalopathies, they also have a bad prognosis (97,98). Phenobarbital acts on GABAA receptors, gamma-Aminobutyric acid (GABA), the principal inhibitory neurotransmitter in the cerebral cortex to inhibit neuronal excitation and then antiseizure effect. It can also inhibit calcium channels, resulting in a decrease in excitatory transmitter release. Furthermore, its sedative-hypnotic effects may be the result of its effect on the polysynaptic midbrain reticular formation, which controls CNS arousal (99,100). Hence, the extent of brain

damage due to hypoxic-ischemic encephalopathy may be exhibited by seizure severity and type so does the pharmacologic effect may vary accordingly.

The foremost limitation was the lack of use of multi-channel video EEG as an additional (to clinical) diagnostic tool in clinical setup. Electrical seizures (as confirmed by EEG) without clinical seizures may be under diagnosed. While using the EEG, the degree to which electrical seizures need to be suppressed, is again an unresolved issue. This electro-clinical dissociation can be resolved by combining both approaches. Besides, we did not included maternal factors and neonatal seizures may not be recognized by health care worker quickly and easily causing progression of insults affecting the response rate. The study did not assess therapeutic drug monitoring factors like hepatic, renal function, serum bilirubin, proteins that might affect serum phenobarbital level and response rate.

However, the study has significant strengths. First, it utilized four years data for establishing better rigor. As neonates are special and vulnerable groups, the study objective to examine the response rate of this widely used(recommended) anti-seizure drug would stimulate further scientific endeavor for its therapeutic monitoring and efficacy study in national context.

CHAPTER SIX: CONCLUSION AND RECOMMENDATION

6.1. Conclusion

In the current study, the response of neonates to phenobarbitone therapy was low with median response time of 29hours. Overall incidence rate of response was also low. Having history of twice/more frequency of seizure insult, severe hypothermia and low birth weight (LBW) decreased the response rate while subtle type of seizure increased likelihood of response to phenobarbitone therapy.

6.2. Recommendations

Federal and regional authorities should work on availing critical equipment like electroencephalogram (EEG) for seizure diagnosis and management, and various phenobarbitone preparations to the hospitals for improving neonatal intensive care service quality.

Health workers should practice and provide prompt action on essential newborn care that includes identifying high risk neonates like hypothermic and low birth weight newborns. Consistent recording of APGAR scores, seizure classifications for each newborn by attending healthcare worker is also recommended. Strong pharmaceutical supply chain management is needed to avail the IV preparation of phenobarbitone because current tablet preparations would not be always feasible to administer (e.g. half doses of the preparation) may cause inconveniences. Special emphasis and close follow up should be given to neonates admitted with high risk of neonatal seizure secondary to HIE.

Both zonal health office and hospital management should focus on implementing protocols and SOPs for risk factor identification and management to increase the response to phenobarbitone and survival of neonates during the neonatal period. The findings of this study will be used by the Maternal and Child Health Service Programs for quality improvement initiatives in NICU services as well as advancing NICU services level of the hospital. Efforts should be made to procure and avail the IV preparation of phenobarbitone for easy administration. Strengthening essential newborn care strategies are critical strategy in enhancing treatment response and thereby saving the lives of newborns.

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Appendix I: Information Sheet

This study aims to assess time to response and predictors of seizure response to phenobarbital therapy among neonates admitted with hypoxic-ischemic encephalopathy at Nekemte comprehensive specialized hospital, Ethiopia.

My name is _____. I am the data collector of this study to be conducted using randomly selected medical record of newborns admitted with neonatal care and treated with phenobarbitone. The researcher is called Wase Benti, a student of Masters of Science in Clinical Trials at CDT-Africa, Addis Ababa University, as you may get his address below.

I would like to review and record some data from neonates admitted with neonatal seizure and treated with Phenobarbitone loading or two repeated doses befoe addition of other anti-epileptic or any other even occurred. It will use systematic random sampling to select 282 records of patients treated during January 1, 2020 to December 31st, 2022. The study has profound benefit to improve evidence-based clinical practice in the neonatal care , as baseline for further experimental studies and development or update of care guideline for this specific illness. Further the study findings will be an input to regional bealth bureau, ministry of health and other development partners initiative of improving the care to reduce neonatal mortality.

The medical record of the neonates will be kept strictly confidential and any data obtained from it will be anonymized.

Your willingness to cooperate in permitting ease of access to record and possibly other support related to study is warmly appreciated. For any information , you may contact the principal investigator here under.

Date if interview_____, starting time _____; finishing time_____

Interviewer's name and code_____, Signature_____

Supervisor's name _____, Signature_____

Tele: +251975886808

Email: wasben333@gmail.com

Thank you for your cooperation!

Appendix II: Data Extraction Checklist

Code: _____

Part I: Neonatal characteristics

Code	Variables	Response	Remark
101.	Fetal presentation	1. Cephalic 2. Brow 3. Breech 4. Others -------	
102.	Gestational Age	1. Post term 42 0/7 wks 2. Term ≥37 0/7wks 3. Late preterm [34-37) 4. Moderate Preterm [32 to 34) 5. Very preterm [28-32) 6. Extremely preterm <28 weeks	
103.	Birth weight in gm	CDC Classifications 1. High birth weight >4000g 2. Normal birth weight [2500g-4000g] 3. Low birth weight [1500g-2500g) 4. VLBW [1000g-1500g) 5. ELBW <1000g	
104.	Postnatal age	_____(hours)	
105.	Sex of neonate	1.Male 2. Female	
106.	Type of Birth	1. Vaginal 2. Caesarean Section 3. Instrumental	
107.	Place of delivery	1. Home 2. Health Institution	
108.	Residence	1. Urban 2.Rural	
109.	Type of admission	1. Inborn 2. Referred from health institution 3. Home	

Part II. Clinical profile

Code	Variables	Response	Remark
201.	What was Apgar score at 5th minute?	_____	
202.	What was Apgar score at 10th minute?	_____	
203.	What was temperature at admission?	_____^{o}c	
204.	What was respiratory rate at admission at admission?	_____Breathes/min	
205.	What was pulse rate at admission at admission?	_____beats/min	
206.	What was oxygen saturation at admission?		
207.	What is the amount of hemoglobin level?	_____	
208.	What is the amount of blood glucose level?		
209.	Where is the newborn lies compared for its birth weight to its gestational age?	1. SGA 2. AGA 3. LGA	
210.	Where is the newborn lies compared for its gestational age?	1.Extremely preterm (<28wks) 2.Very preterm (28-<32wks) 3.Moderate preterm (32-<34wks) 4.Late preterm (34-<37wks) 5.Term (37-42wks) 6.Post-term (>42wks)	

Part III: Seizure related

Code	Variables	Response	Remark
301.	Seizure frequency before medication	1. Once 2. Twice 3. > 3 times	
302.	Seizure type	1. Subtle seizures 2. Tonic seizures 3. Clonic seizures 4. Myoclonic seizures 5. Not mentioned	
303.	Seizure time of onset after birth (how many days after birth)	_____	
304.	Seizure controlled	1.Yes 2. No	

Part IV: Medication Related

Code	Variables	Response	Remark

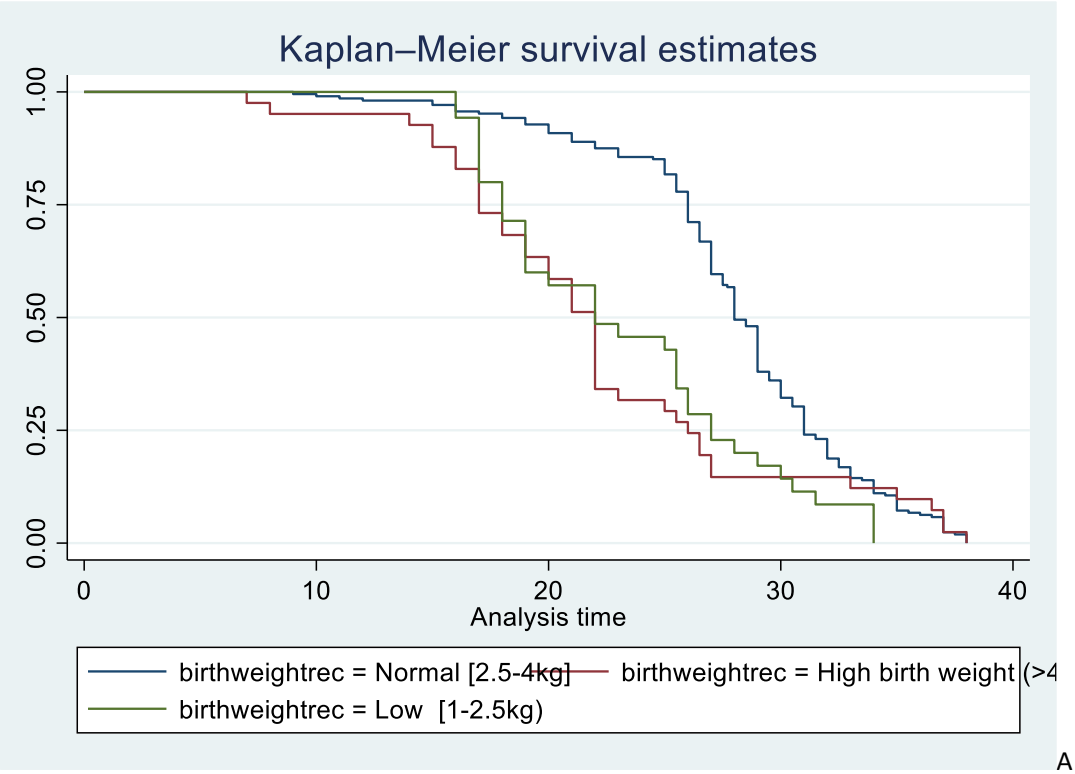
401.	Total dose of phenobarbitone given	_____mg	
402.	Dose at complete seizure response	1. After initial loading dose 2. First repeated dose 3. Second repeated dose	
403.	Co-administered drug	1. Yes 2. No	
404.	Name of Coadimintered medications	_____	
405.	Dose of co-administered medications	_____mg_ _____	

Part V: Comorbidity

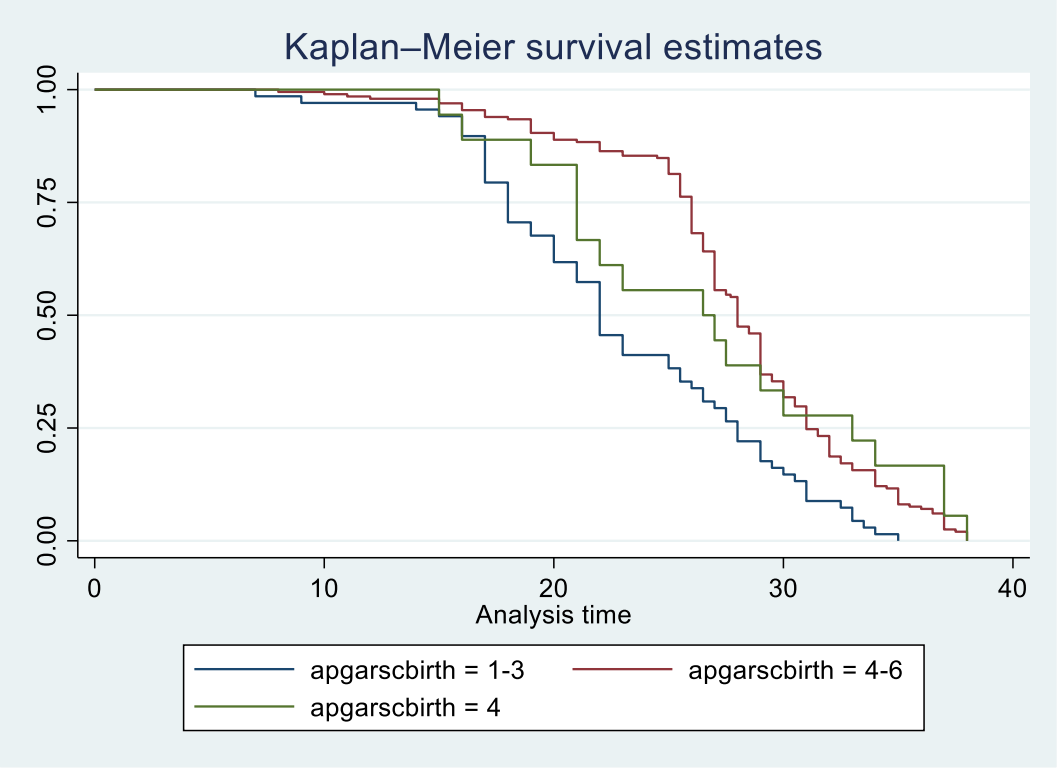
501.	Any major comorbidity	_____	

APPENDIX III: Comparison of Kaplan Meir for variables

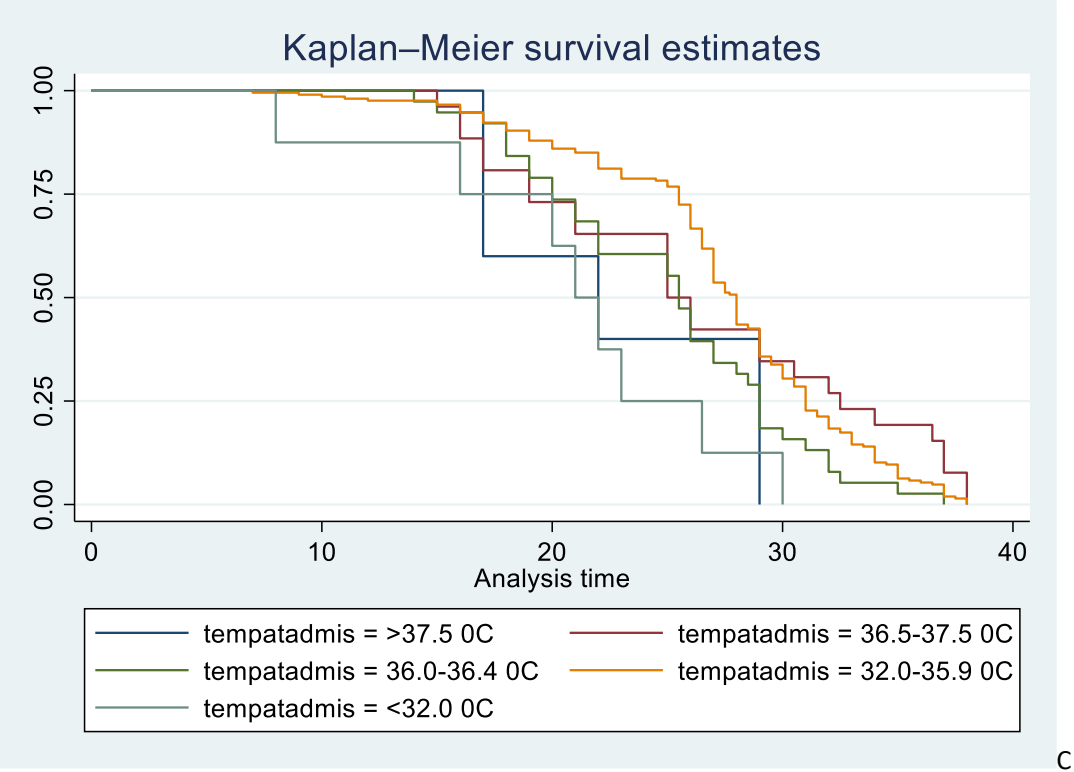
The Kaplan-Meier survival curves comparing response time of neonate with categories of birth weight, APGAR score at first minute of birth, body temperature, birthweight to gestational age and seizure frequencies in Nekemte comprehensive specialized hospital, Ethiopia 2023



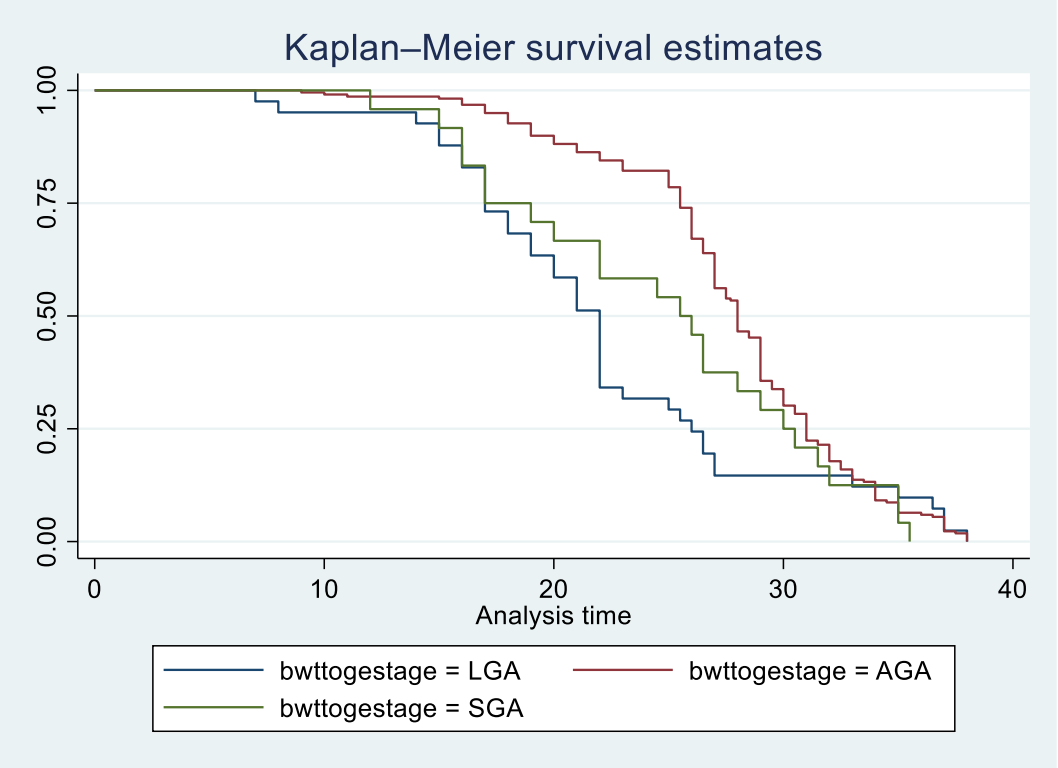
A



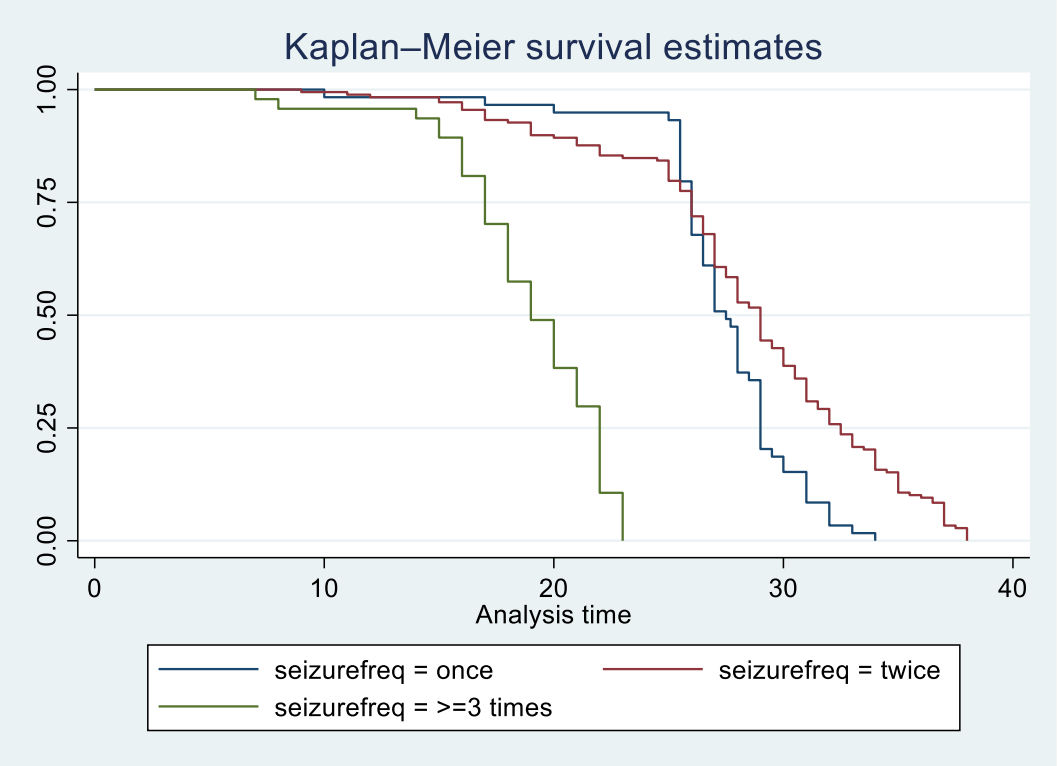
B


Kaplan–Meier survival estimates
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
Analysis time
tempatadmis = >37.5 0C
tempatadmis = 36.5-37.5 0C
tempatadmis = 36.0-36.4 0C
tempatadmis = 32.0-35.9 0C
tempatadmis = <32.0 0C

C


Kaplan–Meier survival estimates
0.00
0.25
0.50
0.75
1.00
0
10
20
30
40
Analysis time
bwttogestage = LGA
bwttogestage = AGA
bwttogestage = SGA

D


Kaplan–Meier survival estimates
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
Analysis time
seizurefreq = once
seizurefreq = twice
seizurefreq = >=3 times

E

APPENDIX IV: Schoenfeld Residuals test for proportionality assumption of each covariate and overall model of cox proportional hazard

Covariates	**Rho**	**X^2**	**df**	**P-value**
Fetal presentation	-0.06414	0.90	1	0.3437
Birth weight	-0.02268	0.11	1	0.7377
Gestational age	0.06287	0.87	1	0.3498
Sex	-0.02256	0.12	1	0.7261
Type of birth	-0.05584	0.79	1	0.3754
Place of delivery	-0.07485	1.19	1	0.2752
Residence	-0.00248	0.00	1	0.9700
Admission type	0.09049	1.96	1	0.1615
Apgar score at 1st minute	-0.05613	0.68	1	0.4104
Body temperature at admission	0.04989	0.59	1	0.4436
Birth weight to gestational age	0.10354	2.43	1	0.1191
Seizure frequency	-0.02016	0.09	1	0.7589
Seizure type	-0.08452	1.62	1	0.2028
Respiration rate	0.01893	0.08	1	0.7751
Pulse rate	0.01563	0.06	1	0.8096
Hemoglobin	0.08799	1.82	1	0.1769
Global test		13.12	16	0.6638

NB: Rho is the correlation coefficient between the residuals and time

APPENDIX V: VIF result of multi-collinearity test for predictors on response to phenobarbitone therapy, 2024

Variable	VIF	1/VIF
Fetal Presentation	3.17	0.315748
Place of delivery	3.15	0.317426
APGAR score at 1st Minute of birth	3.15	0.317131
Birth weight	1.64	0.610513
Birth weight to gestational age	1.32	0.757988
Type of birth	1.27	0.789407
Gestational age	1.20	0.835303
Seizure number	1.19	0.838927
Seizure type	1.13	0.888208
Hemoglobin level	1.11	0.902615
Respiration rate	1.10	0.906539
Admission type	1.10	0.912585
Pulse rate	1.08	0.926757
Body temperature at admission	1.04	0.959852
Place Residence	1.03	0.966968
Sex	1.03	0.971580
Mean VIF	**1.43**	

Nb: VIF-Variance Inflation Factor