POPULATION PHARMACOKINETICS OF GENTAMICIN AMONG NEONATES ADMITTED AT MWANANYAMALA REGIONAL REFERRAL HOSPITAL, DAR ES SALAAM TANZANIA

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Master of Science in Clinical Pharmacology Dissertation

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Muhimbili University of Health and Allied Sciences
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A dissertation submitted in (partial) fulfillment of the requirements for the Degree of Master of Science in Clinical Pharmacology of the Muhimbili University of Health and Allied Sciences

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CERTIFICATION

The undersigned certify that they have read and hereby recommend for acceptance by Muhimbili University of Health and Allied Sciences a dissertation titled; **Population Pharmacokinetics of Gentamicin among Neonates Admitted at Mwananyamala Regional Referral Hospital, Dar es salaam Tanzania**, in (partial) fulfillment of the requirements for the degree of Master of Science in Clinical Pharmacology of the Muhimbili University of Health and Allied Sciences.

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DECLARATION AND COPYRIGHT®

I, Masanyiwa E James, hereby declare that this dissertation is my original work, and it has not been presented and will not be presented to any other University for a similar or any other degree award.

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DEDICATION

This dissertation is dedicated to my family and friends. Thank you for your love, prayers, tireless support and understanding.

ABSTRACT

Background: Gentamicin is an important drug in the class of aminoglycosides, most commonly used to treat resistant gram-negative organisms. Due to its rapid bactericidal activity, gentamicin is widely used in empirical antimicrobial regimens to treat neonatal infections caused by both gram-negative and gram-positive bacteria. Although highly effective, reservations concerning potential otovestibular toxicity and nephrotoxicity have often limited the use of this agent. The recommended use of gentamicin by the World health organization (WHO) requires therapeutic drug monitoring. However, because of limited resources, therapeutic drug monitoring is not commonly done in our country. By not monitoring the serum levels of gentamicin there is a possibility that a considerable number of neonates may be suffering from acute kidney injury after high gentamicin exposure and may later develop chronic kidney disease and or permanent hearing loss.

Objectives: The objectives of this study were to determine the peak and trough serum levels of gentamicin achieved by the neonates following once daily dosing of intravenous gentamicin. We also determined the population gentamicin clearance, volume of distribution and discerned whether there was a significant increase in serum creatinine levels after gentamicin therapy for more than 48 hours.

Materials and methods: This was a population pharmacokinetic study (popPK) in which term neonates admitted and prescribed to receive gentamicin for more than 48 hours were included in the study. The study excluded very sick term neonates in decompensate state and requiring resuscitation, term neonates who had severe congenital malformation and term neonates who were on gentamicin therapy in the past 72 hours before the study. Sparse sampling was employed where 2 to 3 participants contributed serum samples for gentamicin concentration at each time point after the initial intravenous gentamicin dose, using a predetermined sampling schedule. However, serum samples for peak gentamicin concentration were obtained from 12 participants 30minutes after the first dose and steady state trough gentamicin concentration were obtained from each participant 20hours after the second dose. Serum gentamicin concentration was determined using chemiluminescent microparticle immunoassay (CMIA) technology (Abbott architect ci4100 analyzer, Abbott diagnostics in Illinois, United States)**.**In addition, serum creatinine levels before and seven days after start of

gentamicin treatment were determined for each participant using Erba XL-100 analyzer (Erba Mannheim Diagnostics Company, India).

Data analysis: The serum gentamicin concentrations-time profile was fitted using stata version 14.2 software (StataCorp LLC in California, United states) with the assumption of one compartment model where popPK parameters (elimination rate constant, elimination half-life and area under the curve) were directly estimated and clearance together with volume of distribution were calculated from these estimates. Categorical data were presented as proportions and compared using McNemar test; continuous data were expressed as mean ± S.D (standard deviation) and were compared using one sample t-test and paired samples t-test. The results were considered statistically significant at *P* values *<0.05*.

Results: In this population, peak and steady state trough gentamicin concentration was higher than the recommended upper limit of normal peak and trough serum levels for the typical term neonate (M=16.669, SD ± 0.646, *P*< 0.001) and (M=3.283, SD ± 0.707, *P*< 0.001) for peak and trough concentration respectively. By contrast, gentamicin clearance (0.40 $mLmin^{-1}kg^{-1}$) and volume of distribution (0.31Lkg^{-1}) estimated from PK data in our study were lower than the lower limit of the reported reference range. Overall, there was a significant increase in serum creatinine level after gentamicin treatment, suggesting occurrence of renal injury, in most of the study participants.

Conclusions: Neonates achieved high peak and trough serum gentamicin levels which could be explained by the population pharmacokinetics estimates (lower clearance and volume of distribution). Also, high gentamicin concentration may have caused significant renal injury leading to a significant increase in serum creatinine in the majority of study participants.

Recommendations: It is important that the Ministry of Health, Community Development, Gender, Elderly and Children considers therapeutic drug monitoring practice in our health facilities as an important tool in maximizing therapeutic outcomes and minimizing the potential toxic effects associated with higher serum levels when neonates are on gentamicin treatment. Also, kidney function test before, during and after treatment with gentamicin, should be made a routine practice in our health facilities to ensure that doses are given according to the patient's renal function in order to attain serum gentamicin levels which are within the recommended ranges thereby preventing toxicity (nephrotoxicity and ototoxicity).

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LIST OF ABBREVIATIONS

AGAs	Aminoglycoside antibiotics
AKI	Acute kidney injury
AKIN	Acute kidney injury network
AUC	Area under the curve
CBS	Capillary blood sampling
CL	Clearance
C_{max}	Peak concentration
CMIA	Chemiluminescent microparticle immunoassay
CNS	Central nervous system
CPL	Central pathology laboratory
CrCl	Creatinine clearance
C_{trough}	Trough concentration
ED_{50}	Therapeutic dose
eGFR	Estimated Glomerular filtration rate
EMIT	Enzyme multiplied immunoassay technique
GA	Gestational age
GFR	Glomerular filtration rate
HL	Heel lance
HPLC	High performance liquid chromatography
Ht	Height
IMCI	Integrated management of childhood illness
KDIGO	Kidney disease improving global outcomes
LD_{50}	Lethal dose
MIC	Minimum inhibitory concentration
MNH	Muhimbili National Hospital
MRRH	Mwananyamala Regional Referral Hospital
MUHAS	Muhimbili University of Health and Allied Sciences
NONMEM	Nonlinear Mixed Effect Modeling

PK	Pharmacokinetics
PNA	Post natal age
PopPK	Population pharmacokinetics
pRIFLE	Pediatric risk injury failure loss end stage
PTECs	Proximal tubule epithelial cells
SCr	Serum creatinine
t½	Elimination half life
TDM	Therapeutic drug monitoring
TI	Therapeutic index
UDOM	University of Dodoma
VD	Volume of distribution
WHO	World Health Organization

DEFINITION OF KEY TERMS

Peak concentration is the maximum serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and before the administration of a second dose. Usually is measured in mg/L or µg/mL

Trough concentration is the lowest serum concentration reached by a drug before the next dose is administered, often used in therapeutic drug monitoring. Usually is measured in mg/L or µg/mL

Pharmacokinetics is the study of how the body interacts with administered drugs for the entire duration of exposure (study of movement of drugs in the body); it includes absorption, distribution, metabolism, and excretion (ADME).

Population pharmacokinetics can be defined as estimating the pharmacokinetic similarity and differences between individuals from measurements of drug levels in biological fluids (often blood) of subjects or patients arising from some population of interest.

Elimination half-life (t½) is a pharmacokinetic parameter that is defined as the time it takes for the concentration of the drug in the plasma or the total amount in the body to be reduced by 50%.

Volume of distribution (VD) is the volume of fluid into which the total drug administered would theoretically have to be diluted to produce the observed drug concentration in the blood plasma.

Therapeutic drug monitoring (**TDM**) is the clinical practice of measuring specific drugs at designated intervals to maintain a constant concentration in a patient's bloodstream, thereby optimizing individual dosage regimens. TDM is used to determine the dose at which a drug will be the most safe and effective.

Therapeutic index (TI) is a quantitative measurement of the relative safety of a drug. It is the ratio of the dose that produces toxicity (Lethal Dose- LD_{50}) to the dose needed to produce the

desired therapeutic response (Effective dose- ED_{50}). Drugs with low TI like aminoglycosides are toxic while drugs with high TI like penicillins are considered to be safe.

Lethal dose (LD_{50}) is the dose required to kill half the members of a tested population after a specified test duration. LD_{50} figures are frequently used as a general indicator of a substance's acute toxicity. A lower LD_{50} is indicative of increased toxicity.

Effective dose (ED_{50}) is the dose that produces a quantal effect (all or nothing) in 50% of the population that takes it (median referring to the 50% population base). The ED50 is commonly used as a measure of the reasonable expectancy of a drug effect, but does not necessarily represent the dose that a clinician might use.

Minimum inhibitory concentration (**MIC**) is the lowest concentration of a drug, which prevents visible growth of a bacterium or bacteria. MIC depends on the microorganism, the affected human being (in vivo only), and the antibiotic itself. It is often expressed in micrograms per milliliter (μg/mL) or milligrams per liter (mg/L).

Area under the plasma concentration time curve (AUC) is the plot of plasma concentration of a drug versus time after dosage (called "area under the curve" or AUC) gives insight into the extent of systemic exposure to the drug and its clearance rate from the body.

Clearance is a pharmacokinetic measurement of the volume of plasma from which drug is completely removed per unit time. Usually, clearance is measured in L/h or mL/min. It can be renal or non-renal clearance (including hepatic clearance).

Creatinine is the chemical waste molecule that is generated from muscle metabolism. Creatinine is produced from creatine, a molecule of major importance for energy production in muscles. Approximately 2% of the body's creatine is converted to creatinine every day. Creatinine is transported through the bloodstream to the kidneys. The kidneys filter almost all of the creatinine and dispose of it in the urine. Creatinine is the measure of the kidneys function; the elevated creatinine level signifies impaired kidney function or kidney disease. As the kidneys become impaired for any reason, the creatinine level in the blood will rise due to poor clearance of creatinine by the kidneys. Normal levels of creatinine in the blood are

approximately 0.6 to 1.2 milligrams (mg) per deciliter (dL) in adult males and 0.5 to 1.1 milligrams per deciliter in adult females. The baseline normal serum creatinine levels for children under age of 3 years are 0.3 to 0.7mg/dL.

Creatinine clearance (CrCl) is the volume of blood plasma cleared of creatinine per unit time. It is a rapid and cost-effective method for the measurement of renal function. Both CrCl and GFR can be measured using the comparative values of creatinine in blood and urine. Creatinine clearance in a healthy young person is about 95 milliliters (mL) per minute for women and 120 mL per minute for men.

Glomerular filtration rate (GFR) is the volume of fluid filtered from the renal (kidney) glomerular capillaries into the Bowman's capsule per unit time. It is used to check how well the kidneys are working.

Estimated glomerular filtration rate (eGFR) is the best test to measure the level of kidney function and determine the stage of kidney disease. In neonates, health practitioners (clinicians) can accurately calculate it from the results of the serum creatinine test. If the eGFR number is low, the kidneys are not working as well as they should. The earlier kidney disease is detected, the better the chance of slowing or stopping its progression. In neonates, eGFR is calculated using Schwartz and Brion serum creatinine levels-based GFR-estimation formula, which gives accurate estimation of GFR than the other present formulas

eGFR (mL/min/1.73m^2) = k x H (cm)/SCr (mg/dL)

Where k is the constant for association of GFR, laboratory measurements, and anthropometric measurement and its value is 0.33 for preterm neonates, and 0.45 for term neonates; H is the body height in cm and SCr is the serum creatinine levels in mg/dL.

A serum creatinine test measures the level of creatinine in the blood and provides an estimate of how well the kidneys filter (glomerular filtration rate).

Acute kidney injury (acute renal failure) is a condition in which the kidneys suddenly cannot filter waste from the blood.

Nephrotoxicity is a rapid deterioration in the kidney function due to toxic effect of medications and chemicals.

Ototoxicity is the property of being toxic to the ear (oto-), specifically the cochlea or auditory nerve and sometimes the vestibular system.

Audiometry is a branch of audiology and the science of measuring hearing acuity for variations in sound intensity and pitch and for tonal purity, involving thresholds and differing frequencies. Degree of hearing loss is categorized as; Normal<25dB, mild hearing loss 25dB - 40dB, moderate 50dB – 70dB, severe hearing loss >80 dB and profound hearing loss >90 dB.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Infections causing sepsis, meningitis, or pneumonia contributed directly to around 0·6 million neonatal deaths worldwide in 2016 and indirectly to many more through pathways leading to preterm birth and neonatal encephalopathy [1]. Despite this knowledge, understanding of the causes of neonatal infection, particularly in resource-poor settings, is limited [2]. Treatment in these settings usually relies on the sensitive but non-specific clinical diagnosis of possible serious bacterial infection [3].

1.1.1 Neonatal infections: overview

Newborns are particularly more susceptible to infections than older children and adults, because their new immune system is immature to fight the bacteria, viruses, and parasites that cause these infections [4]. They can acquire infections during prenatal development or in the first four weeks of life [5]. Neonatal infections may be contracted through mother to child transmission, in the birth canal during childbirth, or contracted after birth. Thus neonatal infections can present as early as between 6- 72 hours post-delivery (early onset sepsis) while late onset sepsis may develop after 72hrs [6]. Some neonatal infections such as HIV, hepatitis B and other intrauterine infections may not become apparent at birth but present much later [7].

Group B streptococcus and gram-negative enteric organisms (predominantly *Escherichia coli*) are responsible for most cases of early onset neonatal sepsis. While staphylococci spp and to some extent gram-negative organisms are responsible for late-onset neonatal sepsis usually acquired from the environment [8].

1.1.2 Neonatal deaths

The first 28 days of life is the most vulnerable time for a child's survival. Children face the highest risk of dying in their first month of life at an average global rate of 17 deaths per 1,000 live births in 2019, down by 52% from 38 deaths per 1,000 in 1990 [9]. According to the World Health Organization (WHO), four million newborn children die each year during the first four weeks of their lives [10]. Of these, 75% die prematurely during the first week of life [11]. The major causes of neonatal deaths worldwide are neonatal sepsis (30-50%), prematurity (28%) and birth asphyxia (23%) [11]. Clearly, sepsis is the commonest cause of neonatal morbidity and total neonatal deaths each year worldwide [12]. However, there is some variation between countries depending on their care configuration. For instance, in Tanzania, where current neonatal mortality is 20.3 deaths per 1,000 live births, it is estimated that neonatal sepsis accounts for 29% of the neonatal deaths and it varies between regions, ranging from 25% in Mwanza to 38.9% in Dar es Salaam [13].

1.1.3 Management of neonatal sepsis

Based on the common antibiotic susceptibilities of the predominant organisms causing neonatal sepsis, the WHO recommends initial empiric therapy for a neonate with suspected bacterial sepsis to include ampicillin and an aminoglycoside [14].

Thus, for the management of neonatal sepsis, clinicians in many settings make a tentative diagnosis and empirical treatment of neonatal sepsis based on the new neonatal WHO Integrated Management of Childhood Illnesses (n-IMCI) guidelines [15]. The IMCI recommends hospitalization and intramuscular (IM) or intravenous (IV) antibiotic therapy with a combination of gentamicin and benzylpenicillin or ampicillin for at least 7–10 days in infants aged <2 months [16]. The WHO recommends the use of gentamicin as the first choice drug in newborns to treat sepsis not only because of its reliability, rapid bactericidal activity, relatively low rates of resistance, but also because of the long experience with its use [17].

Currently in Tanzania, neonatal sepsis is managed according to the standard treatment guideline (STG) which recommends the use of ampicillin, cloxacillin and gentamicin as the

first line antibiotics [18]. Gentamicin is often used as part of empirical therapy for sepsis in newborns to treat infections caused by gram-negative bacteria[5]. It is usually administered as a bolus injection at a once-daily dosing of 5mg/kg every 24 hours for a period of 7-10 days. Neonatal sepsis is well managed using gentamicin in combination with β-lactam antibiotics like ampicillin and cloxacillin[19]. Gentamicin kills bacteria by inhibiting protein synthesis through binding to the 30S of the prokaryotic ribosomes and to some extent by lysing the cell [19]. However, gentamicin like all other aminoglycosides exhibits a narrow range between toxic and therapeutic dosages and is known to be ototoxic and nephrotoxic when is used for more than 48 hrs without monitoring [20]. Many studies of gentamicin-associated toxicity in neonates show that, it has the potential to cause toxicity, particularly ototoxicity and nephrotoxicity [21]. The recommended use of gentamicin by the World health organization (WHO) requires therapeutic drug monitoring (TDM) to guide dosage adjustments to maximize the efficacy and minimize toxicity which are dose dependent [22].

It is estimated that, up to 25% of all patients who receive aminoglycoside therapy for more than 48 hours without monitoring develop nephrotoxicity and ototoxicity, when trough concentration is >2 µg/mL [23]. A study by Selby *et al* on gentamicin-associated acute kidney injury (AKI) on patients who were treated with gentamicin showed that, acute kidney injury occurred in 24.4% patients and 2.4% progressed to kidney failure [24]. However, renal toxicity is rare in patients receiving once-daily dosing aminoglycosides and when regular trough levels are monitored and dosage adjusted accordingly [25]. According to WHO, neonates need monitoring if prescribed gentamicin because of their renal immaturity and should have a kidney function test before starting gentamicin and renal function should be assessed regularly during and after treatment[20]. Yet these safety measures are not commonly practiced in our health facilities and not advocated for in the standard treatment guidelines. Therefore, this study was done to determine whether the current gentamicin dosing practice in neonates might be associated with acute kidney injury, which may lead to development of chronic kidney disease (CKD) later in life. In fact, the worldwide number of deaths

attributable to CKD in children has almost doubled in the past twenty years, ranking it among the top twenty causes of death globally [26].

Chronic kidney disease has begun to gain recognition as an important contributor to the burden of disease not only in high-income countries, but also in low-income countries in regions such as Sub-Saharan Africa [26]. It is regarded as a substantial health burden with risk factors that include communicable, non-communicable diseases and use of nephrotoxic drugs like gentamicin [27]. Although high quality epidemiological data from Sub-Saharan Africa are sparse, many studies on the epidemiology of chronic kidney disease in Sub-Saharan Africa suggest the prevalence of CKD is substantial (about 13.9%) [27].

1.2 Statement of the problem

Gentamicin is an aminoglycoside antibiotic widely used as the first line antibiotic in empirical antimicrobial regimens in neonates due to its efficacy, low cost and availability. Neonatal sepsis remains among the commonest cause of neonatal deaths and is commonly managed using a combination of gentamicin and other antibiotics. Gentamicin can prevent neonatal deaths caused by neonatal sepsis but can lead to potential acute kidney injury and ototoxicity if therapeutic drug monitoring is not done [25]. According to WHO, neonates need monitoring if prescribed gentamicin because of their renal immaturity and should have a kidney function test before starting gentamicin and regular assessment of renal function during and after treatment should be done [20]. However, for various reasons such as lack of equipments and reagents; lack of awareness among health care personnel and high cost of serum gentamicin and serum creatinine monitoring, serum gentamicin concentration and serum creatinine level are not commonly done before, or after initiating gentamicin treatment in neonates in Tanzania. Yet gentamicin use for 5 to 7 days is common practice. By not monitoring the serum levels of gentamicin and serum creatinine before, during and after treatment the number of neonates that may be suffering from acute kidney injury and who may later develop chronic kidney disease and or hearing loss, which are usually associated with serum levels greater than 2µg/mL and 12 µg/mL(trough and peak concentration respectively) may be high[28].

The study was expected to shed light on whether the current practice of gentamicin dosing without monitoring in neonates is safe or not.

1.3 Conceptual framework

Due to high relative blood flow, the kidney is prone to drug-induced damage. Aminoglycoside type antibiotics like gentamicin, which is exclusively excreted in urine, is one of the leading causes of drug-induced nephrotoxicity. When once daily dosing of intravenous gentamicin is used for more than 48 hrs without monitoring there is high possibility of its peak and trough serum levels to build in the body above the recommended (safety) range and cause potential nephrotoxicity and or ototoxicity. Nephrotoxicity is determined by several parameters including elevated serum creatinine levels, decreased gentamicin clearance, increase in elimination half-life of gentamicin and decrease in estimated glomerular filtration rate (Figure 1).

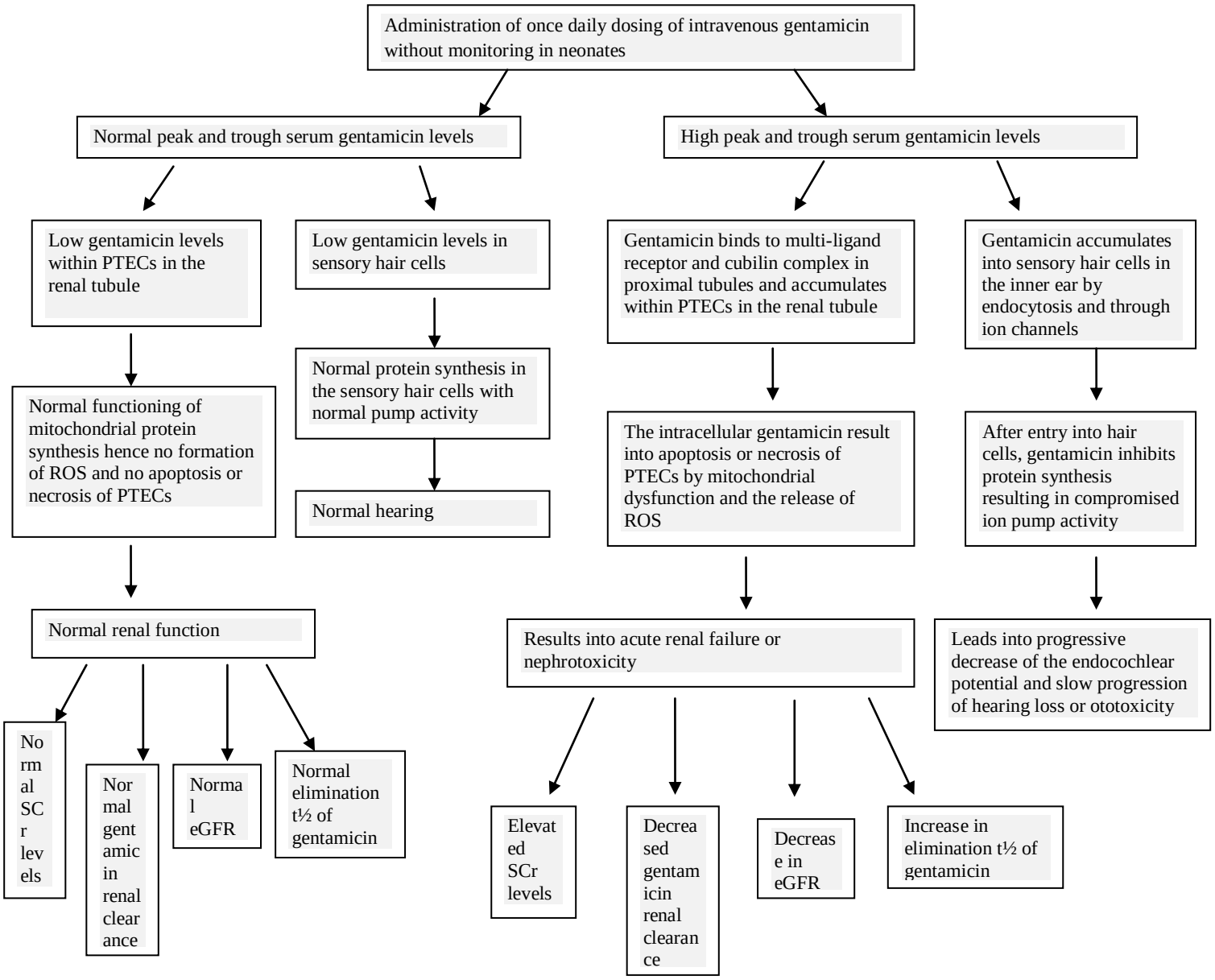

Figure 1: Summarized conceptual framework

Key: eGFR – estimated glomerular filtration rate; ROS – reactive oxygen species; PTECs - proximal tubule epithelial cells; t½ - half-life; SCr – serum creatinine

1.4 Rationale of the study

The findings from this study are essential in determining whether the current practice of gentamicin dosing in neonates without therapeutic drug monitoring is associated with adequate but safe serum concentrations.

Also, the results from this study will help to inform authorities within our health facilities in Tanzania of the safety implication of the current practice and see whether there is a need to change the practice.

Most importantly, if the current practice is proven not safe, will help to recommend to the Ministry of Health, Community Development, Gender, Elderly and Children (MoHCDGEC) the inclusion of TDM as part of standard treatment guideline for the purpose of maximizing therapeutic outcomes and minimizing the potentially toxic effects, especially when the vulnerable population to drug toxicities like neonates are on drugs which are hard to dose.

1.5 Hypothesis

We hypothesized that, neonates on once daily dosing of intravenous gentamicin without monitoring for more than 48 hours achieve high serum gentamicin levels and it may be that there is high proportion of neonates who develop acute kidney injury (AKI) and hearing loss.

1.6 Research questions

1.6.1 What serum levels of gentamicin (peak and trough) are achieved by the neonates after a once daily dose?

1.6.2 What are the population pharmacokinetic parameters (clearance and volume of distribution) of gentamicin in neonates?

1.6.3 Is there a notable change in serum creatinine levels following initiation of once daily dosing of intravenous gentamicin therapy for more than 48 hours in neonates?

1.7 Objectives

1.7.1 General objective

To determine the peak and trough serum levels, population pharmacokinetic parameters and mean change in serum creatinine levels following once daily dosing of intravenous gentamicin among sick neonates in Dar es salaam, Tanzania.

1.7.2 Specific objectives

1.7.2.1 To determine the peak and trough serum levels of gentamicin after the first dose of once daily dosing of intravenous gentamicin

1.7.2.2 To determine the population pharmacokinetic parameters (clearance and volume of distribution) of gentamicin in neonates.

1.7.2.3 To determine the mean change in serum creatinine levels as a marker of AKI after initiation of once daily dosing of IV gentamicin therapy for more than 48 hours in neonates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Pharmacology of gentamicin

Gentamicin is an aminoglycoside antibiotic often used in empirical antimicrobial regimens because of its rapid bactericidal activity and relatively low rates of resistance. It kills bacteria by inhibiting protein synthesis through binding to the 30S of the prokaryotic ribosomes and to some extent by lysing the cell [19]. Gentamicin is frequently used in combination with β-lactam antibiotics and this regimen is recommended for the treatment of sepsis or pneumonia and is also active against *Pseudomonas aeruginosa*, Enterobacter species, Klebsiella species and Serratia species [17]. However, gentamicin is known to be ototoxic and nephrotoxic [29].

Gentamicin is basic in nature and a strongly polar compound; these properties make it highly soluble in water, relatively insoluble in lipids, and have enhanced antimicrobial activity in alkaline rather than acidic environments. As a result, gentamicin is minimally absorbed from the gut when administered orally [19]. To achieve active tissue levels, it is administered intramuscularly or intravenously. It is usually given by slow bolus injection over two to three minutes or via intravenous infusion over 30 minutes [16]. Following administration, gentamicin shows a marked post-antibiotic effect – suppression of bacterial growth which persists for some time (2-6hrs) after the drug is no longer present in the plasma [29]. Gentamicin is distributed freely throughout the vascular and interstitial spaces. It achieves very low intracellular concentrations in most tissues. Its entry into cerebrospinal fluid, bronchial secretions, saliva, prostatic tissue and the vitreous humor also is poor [30]. It is primarily excreted unchanged in urine and is detectable in urine weeks after the cessation of therapy. Excretion is by glomerular filtration, although some active tubular secretion does occur.

Formerly, gentamicin was administered at a dose of 2.5 mg/kg every 12 hours and pharmacokinetics data showed that, this dosing method yields lower peak concentrations which reduce bacterial kill and higher trough concentrations which increases the systemic toxicity [28]. However, studies looking into this reveled that, a once-daily gentamicin dosing of 4-5 mg/kg gives better pharmacokinetic profile, yields higher peak concentrations which enhance bacterial kill and lower trough concentrations which reduces the systemic toxicity in patients with normal renal function [31]. Other studies on pharmacokinetics of once daily dosing of gentamicin in neonates include a study by Faye *et al* [32], a study by Karen *et al* [33] and a study by Dan Miron [34], these studies also showed better pharmacokinetic profile of a once daily dosing of gentamicin with higher peak concentrations and lower trough concentrations which enhance bacterial kill and reduces the systemic toxicity respectively.

Nevertheless, even a once-daily dosing of gentamicin in neonates as it was administered at Mwananyamala regional referral hospital may be associated with potential toxic effects if it is administered for more than 48 hours and if therapeutic drug monitoring is not done [25]**.**

2.2 Peak and trough gentamicin concentrations

Gentamicin shows concentration-dependent activity [35]. A gentamicin peak concentration of 8-10 times the minimum inhibitory concentration (MIC) as determined for the causative microorganism is desirable for effective therapy whilst maintaining a trough level <1 mg/L to avoid toxicity is advised [36]. Gentamicin, like all other aminoglycosides, exhibits a narrow range between toxic and therapeutic dosages. The current practice recommends that, gentamicin should be routinely monitored to avoid any possible toxic effects when treatment extends for more than 48 hours [29].

The peak concentration is usually within the range of 5-12mg/L and should be checked 30 minutes after the end of a 30 minute infusion or immediately after the end of a 1-hour infusion [29]. For some infections of bacteria species, such as *Pseudomonas aeruginosa* and Enterobacteriaceae the dose may need to be increased in order to achieve higher peak levels (15-20 mg/L), however, these peak serum levels are considered toxic [37].The optimal target gentamicin trough concentration is ≤ 1.0mg/L and should be checked four hours before the next dose is due [38]. Trough concentration levels of <1mg/L are required before the next dose

can be given in order to minimize toxicity. If the trough level is above 1mg/L the dose must be omitted until the level falls below 1mg/L [38]. It is generally accepted that peak concentrations >4 µg/mL are necessary for antibacterial efficiency (peak concentration <5 µg/mL is associated with lesser efficacy) and that trough concentrations of >2 µg/mL are a risk factor for nephrotoxicity and ototoxicity [20].

2.3 Potential toxic effects of gentamicin

Most side effects of gentamicin are dose related. The important side effects are damage to the ear and the kidney (ototoxicity and nephrotoxicity respectively).

Ototoxicity, which manifests as high-frequency hearing loss, occurs in a treatment-duration-dependent fashion in the majority [23]. However, an idiosyncratic form of toxicity resulting in profound and irreversible hearing loss even after very low exposures is recognized to affect genetically predisposed individuals such as those with the *A1555G* mitoribosome mutation [39]. Unlike nephrotoxicity, ototoxicity is irreversible [39]. Even short courses of gentamicin therapy in healthy newborn infants can lead to abnormalities of auditory function, therefore neonates on gentamicin should be monitored for ototoxicity by audiometry [29]. And ototoxicity occurs in up to 25% of neonates treated with gentamicin [40].

The recognized risk factors for nephrotoxicity in neonates include preexisting renal dysfunction, concomitant nephrotoxic drugs, prolonged duration of gentamicin therapy and higher doses [23]. Nephrotoxicity is usually reversible [41], although progression to anuric renal failure has been uncommonly observed. Careful serum drug monitoring is advised and an increasing trough level may be an early indication of toxicity [42]. Nephrotoxicity occurs in 10–25% of patients on aminoglycoside [23].

Nephrotoxicity and ototoxicity occur when trough concentration is >2 µg/mL for the treatment that extends more than 48 hours [20].

2.3.1 Mechanism of ear and renal injury by gentamicin

2.3.1.1 Mechanism of ear injury by gentamicin

Gentamicin uptake into sensory hair cells in the inner ear is both by endocytosis and transport through ion channels [43]. After entry into hair cells, gentamicin binds to mitochondrial ribosome and cause misreading in mitochondrial protein synthesis rather than direct inhibition of protein synthesis, leading to a decrease in mitochondrial ATP synthesis, which results into compromised ion pump activity [44]. Reduced ion pump activity in strial intermediate cells ultimately lead to a progressive decrease of the endocochlear potential, this considerably explains the slow progression of hearing loss after exposure to gentamicin [43]. Also, a decrease in mitochondrial ATP synthesis disrupts normal metabolism and resulting in the generation of reactive oxygen species lethal to the inner-ear hair cells [44].

2.3.1.2 Mechanism of renal injury by gentamicin

Gentamicin accumulates within proximal tubule epithelial cells (PTECs) in the renal cortex following glomerular filtration by endocytosis while cells of distal tubules and collecting ducts are significantly less affected by cytotoxic effects [45]. The increased accumulation of gentamicin in proximal tubules is related to expression of transport molecule for proteins and cations (the multi-ligand receptor (megalin) and cubilin complex) in proximal tubules and this is thought to be the key determining the mechanism for the development of toxicity [45]. Intracellular gentamicin can result in apoptosis or necrosis of PTECs by a variety of pathways including mitochondrial dysfunction and the release of reactive oxygen species [46].

2.4 Population pharmacokinetic parameters (Clearance and volume of distribution of gentamicin) in neonates

2.4.1 Volume of distribution of gentamicin in neonates

The physiological conditions of neonates are different from those of adults. Neonates have a larger extracellular fluid volume; they also have immature liver and kidney functions as well as higher plasma concentrations of bilirubin and non-esterified fatty acids. The water content is larger in preterm than in term infants and gentamicin is fairly water soluble and is distributed predominantly in extracellular fluid, which varies inversely with gestational age. The large amount of extracellular body water in neonates and young infants results in lower

plasma concentrations compared with adults for a given body weight-adjusted dosage regimen [30]. Volume of distribution of gentamicin in neonates ranges from 0.4 to 0.7 L/kg; it is larger in preterm than term infants and it is approximated to be 0.40-0.45 L/kg in the first week of life [47].

2.4.2 Gentamicin clearance in neonates

The majority of nephrons are formed in the third trimester of pregnancy and nephrogenesis is complete between 32 to 36 weeks of gestation [48]. In preterm births (below 37 weeks of gestation) and infants born at 25 weeks of gestation, the glomerular filtration rate (GFR) is quite low at birth; this is also typical in term newborns. During the first weeks of life, there is a progressive rise in GFR resulting from an acute increase in cardiac output and renal blood flow induced by birth and a decrease in renal vascular resistance [49]. The renal glomerular filtration and tubular secretion of gentamicin (aminoglycosides) is reduced in neonates. The reduced renal excretory function affects the disposition of gentamicin and its clearance (Cl) is reduced in newborn infants compared to children.

In neonates, the half-life of gentamicin ranges from 5.4 to 10.0 hours and clearance 0.50 to 1.71 mL/min/kg [29]. Preterm neonates have a longer half-life than full-term neonates. Therefore, because of age-associated changes in organ function and body composition, gentamicin treatment regimens must be individualized appropriately to reflect maturation (increase in age) and growth (increase in size). Thus, it is mandatory to monitor gentamicin serum concentrations whenever infants are treated for 48 hours or more [29].

2.5 Therapeutic drug monitoring

Therapeutic drug monitoring involves determining serum concentration after initiating therapy to see whether patients achieved desired concentrations which are determined by the pharmacokinetic profile of a drug. Pharmacokinetics of a drug depends on patient-related factors as well as on the drug's chemical properties. Some patient-related factors (such as renal function, genetic makeup, sex and age) can be used to predict the pharmacokinetic parameters in populations [50]. For example, the half-life (t½) of many drugs may be remarkably long in the elderly due to decreased drug elimination (metabolism and excretion).

Other factors are related to individual physiology. The effects of some individual factors (such as renal failure, obesity, hepatic failure, and dehydration) can be reasonably predicted, but other factors are idiosyncratic and thus have unpredictable effects [51]. Because of individual differences, drug administration must be based on each patient's needs. Traditionally, this was done by empirically adjusting dosage until the therapeutic objective is met. This approach is frequently inadequate because it can delay optimal response or result in adverse effects. Knowledge of pharmacokinetic principles helps prescribers adjust dosage more accurately and rapidly. Application of pharmacokinetic principles to individualize pharmacotherapy is termed therapeutic drug monitoring [50].

Therapeutic drug monitoring (TDM) is the clinical practice that measures the amount of certain medicines in the blood [25]. It is done to make sure the amount of drug the patient is taking is both safe and effective. The prescribing process does not end after writing a recipe; in fact, monitoring the results of the treatment is an important part of the rational prescribing process; this means that clinicians should assess patients' clinical evolution, expected outcomes and potential adverse effects of the prescribed medicines periodically [25]. Routine monitoring is not advocated for most drugs. The therapeutic benefits of many drugs could be easily observed and measured, so it is possible to know if the administered dose is appropriate for that patient or if some dose adjustments are required either to avoid toxic effects or to obtain the desired benefit [51].

Only clinically meaningful tests should be performed. Drug assays are costly, so the reason for monitoring and the additional information to be gained should be carefully considered. So, regular monitoring of many drugs is not required in a clinically stable patient [25]. Notwithstanding this, for a few specific medicines, this is impracticable, while insufficient levels will lead to under treatment or resistance, and excessive levels can lead to toxicity and tissue damage. When there is a large inter-individual variation between dose and effect, individualizing drug dosage is important. This is particularly relevant for drugs with a narrow target range or concentration-dependent pharmacodynamics [51]. Additionally, drug concentration at the site of action cannot be routinely measured, but the desired or adverse

effects may correlate better with plasma or blood concentrations than they do with dose. So for these drugs, concentration measurements are a valuable surrogate of drug exposure, particularly if there is no sensitive or straightforward measure of effect [25].

Therapeutic drug monitoring (TDM) helps the clinician to determine the best dosages for people taking certain types of hard-to-dose medicines (i.e. drugs that can easily be under- or overdosed). Below are some of the most common drugs that should be monitored.

Table 1.1: List of medicines widely analyzed for therapeutic drug monitoring [25].

Type of medicine	**Medicine names**
Antibiotics	Gentamicin, vancomycin, amikacin
Heart drugs	Digoxin, procainamide, lidocaine
Anti-seizure drugs	Phenytoin, phenobarbital
Drugs treat autoimmune diseases	Cyclosporine, tacrolimus
Drugs that treat bipolar disorder	Lithium, valproic acid
Antipsychotics	Pimozide and clozapine

Therapeutic drug monitoring (TDM) as a tool should be used when patients are receiving one of the listed drugs (Table 1.1); these drugs require a measurement of their blood concentration. In most cases, however, the decision to ask for TDM is not so much related to specific drugs, as to factors related with the patient who is being treated with them [51]. Concomitant treatments and potential drug-drug interactions, special conditions (such as pregnancy, neonates, obesity) or comorbidities (such as liver or kidney failure), as well as being admitted to special units (such as intensive care units), are situations in which TDM is advised. However, some medicines requiring TDM may not require monitoring in some situations such as non-complicated patients in primary health care because a close clinical follow-up is enough to anticipate potential dosage problems [52].

Following critical review of the evidences about TDM for a proposed list of drugs (Table 1.1), a three-category scale of prioritization of the value of monitoring (TDM recommendation) has been proposed: “high”, “moderate” and “low”[25]. Low is for those drugs with published evidences of the useless value of TDM in clinical practice, and high is for drugs with a really narrow therapeutic window with published evidences of the clinical benefits of TDM [25]. For those included in the high TDM recommendation, like gentamicin, TDM is useful even for noncritically ill patients.

Therapeutic drug monitoring is based on a known concentration range or target concentration that has been determined to be safe and effective from pharmacokinetic studies (dose-finding studies) during drug development. Pharmacokinetic studies have usually been carried out in small numbers of people, often healthy volunteers (traditional pharmacokinetics). In traditional pharmacokinetic studies, small numbers of people (healthy volunteers) are intensively sampled over a given post-dose period. By contrast, in population pharmacokinetics studies, data are obtained from patients being treated with a drug; these patients are often taking different doses and have blood samples at different times. However, traditional PK studies have limitations in population such as neonates. Tiny blood volume in neonates limits the extensive blood sampling; therefore, sparse-sampling population pharmacokinetics (popPK) is one of the most suitable approaches that can be applied to estimate PK parameters in this population.

2.6 Population pharmacokinetic studies

One of the most critical aspects of a pharmacokinetic (PK) study in neonates is blood sampling in which frequent blood sampling is not practical due to ethical considerations. Under normal circumstances it is possible to draw frequent blood samples from subjects. There are, however, situations under which frequent blood sampling is not practical (critically ill patients, neonates and very young children, rare diseases, and elderly subjects) [53]. Under these circumstances, a limited number of blood samples (also known as sparse sampling) can be taken from subjects for the PK assessment [54].

Tiny blood volume in neonates limits the extensive blood sampling; it is difficult to do intensive sampling in individual subject for this study population for the purpose of obtaining all serum concentration data points to construct serum concentrations-time profiles. Therefore, sparse-sampling population pharmacokinetics (popPK) is one of the most suitable approaches that can be applied to estimate PK parameters in neonates.

For popPK analysis, since the number of blood samples is sparse, it is widely believed that a large number of subjects are required for accurate estimation of pharmacokinetic parameters. As mentioned earlier, there are situations where frequent blood sampling may not be possible and yet estimation of PK parameters, especially clearance, is required for appropriate dosing. There are several situations where not only frequent blood sampling is not possible but sample size is also very small. These situations include neonates, infants, and rare diseases.

Considering that, under such conditions there will be only few subjects (or children) available for popPK study and frequent blood samples will not be available. Weber *et al* performed a popPK study to evaluate whether one or two blood samples each from few selected subjects can be used to accurately estimate the clearance and volume of distribution of the central compartment for a given drug. The study involved five (small sample) subjects only chosen and each subject gave either one or two blood samples. The subjects were selected randomly and blood samples from each subject were taken in a way that they covered the entire range of concentration versus time data profile from extensive sampling (from the beginning till the end of the sampling scheme in the extensive sampling profile). Then, the population analysis was performed using two software programs namely Nonlinear Mixed Effect Modeling (NONMEM) and single subject non-linear fitting module WinNonlin pharmacokinetics software**.** The authors concluded that the accuracy of estimation for clearance and volume of distribution of the central compartment can be improved by taking two rather than one blood sample from each subject; with a small sample size and one or two blood samples, mean population clearance and volume of distribution can be reasonably accurately estimated from

WinNonlin pharmacokinetics software. For clearance, one blood sample from five subjects provided a fairly accurate estimate but two blood samples were required to estimate volume of distribution more accurately. WinNonlin pharmacokinetics software provided a more accurate estimate of clearance from five subjects' data (irrespective of one or two blood samples) than NONMEM. The mean population clearance and volume of distribution obtained from one or two blood samples each from five subjects were comparable with the observed values obtained from the extensive blood sampling. However, the mean population PK parameters obtained from two blood samples from each subject provided a better estimate of PK parameters than one blood sample population PK analysis [53].

Therefore, we designed a population pharmacokinetic study using sparse sampling (two to three samples from each subject) among neonates at Mwananyamala regional referral hospital Neonatal unit, to determine whether the current gentamicin dosing practice is associated with adequate and safe serum concentrations.

2.7 Determination of serum gentamicin concentrations

Chemiluminescent microparticle immunoassay and high-performance liquid chromatography (HPLC) are the techniques commonly used in quantifying serum gentamicin concentrations in PK studies. However, chemiluminescent microparticle immunoassay is more commonly used in clinical laboratories due to the ease of performance (easy for automation), more specific as antigen-antibody involved, tiny serum volumes required (this make it more useful in popPK in neonates), rapidity, and lower cost in comparison to HPLC [55].

The use of a more precise method, such as liquid chromatography-tandem mass spectroscopy (LC-MS/MS), which is considered to be the gold standard reference method, with high specificity, sensitivity, and accuracy makes it more suitable for pharmacokinetic studies compared to immunoassays. However, LC-MS/MS has high instrument costs, greater technical complexity, speed, and turnaround time of sample analysis, are considered as the main disadvantages and limits its use especially in resource poor countries.

HPLC technique offers the highest resolution and best degree of specificity than CMIA. However, the difference in accuracy and precision in determining serum gentamicin concentrations between HPLC and CMIA is not significant [55]. Using CMIA in quantifying gentamicin is cost effective, saves time in analyzing samples (it can analyze 100 samples per hour) and also it analyzes tiny serum volumes compared to HPLC. Therefore, CMIA was considered to be the most appropriate method in our study.

2.7.1 Abbott architect ci4100 analyzer: overview

The Abbott Architect System is a fully automated random access analyser that utilizes a chemiluminescent microparticle immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex. The ARCHITECT *i*Gentamicin assay is an *in vitro* chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of gentamicin in human serum or plasma on the ARCHITECT *i*System with *STAT* protocol capability. The measurements obtained are used in the diagnosis and treatment of gentamicin overdose and in monitoring levels of gentamicin to help ensure appropriate therapy.

The ARCHITECT *ci*4100 offers a maximum throughput of up to 100 tests per hour and the required sample for analysis is the sum of 10-150 µL sample volume (Average of 62 µL) and the sample cup (dead volume) of 50µL [56].

2.7.2 Biological principles of chemiluminescent microparticle immunoassay applied in the Abbott architect ci4100 analyzer

The ARCHITECT *i*Gentamicin assay is a one-step immunoassay for the quantitative determination of gentamicin in human serum or plasma using CMIA technology with flexible assay protocols, referred to as Chemiflex[56]. In the ARCHITECT *i*Gentamicin assay, sample, anti-gentamicin coated paramagnetic microparticles, and gentamicin acridinium labeled conjugate are combined to create a reaction mixture. The anti-gentamicin coated microparticles bind to the gentamicin present in the sample and to the acridinium-labeled conjugate. After washing, Pre-trigger and Trigger Solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light unit (RLU). A direct

relationship exists between the amount of gentamicin in the sample and the RLUs detected by the ARCHITECT *i*System optics [56].

2.8 Estimation of GFR using serum creatinine

Renal function assessment in neonates is of the utmost importance in predicting drug dosing, ensuring safe drug therapy, and detecting acute kidney injuries early. However, measuring GFR in young infants is burdensome and highly impractical as a result of the difficulties of urine and blood collection and administration of exogenous markers in this vulnerable population. Therefore, estimation of GFR utilizing endogenous renal biomarkers (serum creatinine, cystatin C, blood urea nitrogen) and anthropometric measurements (body height, weight, muscle mass) has become a useful tool in pediatric clinical practice [57].

Serum creatinine (SCr) is the most extensively studied and therefore most widely used descriptor for evaluating GFR and it is also a reasonable estimator of GFR in neonates [58]. In neonates serum creatinine is reported to be inversely correlated with body weight and gestational age (GA), because of variability in body weight this correlation lead to high interindividual variability in serum creatinine estimates in neonates [58]. In addition, SCr has also been validated in neonates with inulin clearance (Inulin clearance is considered the gold standard method for measuring GFR) and found to be a good estimate of GFR [59].

Several SCr-based GFR-estimation formulas have been developed for pediatric use including the Leger and the Flanders metadata formulas, however, Schwartz *et al* and Brion *et al* studies conducted in the 1980s in both term and preterm infants led to the development of GFR estimation formula which gives excellent estimate of GFR in neonates:

$$eGFR\ (mL/min/1.73m^2) = k \times Ht\ (cm)/SCr\ (mg/dL)$$

Where k is the constant for association of GFR, laboratory measurements, and anthropometric measurement and its value is 0.33 for preterm neonates, and 0.45 for term neonates. Of note,

both of these studies used the Jaffe assay methodology (a colorimetric method used in clinical chemistry to determine creatinine levels in blood and urine) to measure serum creatinine (SCr) values, and single-injection inulin was utilized to measure GFR [60]. The authors concluded that among the variables of body size tested, body height divided by SCr provided the best correlation with GFR, allowing accurate estimation of GFR without the need for any urinary marker measurement and also they did not find any significant differences between males and females or between prepubertal or post pubertal children. In addition, the authors claimed that because the constant 0.45 in term neonates and SCr do not change significantly during the first year of life, GFR can be approximated at the bedside from a single measurement of body length of the healthy full-term neonates (GFR = 0.45 × L/0.39 = 1.1 × Ht) [61].

Despite its well-recognized limitations, SCr remains the renal biomarker upon which Clinical Pharmacologists and Clinicians have primarily relied for estimating GFR in neonates. Of the SCr-based GFR-estimation formulas that have been developed for pediatric use, only one was derived from preterm and term neonates (Schwartz and Brion formula in 1986). In light of this, the Schwartz and Brion formula (eGFR = k × Ht/SCr, k = 0.33 [preterm], k = 0.45 [term neonates]) is considered the most appropriate for estimating GFR in the neonatal population and was used in the estimation of GFR of the neonates participated in this study.

2.9 Serum creatinine levels as an indicator of gentamicin induced renal toxicity

2.9.1 The baseline serum creatinine levels and dosage adjustments for term neonates

For term neonates, the baseline normal serum creatinine levels is 0.5 (Normal low) to 1.5 (Normal high) mg/dL where serum creatinine levels that reach 2.0mg/dL or more in babies indicate severe kidney impairment [62]. All patients should have a kidney function test before starting gentamicin and renal function should be assessed regularly, premature infants and neonates need extensive monitoring if prescribed gentamicin because of their renal immaturity. If renal function is reduced during treatment, the dose of gentamicin should be adjusted accordingly [29].

A study by Pacifici GM *et al* on regimen, toxicology and pharmacokinetics of gentamicin in neonates recommended that, once daily gentamicin dosing should involve giving a patient (term neonate) an intravenous dose of gentamicin of 5mg/kg every 24 hours, unless creatinine clearance is <15ml/min/1.73m^2, when a reduced dose (e.g. 2–3mg/kg) should be used. Furthermore, the study showed that, using lower doses of gentamicin in patients with renal impairment to reduce toxicity has no negative effect on the efficacy of treatment, and the frequency of dosing and timing of the next dose should depend on the patient's renal function [29].

Another study by Bourguignon L *et al* on evaluation of various gentamicin dosage regimens in pediatric patients suggested that, if the patient's baseline renal function is normal (eGFR is >15ml/min/1.73m^2), the patient should be monitored twice weekly and if the patient's baseline renal function is deranged (eGFR is <15ml/min/1.73m^2), or renal function deteriorates, the patient should be monitored daily whilst on treatment and should have gentamicin levels taken every 48 hours, and receive their next dose when levels fall to <1µg/mL [63]. Findings from these studies have been adopted by WHO and form basis of recommendations used in many countries.

2.9.2 Use of serum creatinine levels as an indicator of gentamicin induced renal toxicity

Kidney Disease Improving Global Outcomes (KDIGO) in neonates, defines AKI as the increase in serum creatinine by 0.3mg/dL or more within 48 hours or increase in serum creatinine to 1.5 times baseline or more within the last 7 days[64].There is an 8 to 30% incidence of nephrotoxicity (absolute increase in serum creatinine level of 0.5 mg/dL) associated with gentamicin exposure in older age-groups but there is paucity of studies in neonates regarding gentamicin-induced nephrotoxicity [45]. However, studies on gentamicin induced nephrotoxicity in animals suggest that, nephrotoxicity is greatest, and occurs earlier, in the most immature animals [65].

Current strategies for the prevention of gentamicin-induced nephrotoxicity include extended-interval dosing, and drug trough level monitoring with dose adjustment [65].The traditional indicator of acute kidney injury (AKI) is a rise in serum creatinine concentration, which forms

the basis of all current AKI definitions (Table 2.1). A rise in serum creatinine concentration as an indicator of GFR has long been established in clinical practice and is simple to measure. In addition, the test is very widely available [66]. However, an elevation of serum creatinine is a delayed response, with levels rising significantly above baseline only when 25–50 % of renal function has been lost [66]. Reliance upon this measurement means that AKI is frequently not identified early and that the degree of damage may be underestimated [66]. Furthermore, an increased level of serum creatinine is a marker of glomerular filtration and not an indicator of damage at other sites in the nephron [66].

Table 2.1: Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury (AKI) Classification including neonatal modifications [64]

Neonatal		
Stage	Serum creatinine	Urine output
1	≥ 0.3 rise within 48 h or ≥ 1.5–1.9 × rise from baseline (previous lowest value) within 7 days	≤ 1 ml/kg/h for 24 h
3	2.0–2.9 times baseline	≤ 0.5 ml/kg/h for 24 h
3	≥ 3 × rise from baseline or serum creatinine ≥ 2.5 mg/dL or renal replacement therapy initiation	≤ 0.3 ml/kg/h for 24 h

A study by Devarajan P *et al* on progression of chronic kidney disease after acute kidney injury showed that, acute kidney injury (AKI) causes at least 2 million deaths worldwide each year, and its incidence rate is increasing; evidence suggests that patients who have recovered from AKI have a 25% increased risk for developing progressive chronic kidney disease (CKD) and even end-stage renal disease (ESRD); in addition, these patients demonstrate a 50% increase in mortality after 10 years. These clinical data demonstrate that AKI closely correlates with poor long-term patient outcomes and later CKD development [67]. However,

to date, few mechanisms and individualized molecules, that affect the progression from AKI to CKD have been identified [67]. This emphasizes the importance of adherence to the WHO guidelines in clinical practice.

As stated earlier, the WHO recommends that, the use of gentamicin requires therapeutic drug monitoring to guide dosage adjustments to maximize efficacy and minimize toxicity. However, in our health facilities in Tanzania this practice is not done. Therefore, this study was done to determine whether the current practice of gentamicin dosing without monitoring in neonates is safe or not.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

This was a population pharmacokinetic study (popPK) whereby a limited number of blood samples (sparse sampling) for gentamicin concentration were drawn from each participant.

3.2 Study area

The study was conducted at the neonatal unit of Mwananyamala regional referral hospital in Dar es salaam, Tanzania. Mwananyamala Regional Referral Hospital is one of the three regional referral hospitals in Dar es Salaam. It is the designated regional hospital for the Kinondoni administrative district. The hospital has a catchment area of 2.0 million people. The neonatal unit of Mwananyamala regional referral hospital is a busy unit, which admits about 250-400 neonates (preterm and term) per month. The unit is divided into term baby unit, preterm baby unit and neonatal intensive care unit (NICU) all of which care for newborn babies up to 28 days of life. The term and preterm baby units are further divided into different special rooms and babies are kept in these rooms depending on the diagnosis or underlying problem. The rooms are provided with necessary equipment depending on the needs such as infusion pumps and phototherapy machine.

Services provided in the neonatal unit include: care of low-birth-weight babies including premature and small for gestational age babies who may be normal or sick, normal term babies whose mothers are sick, died or not able to care for their babies, babies with asphyxia and babies with infections such as pneumonia and those with septicemia. The unit has enough staffs, about two pediatricians, six clinicians and seven enrolled nurses who work in shifts (morning and evening shifts). Clinicians at MRRH usually make a tentative diagnosis and initiate empirical treatment of neonatal sepsis before obtaining results of blood culture tests. The hospital laboratory unit has only one biotech analyzer for performing blood culture tests, the biotech analyzer is capable of analyzing 60 blood samples at once, however, results from the analyses are obtained after3 to 4 days.

Neonatal sepsis at MRRH is managed empirically by 5mg/kg once daily dosing of intravenous gentamicin administration in combination with ampiclox (combination of ampicillin and cloxacillin) as the first line antibiotics for 5 to 7days. However, these neonates receiving first line antibiotics (gentamicin, ampicillin and cloxacillin) can be shifted to the second line antibiotics (Ceftriaxone and Ciprofloxacillin) regimens when no or poor therapeutic responses are observed within 3 days.

3.3 Study population

The study included only term neonates, 1 to 28 days of age admitted at Mwananyamala regional referral hospital and prescribed with antibiotics one of them being once daily dosing of intravenous gentamicin for more than 48 hours.

3.3.1 Inclusion criteria

The inclusion criteria were term neonates aged 1 to 28 days admitted at Mwananyamala neonatal unit; prescribed with antibiotics one of them being a once daily dosing of intravenous gentamicin for more than 48 hours; and a written consent from the mother or caretaker for the baby to participate in the study.

3.3.2 Exclusion criteria

The exclusion criteria included very sick term neonates in decompensate state and requiring resuscitation; term neonates who had severe congenital malformation such as anencephaly; term neonates who were receiving other medications that have potential nephrotoxicity or ototoxicity during the course of treatment for example diuretics; term neonates who were receiving other medications that have potential pharmacokinetics interaction by either increasing or decreasing gentamicin serum levels for example phenobarbital. Term neonates who were on gentamicin therapy within the past 72 hours before the study were also excluded.

3.4 Selection of study participants

Term neonates who participated in the study were enrolled consecutively and each participant was assigned a participation number.

3.5 Sample size of the study

In popPK studies, estimation of sample size to determine the minimum number of subjects required to make statistical inference with enough power depends on the parameter of choice (For example clearance and volume of distribution), the sampling designs and the method for the analysis of the collected data among other things and this estimation is done using pharmacokinetic modeling and simulation [68]. The number of subjects in a popPK study is directly related to power of the study, therefore, the power to estimate the confidence interval of a parameter of choice to a particular precision limit is determined by making stepwise increases in sample size until the power is achieved [69].

This work was a population pharmacokinetics study and thus, estimating the 95% confidence interval on the clearance (CL) and volume of distribution (VD) estimated with a 20% precision and a power of 0.8 the required sample size was calculated to be 20-24 participants. Therefore, twenty-four (24) term neonates who met the inclusion and exclusion criteria admitted at Mwananyamala regional referral hospital were included in this study. Although the primary objective was to estimate gentamicin popPK parameters, assessment of change in renal function after gentamicin exposure was also carried out.

3.6 Study procedures

Term neonates admitted at the neonatal unit participated in the study. After obtaining consent from mothers or caretakers, a pediatrician at the unit assessed the neonates to exclude those who did not meet the inclusion criteria. Eligible participants were then assigned a participation number followed by the task of collecting blood samples as described below (Table 3.1). Participants who died or withdrew in the middle of the study were replaced by other term neonates who met the inclusion criteria during the study (Figure 2). This study did not interfere with any of the routine activities which were performed by clinicians as well as nurses at the unit during the entire study.

3.6.1 Blood sample collection for the estimation of clearance and volume of distribution and determination of peak serum gentamicin levels

A blood sample,0.5mLwas drawn twice via a heel prick from each participant at different randomly scheduled sampling time (hours) within 24 hours after administration of the first dose of intravenous gentamicin (see Table 3.1) in a way that covered the entire range of serum gentamicin concentrations versus time data profile from extensive sampling for the estimation of popPK parameters. After intravenous administration of gentamicin, peak serum concentrations usually occur after 30 minutes, so the blood samples for the determination of peak serum gentamicin achieved by the neonates in the study were collected 30 minutes from the time the first dose of IV gentamicin was administered as depicted in Table 3.1.

Table 3.1: Population pharmacokinetic sampling schedule

Participant Number	**Sampling schedule (hours)**															
	¼	½	1	2	3	5	6	8	10	12	14	16	18	20	23	24
01		**x**				**x**										
02					**x**										**x**	
03		**x**					**x**									
04		**x**		**x**												
05		**x**						**x**								
06			**x**											**x**		
07		**x**											**x**			
08		**x**							**x**							
09	**x**							**x**								
10										**x**			**x**			
11			**x**								**x**					
12		**x**										**x**				
13				**x**										**x**		
14					**x**										**x**	
15		**x**								**x**						
16		**x**						**x**								
17		**x**														**x**
18		**x**									**x**					
19		**x**										**x**				
20									**x**							**x**
21	x							x								
22							x									
23						x										
24	x															
25	x															

3.6.2 Blood sample collection for determination of the trough serum gentamicin concentration.

According to the literature, blood samples for the determination of trough serum concentrations of gentamicin are taken four (4) hours before the next dose is due, so 0.5mL of blood sample from each participant was drawn once via a heel prick 20 hours from the time the second dose of intravenous gentamicin was administered.

3.6.3 Handling and analysis of blood samples

Blood samples from each participant at each scheduled time collected within 24 hours following administration of the first dose of intravenous gentamicin for estimation of clearance, volume of distribution and determination of peak serum gentamicin levels were collected in separate serum separator tubes (red topped tubes of 5mL total volume) and labeled by the three names of the mother or caretaker of the baby, date the sample was collected, time the sample was collected and the study number of the baby. Thereafter, samples were immediately taken to the laboratory for storage at -20^0C at Mwananyamala regional referral hospital and were stored at that temperature for not more than an hour before being transported in a Cool ice box ($2\text{-}8^0$C) to central pathology laboratory (CPL) of Muhimbili National Hospital for analysis using Abbott architect ci4100 analyzer.

At CPL, serum was transferred to another tube and centrifuged at 3000 Relative Centrifugal Force (RCF) for 10 minutes to obtain serum and stored frozen ready for being analyzed on the next day. The frozen serums were put on the working bench at room temperature ($15\text{-}30^0$C) for about 30 minutes on the next day for thawing before the analysis was performed.

The preliminary procedures which were performed on the machine before running test of the patient samples were calibration and quality control. The calibrators were tested in duplicate at the calibration range of 0.0 – 10.0µg/mL to verify if the system gives the correct results, the status of the calibration was accessed from calibration status screen which clearly showed whether the calibration passed or failed. For quality control, a single sample of each control level was tested once every 24 hours each day of the machine use to measure whether a given

method is providing the same results day after day. After calibration and quality control procedures were performed and ensuring that the system is able to give the correct results, the step which followed was loading the reagents (anti-gentamicin coated paramagnetic microparticles and gentamicin acridinium-labeled conjugate), patient samples, consumables (pre-trigger and trigger solution) and the reaction vessels into the machine.

The reagent bottles were placed on the matching-colored sections on the reagent carrier and loaded into the machine; the carrier transport in the machine then took up the carrier with the reagents and moved it past the bar code reader. The bar code reader was able to identify the reagents kit and enabled the carrier transport to load the carrier with reagents on the reagent carousel in the processing module. After loading reagents into the machine, the followed step was loading sample tubes in the sample carriers. Before these sample carriers containing sample tubes to be loaded into the priority section or routine section of the machine, information on sample ID and sample tubes position on the sample carriers were entered on the screen. The sample ID information entered were the three names of the mother or caregiver of the baby, date and time the sample was collected for each sample tube.

The sample carriers were loaded into the priority section by pushing them in until the indicator illuminated on the machine. The Ci4100 has 36 positions for sample carriers and one sample carrier can take 5 sample tubes, therefore about 180 sample tubes containing patient samples can be loaded into the machine at once for analysis. After loading the sample carriers into the priority positions, the robotic sample handler (RSH) present in the machine took the sample carriers to the processing module. Reaction vessels were picked by arms and dropped into the processing module through the reaction vessel loader and hoper assembly of the machine.

After all required materials were loaded into the analyzer; the final step was running tests of the patient samples and printing the results. The reaction (sandwich immunoassay reaction) between gentamicin in the sample and the reagents take place in the processing module (a covered circular track part of the analyzer that provides incubation temperatures, liquid aspiration and wash points in the machine). This reaction involve the following automated

procedures; the probe (part of the analyzer which is supported by the syringe assembly for aspiration and dispensing) aspirates specific volume of the patient samples (50µL) and dispenses it into the reaction vessels in the processing module, then the probe is washed by the washing pump present in the machine before aspirating the anti-gentamicin coated paramagnetic microparticles (the paramagnetic and coated with capturing molecules-Immobilized antibody reagent) and dispenses it into the reaction vessels containing the patient samples in the processing module. This reaction mixture is mixed with the help of vortexer present in the machine and incubated for 15 minutes.

In the reaction mixture, gentamicin present in the patient samples bind to corresponding capturing molecules on the microparticles and form immune complex. The reaction vessels are washed and this stage removes all unbound materials. After undergoing another wash, the probe aspirates gentamicin acridinium labeled-conjugate and dispenses it into the washed reaction vessels containing the reaction mixture. The solution mixture is again mixed, a chemiluminescent acridinium labeled-conjugate added binds to the immune complex to complete the reaction mixture.

After a period of incubation (15 minutes), the reaction mixture is again washed to remove the unbound materials. Pre-trigger (Hydrogen peroxide) is added next which creates an acidic environment to prevent early release of energy or light emission (to allow completion of the reaction mixture). Lastly, the trigger solution (sodium hydroxide) is added to complete the reaction. This will trigger the emission of light which is measured in relative light units with the help of system optics in the machine. Relative light units are converted into the optical densities by the system and reading is given for the analyte. A direct relationship exists between the amount of analyte in the sample and the RLUs detected by the Architect System optics.

Patient samples which had gentamicin concentration of > 10µg/mL were flagged as "> 10µg/mL" and needed dilution to get their concentration values. The manual dilutions of such samples were performed using the dilution factor of 1:5 (the suggested dilution factor for the architect *i*Gentamicin assay) and this involved manual addition of 50µL of the patient samples with gentamicin concentration flagged as "> 10µg/mL" to 200µL of the architect *i*Gentamicin calibrator A. The resulting mixtures were loaded into the machine for analysis following same procedures as explained above. This dilution factor was entered in the patient order screen before running the test of the diluted patient samples and the machine used this dilution factor to automatically calculate the concentration of the sample.

The same handling and analysis procedures were done on the second day of the study following administration of the second dose of intravenous gentamicin where all blood samples collected from each participant in separate and labeled serum separator tubes for the determination of trough serum gentamicin concentration were stored and transported to CPL for analysis.

3.6.4 Blood sample collection for serum creatinine levels measurement

Serum creatinine levels (SCr) to assess renal function for all participants in the study were measured before initiation of once daily dosing of intravenous gentamicin therapy to get the baseline serum creatinine levels for each participant and after cessation of intravenous gentamicin therapy to see if there is any significant change from the baseline values.

Blood sample (0.5mL) was drawn via a heel prick from each participant before initiation of once daily dosing of intravenous gentamicin treatment and the sample was taken immediately to the laboratory for analysis using a fully automated Erba XL-100 analyzer at Mwananyamala regional referral hospital. Also, at day seven of treatment with IV gentamicin; 0.5mL of blood sample from each term neonate participating in the study was drawn and immediately taken to the same laboratory to measure serum creatinine levels after treatment.

3.6.5 Determination of Acute Kidney Injury (AKI)

Acute kidney injury was determined using the Kidney Disease Improving Global Outcomes (KDIGO) in neonates and AKI was defined as an increase in serum creatinine by 0.3mg/dL or more within 48 hours or increase in serum creatinine to 1.5 times baseline or more within the last 7 days (Table 2.1) [64]. Also, the estimation of GFR (mL/min/1.73m^2) values before and after treatment (at day seven) for each participant were calculated from serum creatinine levels (mg/dL) measured before and after initiation (at day seven) of once daily dosing of intravenous gentamicin treatment using Schwartz and Brion serum creatinine levels-based GFR-estimation formula. This formula gives accurate estimation of GFR in neonates than the other present formulas [61].

3.7 Data analysis

Assuming a one compartment model, the serum gentamicin concentrations versus time profile was fitted using stata version 14.2 software to give the characteristic serum concentration time curve following an intravenous dose. From this curve, population pharmacokinetic parameters were directly estimated (elimination rate constant, elimination half-life and area under the curve). Using these popPK parameters which were directly estimated from the profile, other important popPK parameters (clearance and volume of distribution) were calculated.

The Kolmogorov-Smirnov and Shapiro Wilk test was used to determine the normality of data distribution. Categorical data were presented as proportions and compared using McNemar tests; other categorical data for defining baseline characteristics and clinical records of the study participants were presented in frequency tables. Continuous data were expressed as mean ± S.D (standard deviation) and were compared using one sample t-test (comparison of the observed mean peak or trough serum gentamicin concentration and the known reference) and paired samples t-test (comparison of the mean serum creatinine concentration before and after treatment with gentamicin). The results were considered statistically significant at P values <0.05.

3.7.1 Variables

3.7.1.1 Dependent variables

The dependent variables were peak and trough serum gentamicin concentrations, serum creatinine levels, gentamicin renal clearance, volume of distribution of gentamicin, IV gentamicin dose administered (mg), patient's renal function and eGFR

3.7.1.2 Independent variables

The independent variables were Gentamicin IV dose administered (5mg/kg), patient's age, sex, height, body weight when the test was taken, clinical diagnosis and the time scheduled for collecting blood samples for each participant.

3.8 Ethical consideration

The study obtained approval from the MUHAS Ethical Review Committee (Appendix VI) and commenced after receiving ethical approval of the ethics committee board and after obtaining permission to do a study from the Medical Officer In-charge of Mwananyamala regional referral hospital (Appendix VII). Patients were enrolled in the study only after obtaining the informed parental consent and the numbers of pricks were limited between three pricks within 24 hours. Blood sampling procedures were done by a nurse (research assistant) and followed aseptic techniques, so, no participant was harmed by these blood sampling procedures during the study.

3.9 Study limitations and mitigation

Chemiluminescent microparticle immunoassay specificity to gentamicin tends to slightly or negligibly decrease when blood samples do contain β-lactam antibiotics. Since all neonates who were admitted at Mwananyamala regional referral hospital were treated with gentamicin in combination with ampiclox (combination of ampicillin and cloxacillin), so some slight or negligible falsely low gentamicin levels on analysis with chemiluminescent microparticle immunoassay (CMIA) were expected. However, this effect was mitigated by storing the blood samples frozen at CPL when delay in analysis of more than 8 hours was anticipated.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSIONS

4.1 RESULTS

A total of two hundred and twenty-six (226) neonates were screened during the study whereby 115(50.9%) were male and 111(49.1%) were female. One hundred and ninety-seven (197) which is 87.2% of all screened neonates were not eligible to participate in the study and twenty-nine (29) which is 12.8% were eligible to participate. Majority of the neonates screened (62.4%) had already been initiated on a gentamicin based treatment before their referral to Mwananyamala regional referral hospital (MRRH); however we were able to get the required number of the study participants (24) as per protocol. In the middle of the study, one (1) out of twenty-four (24) participants who were selected to participate in the study died and was replaced by another term neonate who met the inclusion criteria, to make a total of 25 term neonates who participated in this study.

Data of the participant who died during the study were included in some analyses such as analysis of the social demographic and clinical data of the study participants, and in the determination of population pharmacokinetic parameters (clearance and volume of distribution). However, data of serum creatinine levels after treatment with gentamicin and trough serum gentamicin concentration of this participant were not available, therefore, this participant was not included in the analysis of serum creatinine levels before and after treatment and in the determination of trough serum gentamicin levels.

Patients' recruitment and participation in the study is summarized in Figure 2.

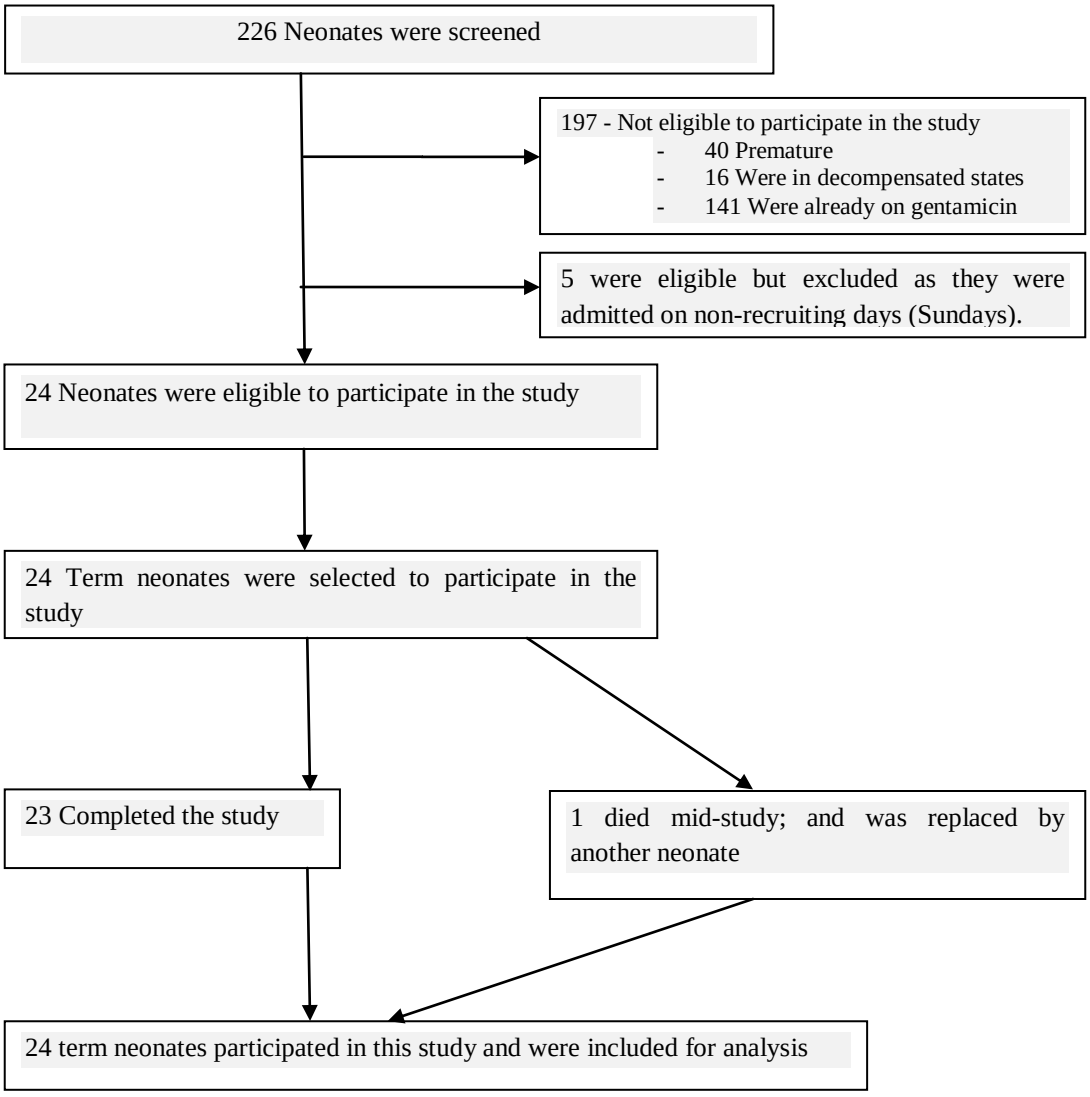

Figure 2: Patients' recruitment and participation in the study.

Majority of the study participants were male (56%). The study participants had median gestational age of 38 (Range 37, 40) weeks, which corresponded with their mean birth weight of 3.12 (SD ± 0.61) kg. Most of the study participants (72%) had clinical diagnosis of sepsis and few(4% each) with meconium aspiration and pneumonia. The mean Apgar score of the study participants was 8.0 (SD ± 0.2). Other social demographic and clinical data of the study participants are shown in Table 4.1

Table 3.1: Social demographic and clinical data of the study participants

Characteristics	**N = 25**	**%**
Median age in days (Range)	2 (1, 24)	
Sex		
Male	14	56.0
Female	11	44.0
Mean body weight (kg) (±SD)	3.23 ± 0.01	
Mean height (cm) (± SD)	50.16 ± 3.03	
Median gestational age in weeks (Range)	38 (37, 40)	
Mean Apgar score (±SD)	8.0 ± 0.2	
Clinical diagnosis		
Neonatal sepsis	18	72.0
Neonatal jaundice	3	12.0
Meconium aspiration	1	4.0
Pneumonia	1	4.0
Birth asphyxia	2	8.0
Other clinical records		
Diarrhoea	5	20
Unable to suck	3	12

4.1.1 Peak and trough serum gentamicin levels

Twelve (12) participants contributed blood samples for the peak serum gentamicin concentrations time point of the popPK profile. All 12 blood samples were found to have high peak serum gentamicin levels (>12µg/mL) compared to the reported upper limit of normal peak serum gentamicin concentration for the typical term neonates. As a result, the overall peak serum gentamicin concentrations achieved by the neonates in this study was high (one sample t-test) with a mean peak concentration (SD) of 16.669, (± 0.646); 95% CI= 16.259, 17.079 µg/mL; t (11) = 25.05; *P*< 0.001 (Figure 3).

In the analysis for peak serum concentration (figure 3), the upper limit of normal peak serum gentamicin concentration (12µg/mL) reported in literature for the typical term neonates was used as the null hypothesis (Ho =12µg/mL) and was compared with the mean peak serum gentamicin concentration observed in our study (16.669 ± 0.646). In this comparison, the estimated mean of the study participants was significantly higher than the upper limit of the normal gentamicin concentration, *P*< 0.001.

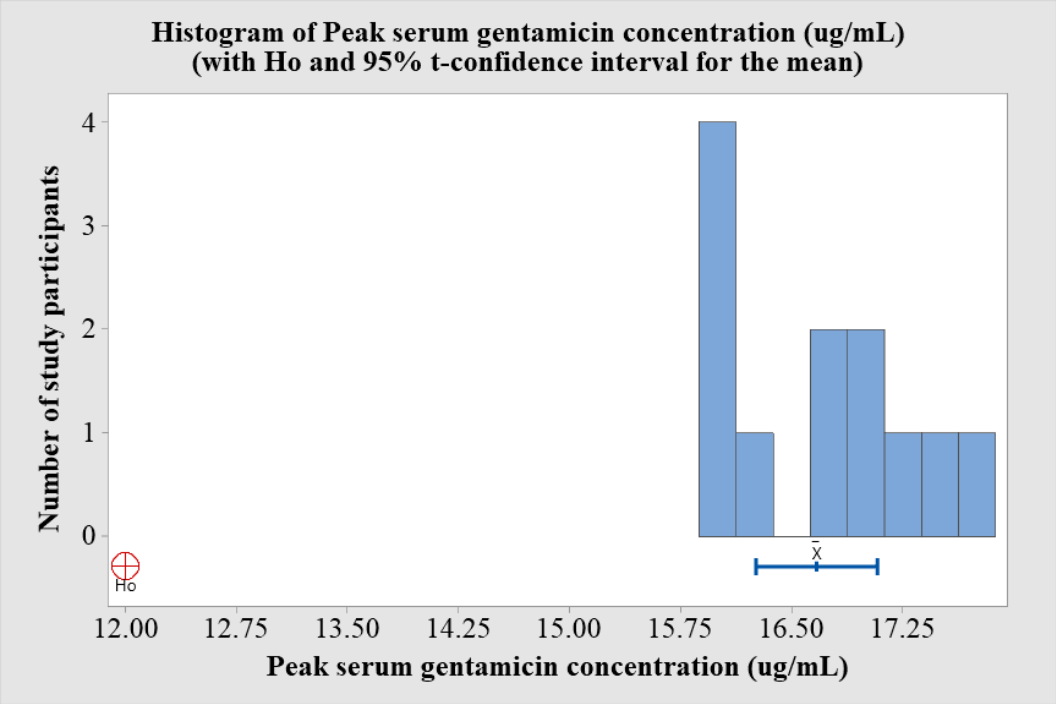

Figure 3: Peak serum gentamicin concentrations

A blood sample for trough serum gentamicin concentration was collected from each of the 24 participants and analyzed. All 24 participants were found to have high trough serum gentamicin levels (>2µg/mL) compared to the reported upper limit of normal trough serum gentamicin concentration for the typical term neonates. As a result, the overall trough serum gentamicin concentrations achieved by the neonates in this study was high (one sample t-test) with a mean trough concentration (SD) of 3.283, (±0.707); 95% CI= 2.984, 3.581 µg/mL; t (23) = 8.893; *P*< 0.001 (Figure 4).

Like for peak concentration above, the upper limit of normal trough serum gentamicin concentration (2µg/mL) reported in literature for the typical term neonates was used as the null hypothesis (Ho = 2µg/mL) and was compared with the mean trough serum gentamicin concentration observed in our study (3.283 ± 0.707). In this comparison, the estimated mean of the study participants was significantly higher than the upper limit of the normal gentamicin concentration, *P*< 0.001 as depicted in Figure 4.

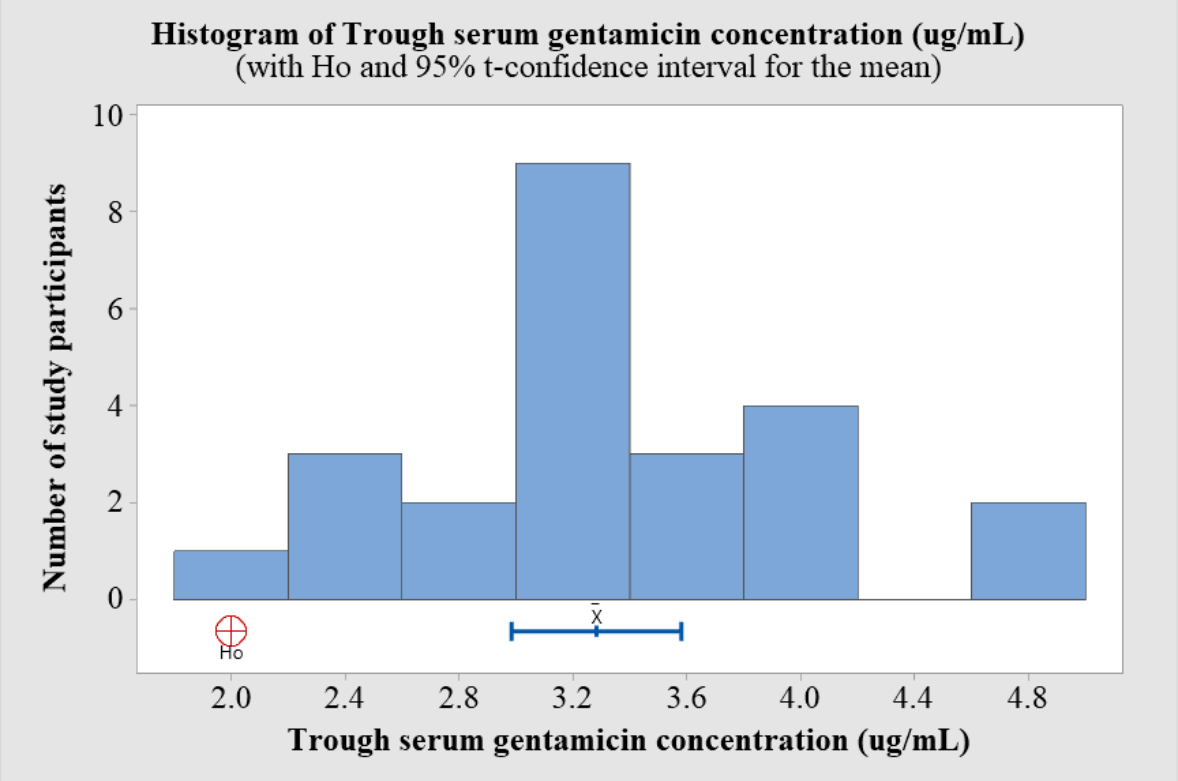

Figure 4: Trough serum gentamicin concentrations

4.1.2 Population pharmacokinetic parameters (clearance and volume of distribution)

For the popPK profile, a total of 45 serum samples contributed by participants at the different time points were measured for serum gentamicin concentrations and the averages of serum gentamicin concentrations at each time point (table 3.1) were used to plot the serum gentamicin concentrations versus time profile. The profile was fitted using stata version 14.2 software to give the characteristic serum concentration time curve following an intravenous dose assuming one compartment model (Figure 5). From this curve, population pharmacokinetic parameters were directly estimated where elimination rate constant was found to be $0.0793h^{-1}$; elimination half-life (t½) 8.7384 hours and area under the curve (AUC) $206.11\mu gmL^{-1}h^{-1}$. Using these popPK parameters, clearance and volume of distribution were calculated and were 0.40 $mLmin^{-1}kg^{-1}$ and $0.31Lkg^{-1}$ respectively.

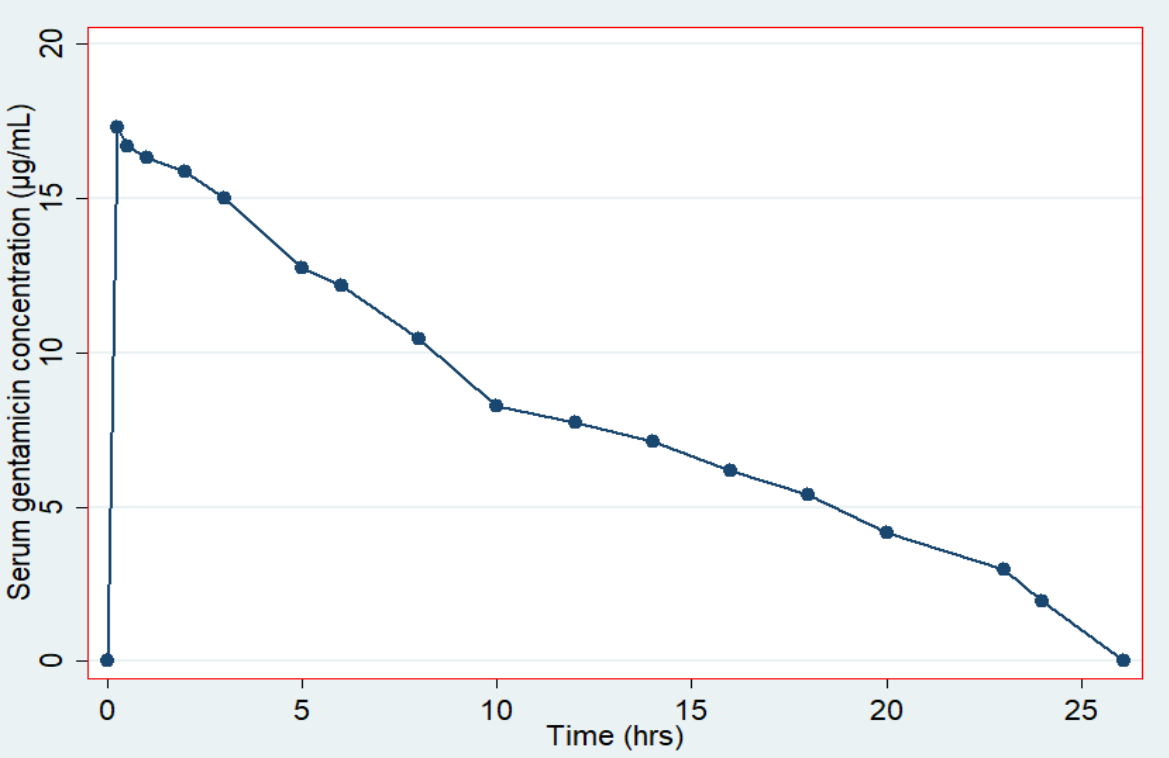

Figure 5: Serum gentamicin concentrations (µg/mL) versus time (hours)

4.1.3 Mean change in serum creatinine after gentamicin treatment.

A total of 48 blood samples, one before and one after treatment with gentamicin from each of the 24 participants were collected and measured for serum creatinine levels. Before treatment 2(8.3%) participants had high serum creatinine levels (M= 137.91 ± 0.01 µmol/L) whereas 20(83.3%) participants had high serum creatinine (M= 228.28±6.04 µmol/L) levels after treatment with gentamicin (Figure 7). This shows a tenfold increase in the number of participants with high serum creatinine levels seven days from start of gentamicin treatment. A paired sample t-test indicated that, the overall mean serum creatinine levels after treatment with gentamicin (209.7±70.4µmol/L) were significantly higher than mean serum creatinine levels before treatment (103.3±23.6 µmol/L) with a statistically significant mean difference (106.4± 67.1; 95% CI :78.1, 134.7µmol/L), t (23) = 7.77, $P< 0.001$ (Figure 6).

For analysis of mean change in serum creatinine concentration after treatment with gentamicin we hypothesized that the difference between the mean serum creatinine levels after treatment and before treatment was zero (Ho= 0). However, when the two means were compared (paired samples t-test), the difference in the means was found to be significantly higher than the hypothesized mean difference (Ho= 0 versus 106.4 ± 67.1), $P< 0.001$ (Figure 6).

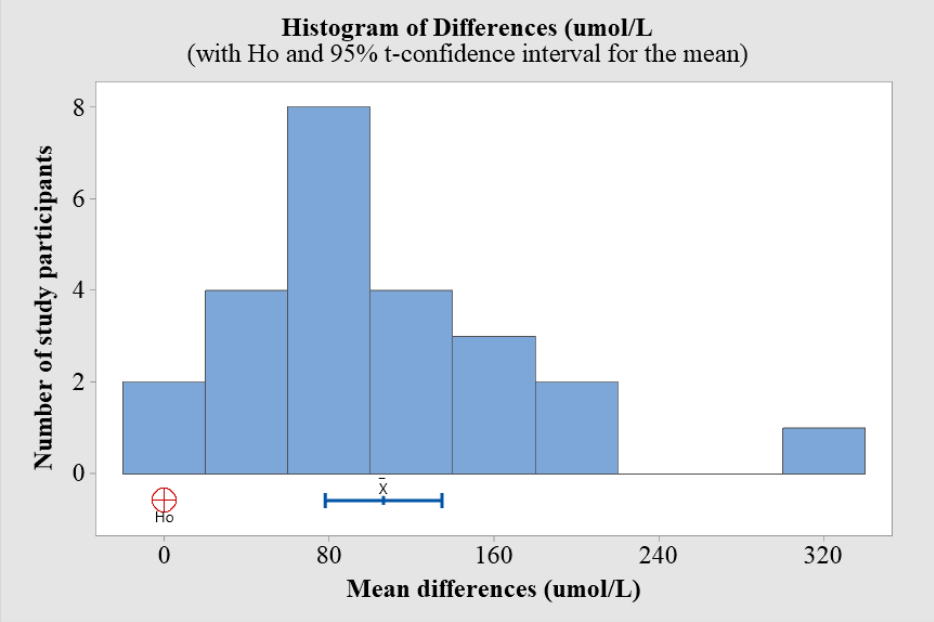

Figure 6: Mean differences in serum creatinine levels after gentamicin treatment

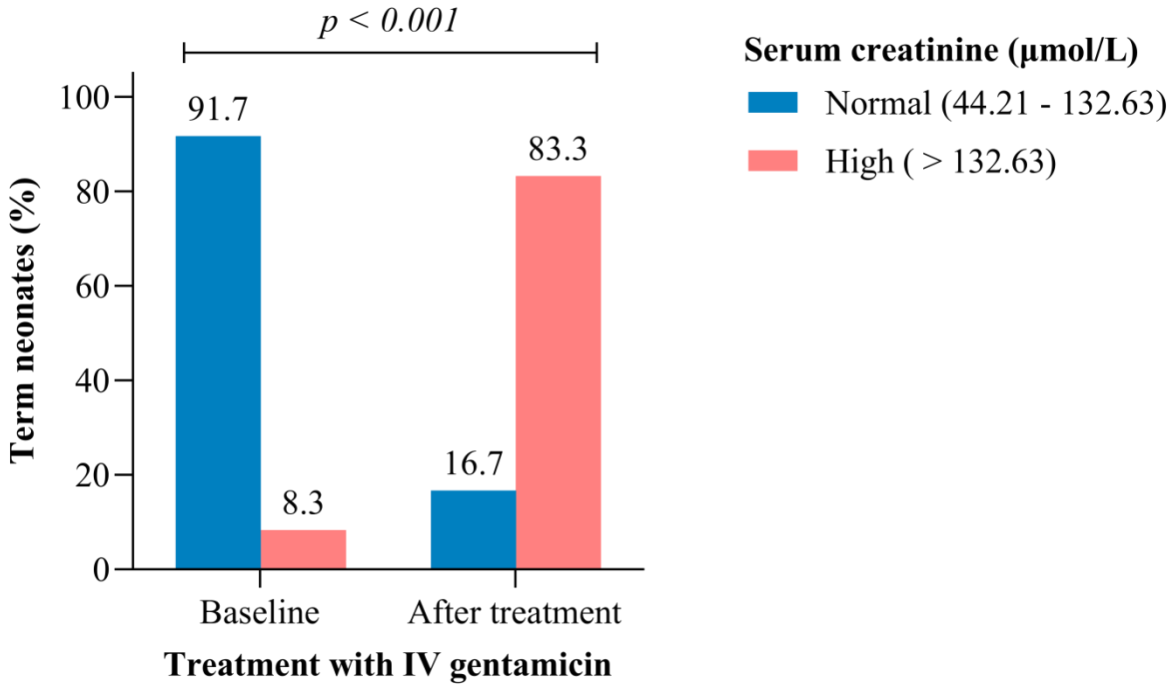

Figure 7: Percentage of high serum creatinine levels before and after treatment with gentamicin

In line with change in serum creatinine levels before and after treatment with gentamicin, 2(8.3%) participants had low eGFR before initiation of IV gentamicin whereas 20(83.3%) participants had low eGFR after treatment with intravenous gentamicin (Figure 8); this shows a tenfold increase in the number of participants with low eGFR seven days from start of gentamicin treatment. Therefore, in our study, change in serum creatinine after treatment with gentamicin correlated well with change in eGFR. The calculated values of estimated glomerular filtration rate (eGFR) of each study participant are shown in table 4.2

Table 4.1: Calculated eGFR of the study participants before and after treatment

Participant number	Calculated eGFR before treatment		Calculated eGFR after treatment	
	Value (mL/min/1.73m^2)	Status	Value (mL/min/1.73m^2)	Status
01	15.18	Normal	9.38	Low
02	17.13	Normal	8.79	Low
03	15.42	Normal	10.48	Low
04	19.61	Normal	11.31	Low
05	18.62	Normal	16.79	Normal
06	18.99	Normal	10.17	Low
07	28.37	Normal	9.05	Low
08	31.33	Normal	14.69	Low
09*	5.22	Low	-	
10	18.93	Normal	14.91	Low
11	15.53	Normal	8.58	Low
12	16.72	Normal	7.79	Low
13	16.41	Normal	8.43	Low
14	21.71	Normal	8.36	Low
15	17.17	Normal	9.50	Low
16	24.31	Normal	6.56	Low
17	20.87	Normal	19.10	Normal
18	36.17	Normal	17.85	Normal
19	26.25	Normal	7.37	Low
20	26.96	Normal	9.00	Low
21	24.74	Normal	15.40	Normal
22	15.55	Normal	8.45	Low
23	13.82	Low	9.52	Low
24	17.23	Normal	8.25	Low
25	14.80	Low	4.45	Low

*The participant was not included in this analysis, because the eGFR after treatment was missing (died in the middle of the study)

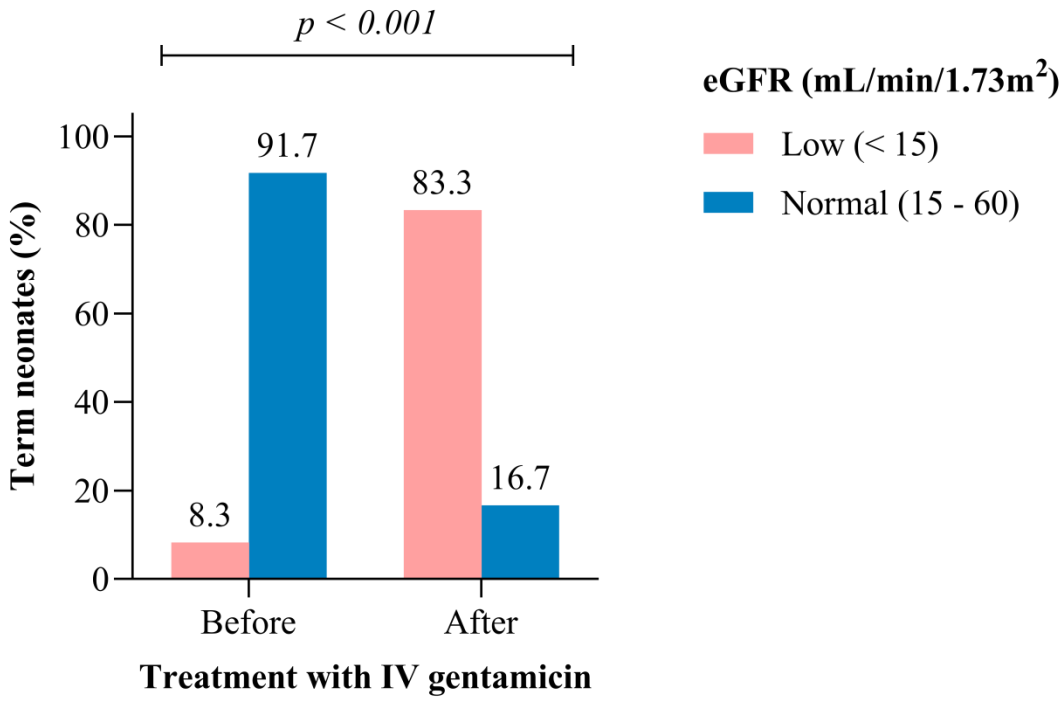

Figure 8: Percentage of high estimated GFR before and after treatment with gentamicin

4.1.4 Acute kidney injury (AKI) staging based on updated KDIGO criteria

Based on updated KDIGO criteria, 14(58.33%) of the study participants had baseline serum creatinine levels increased by a factor of 1.5 to 2 after gentamicin treatment and were considered as having developed AKI stage I, 10(41.67%) of the study participants had baseline serum creatinine levels increased by a factor of 2 to 3 and were categorized as having developed AKI stage II and there were no study participants who developed AKI stage III as shown in Figure 9. Therefore, more than 90% of participants in our study developed acute kidney injury after gentamicin treatment.

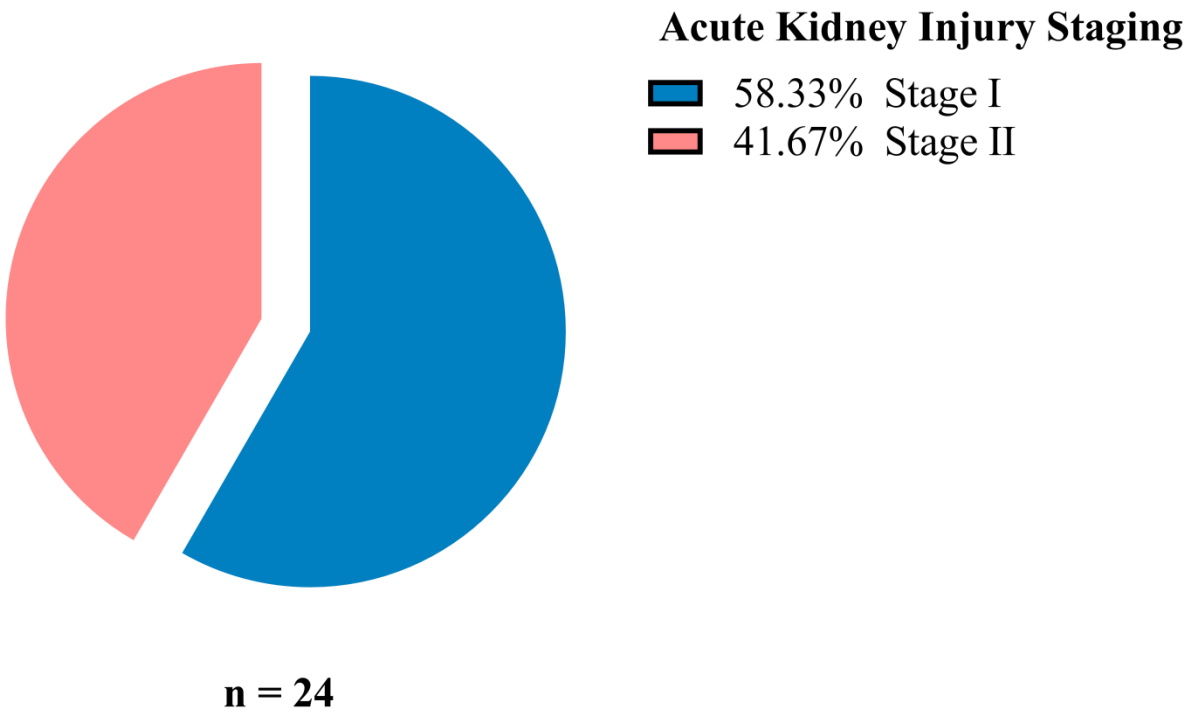

Figure 9: Acute kidney injury staging based on KDIGO criteria

4.2 DISCUSSION

The aims of this study were to determine the peak and trough serum levels of gentamicin achieved by the neonates following once daily dosing of intravenous gentamicin, to determine the population gentamicin clearance and volume of distribution and to determine whether there was a significant increase in serum creatinine levels after gentamicin therapy for more than 48 hours.

4.2.1 Peak and trough serum gentamicin levels

Different studies on clinical pharmacokinetics of gentamicinin neonates including a study by Pacifici GM *et al* shown that, neonates who achieved peak serum gentamicin levels above 12µg/mL and trough serum gentamicin levels above 2µg/mL during the study, developed acute kidney injury and permanent hearing loss [28]. In our study, the pharmacokinetic data of gentamicin in the studied term neonates indicate that, the peak and trough serum gentamicin concentrations exceeded the potential toxic concentrations of >12µg/mL and >2µg/mL respectively. These data lend support for the recommendation to perform routine baseline serum creatinine test before starting gentamicin treatment, renal function assessment during and after treatment and serum gentamicin levels monitoring [28]. Indeed, recent studies, including a study by Pacifici GM *et al*on gentamicin-induced nephrotoxicity in neonates have shown that, in circumstances of no baseline serum creatinine monitoring, regular renal function assessment during treatment and gentamicin levels monitoring, patients achieve higher peak and trough serum gentamicin levels which increase the risk of nephrotoxicity and ototoxicity, highlighting the need for serum creatinine levels and gentamicin concentration monitoring during treatment with gentamicin [29].

In our study it was observed that, 8.3% of the study participants had deranged eGFR (eGFR <15ml/min/1.73m^2) at baseline and this number increased tenfold (83%) within seven days from start of gentamicin treatment. This implies the potential nephrotoxicity of gentamicin in these neonates and the need to check renal function test before and after initiation of gentamicin. These findings show that, more than 90% of participants developed acute kidney injury after treatment with gentamicin. In comparison with other studies done on gentamicin-

associated acute kidney injury (AKI),our study resulted into large proportion of study participants who developed AKI. For-example, a study by Selby *et al* on gentamicin-associated acute kidney injury (AKI) on patients who were treated with gentamicin for more than 48 hours without monitoring showed that, acute kidney injury occurred in 24.4% patients, this difference with our study findings maybe contributed by the difference in trough serum gentamicin levels achieved by the study participants. In this study, the overall trough serum gentamicin concentration was 2.37 (±0.24) µg/Ml while in our study was 3.283 (±0.707) µg/mL, this difference in trough serum gentamicin concentration can explain the observed difference because the higher the trough serum concentration achieved, more damage to the kidneys occur.

Studies on regimen, toxicology and clinical pharmacokinetics of gentamicin in neonates including a study by Pacifici GM *et al* recommend that, the estimated GFR of the neonates should be checked before initiating gentamicin treatment. In addition, findings from this study highlighted that, if the patient's baseline renal function is normal (eGFR is >15ml/min/1.73m^2), patient should be monitored twice weekly and if the patient's baseline renal function is deranged (eGFR is <15ml/min/1.73m^2) or renal function deteriorates, the patient should be monitored daily whilst on treatment and should have gentamicin levels taken every 48 hours, and receive their next dose when levels fall to <1µg/mL to prevent gentamicin induced nephrotoxicity [29].

The normal and safe practice requires that baseline creatinine clearance be checked for all patients and the dose to be given, frequency of dosing and timing of the next dose to depend on the patient's renal function [29]. Therefore, the practice of starting treatment with gentamicin in neonates without monitoring their baseline serum creatinine and drug levels in our health facilities appears to be unsafe and the need to seriously consider change of practice is required to ensure that doses are given according to the patient's renal function and serum levels (peak and trough) attained are within the recommended range thereby preventing toxicity (nephrotoxicity and ototoxicity).

Another medication error which is considered to cause elevated serum concentration of gentamicin in many settings is a failure to strictly adhere to the right time of administering

gentamicin between doses (24 hours dosing interval), this medication error was observed during this study, most of gentamicin doses in neonates were administered between 16-18 hours before the 24 hours interval was due. Gentamicin can accumulate in the body when administered at shorter intervals than 24 hours; so, also, this practice probably contributed to the observed higher levels in our study.

4.2.2 Population pharmacokinetic parameters (clearance and volume of distribution)

The population pharmacokinetic parameters (clearance and volume of distribution) in our study were lower compared to the recommended normal ranges in term neonates. Clearance was 0.40 $mLmin^{-1}kg^{-1}$ and volume of distribution was 0.31Lkg^{-1} whereas the recommended normal ranges are 0.50 to 1.71 $mLmin^{-1}kg^{-1}$ for clearance [29] and 0.4 to 0.7 Lkg^{-1} for the volume of distribution [47]. These findings show that the population pharmacokinetics following the initial dose correlate with the steady state pharmacokinetics achieved 20 hours from the initial dose; it can be suggested that the higher serum gentamicin levels observed in the study participants indicate lower gentamicin clearance and or volume of distribution. In this study, determinants which alter gentamicin clearance such as sepsis, impaired renal function and birth asphyxia may have contributed to the observed lower gentamicin clearance, because majority of the study participants (72%) had clinical diagnosis of sepsis, 8.3% participants had impaired renal function before starting gentamicin treatment and 8.3% participants had clinical diagnosis of birth asphyxia; these clinical conditions are believed to lower renal clearance of gentamicin.

Mathur NB *et al* investigated the effects of sepsis in the changes in renal function in neonates and found that, sepsis can operate through variety of mechanisms in producing renal failure. It can cause renal failure by shock, cardiac failure, hemorrhage, disseminated intravascular coagulation and through acute tubular necrosis and result into reduced renal clearance as it was observed in this study [70]. Also, a study by Pacifici GM *et al* on clinical pharmacokinetics of gentamicin in neonates found that, asphyxiated neonates tend to have prolonged gentamicin half-life and significant decreases in urine output which is an indicator

of reduced renal function [20]. Since all these determinants are believed to reduce the renal function, so, monitoring baseline creatinine clearance before starting treatment remains important because the reduced renal function in the study participants would have been identified before treatment and dosages would have been adjusted accordingly to ensure that serum levels (peak and trough) attained are within the recommended range.

Neonates have a larger extracellular fluid volume and therefore tend to have large volumes of distribution because gentamicin is a fairly water-soluble drug and is distributed predominantly in extracellular fluid. The large amount of extracellular body water in neonates results in lower serum gentamicin concentrations; however, this is contrary to the findings of this study because the serum gentamicin levels were higher and the volume of distribution was lower probably due to the lower extracellular fluid volume (ECF) [30]. Any factor, which reduce the extracellular fluid volume tend to decrease the volume of distribution of gentamicin. In this study, 20% of the study participants had diarrhoea and 12% were unable to suck and were put on IV fluids (DNS); also, these participants had elevated serum creatinine levels when compared to other study participants with mean serum creatinine level of 160 ± 18.5 µmol/L before treatment. Diarrhoea and inadequate feeds in neonates tend to reduce the ECF and lead to reduced volume of distribution of gentamicin and clearance. A study by Pacifici GM *et al* highlighted that, it is important to monitor the fluid balance closely and correct dehydration prior commencing treatment with gentamicin to ensure that the extracellular fluid volume is kept normal thereby attaining serum gentamicin levels which are within the accepted ranges to prevent the risk of nephrotoxicity and ototoxicity [29].

The overall limitation of this study was on pharmacokinetic data analysis. Initially, PK data analysis was proposed to be done using WinNonlin Pharmacokinetics software. However, due to inaccessibility of WinNonlin Pharmacokinetics software, we analyzed the data using stata version 14.2 software. Although the best PK software would have been WinNonlin, we believe stata version 14.2 software gave us fair estimates like that would have been obtained by WinNonlin since it has PK commands which give estimates of PK parameters. Also, the

variation of serum gentamicin concentration among participants which might have influenced the observed results at each time point was not determined. However, we believe this variation was not significant as most of our study participants were from similar geographical location, born with black mothers, had small difference in age and weight; and also majority had similar clinical diagnosis (Neonatal sepsis).

4.2.3 Change in serum creatinine and eGFR after treatment with gentamicin.

We have observed higher serum creatinine levels in neonates on treatment with gentamicin without baseline serum creatinine levels test and without monitoring of serum gentamicin concentrations. The mean serum creatinine levels after treatment was higher by 106.4±67.1µmol/L compared to the mean serum creatinine levels before treatment. Gentamicin serum levels above the recommended ranges tend to accumulate within the proximal tubule epithelial cells (PTECs) in the renal cortex following glomerular filtration by endocytosis [45] thereby causing nephrotoxicity. Although was not part of this study, ototoxicity is another negative impact of gentamicin treatment, gentamicin serum levels above the recommended ranges also tend to accumulate in the sensory hair cells in the inner ear by both endocytosis and transport through ion channels [43] leading to a decrease in mitochondrial ATP synthesis, which results into compromised ion pump activity, progressive decrease of the endocochlear potential and slow progression of hearing loss [44].

Recent studies have shown that, nephrotoxicity occurs in 10–25% of patients on aminoglycosides without monitoring [23] and ototoxicity occurs in up to 25% of neonates [40] when trough concentration is >2 µg/mL for the treatment that extends more than 48 hours [20]. Nephrotoxicity is considered significant when there is mean increase in serum creatinine levels of 44.21µmol/L and above from the baseline levels [45]. In our study, the mean increase in serum creatinine levels was three times this minimum level for nephrotoxicity and can be suggested that, significant renal injury occurred in the study participants as the result of gentamicin accumulation. The traditional indicator of AKI is a rise in serum creatinine concentration, which forms the basis of all current AKI definitions. In our study, based on

updated KDIGO criteria, about 58% of the study participants had baseline serum creatinine levels increased by a factor of 1.5-2 and were considered to have developed AKI stage I whereas about 42% had baseline serum creatinine levels increased by a factor of 2-3 and were categorized to have developed AKI stage II. Evidence from different studies suggest that, patients who have recovered from AKI have a 25% increased risk for developing progressive chronic kidney disease (CKD) and even end-stage renal disease (ESRD). Therefore, 25% of our study participants had increased risk for developing progressive chronic kidney disease (CKD).

In conclusion, it is important to perform kidney function test before, during and after treatment with gentamicin and to monitor serum gentamicin levels to ensure that doses are given according to the patient's renal function and serum levels (peak and trough) attained are within the recommended range thereby preventing toxicity (nephrotoxicity and ototoxicity) as it was observed in this study. In addition, like nephrotoxicity, ototoxicity occurs in up to 25% of neonates treated with gentamicin [40]. Because of its feasibility (lack of equipment), we did not do audiometry (the science of measuring hearing acuity for variations in sound intensity and pitch and for tonal purity, involving thresholds and differing frequencies) in our study. Since we have seen more than 90% of the study participants developed acute kidney injury; therefore, we believe some of these neonates suffered both nephrotoxicity and ototoxicity. Therefore, we recommend other studies to be done to look at the gentamicin-induced ototoxicity in neonates.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

This study shows that, neonates admitted at MRRH who receives once daily dosing of IV gentamicin without serum creatinine levels test before, during treatment and serum gentamicin concentrations monitoring achieve higher peak and trough serum concentrations than the recommended normal upper limit. In addition, neonates in our study had lower clearance and volume of distribution of gentamicin that may explain the observed higher serum gentamicin levels. Lastly, there was a significant increase in serum creatinine concentration and a significant reduction in eGFR following gentamicin treatment which met the KDIGO criteria for acute kidney injury. This suggests that a significant number of neonates receiving gentamicin without serum creatinine and serum gentamicin concentration monitoring are at risk of developing CKD during their life time

5.2 RECOMMENDATIONS

Our findings highlight the need for the Ministry of Health, Community Development, Gender, Elderly and Children to revise the standard treatment guidelines regarding empirical treatment of neonatal sepsis and the use of gentamicin in neonates in general, in view of making serum creatinine testing before, during and after treatment and gentamicin serum concentration monitoring mandatory. The feasibility of this recommended change in practice needs to be studied. However, serum creatinine and serum gentamicin monitoring at district or regional referral hospitals is likely to be feasible. Immunoassay is a relatively cheap high-throughput technology and biochemistry panel analyzers can do both serum creatinine and serum gentamicin testing. Therefore, all this may require minimal strengthening of the district or regional referral level clinical laboratories. Additionally, health practitioners should be made aware or reminded of the importance of strictly adhering to the right time of administering gentamicin between doses (24 hours dosing interval) and be informed on the consequences of not adhering to it because gentamicin is not as safe as it may be thought, it can accumulate in

the body and cause toxicity when administered at shorter intervals than what is recommended by the manufacturer.

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APPENDICES

Appendix I: Case report form

Parameters	Readings
Baseline assessment	
Date:	…… /…… /…….
COIN NO:	…….
I/O ……..	**Physical address** Street ……..…… Street chairperson…….…. Mobile phone ……..……. Other close relative contacts …….
LMP	…..… /……. /……
DOB	……. /……. /……
Gestational Age	…….
Sex:	Male ☐ Female ☐
Birth weight:	……...kg
Height of the neonate:	……...cm
Consent Given	Yes ☐ No ☐
Decompensated requiring resuscitation?	Yes ☐ No ☐
Was on IV gentamicin therapy for the past 24 hours?	Yes ☐ No ☐
Has severe congenital malformation?	Yes ☐ No ☐
Receiving other medications which have potential nephrotoxicity or ototoxicity?	Yes ☐ No ☐
Receiving other medications which have potential pharmacokinetics interaction by either increasing or decreasing gentamicin serum levels?	Yes ☐ No ☐
Is he/she term neonate?	Yes ☐ No ☐
Once daily IV Gentamicin for more than 48 hours prescribed?	Yes ☐ No ☐

Is the patient eligible to participate in the study?	Yes ☐ No ☐
Was the patient selected to the study?	Yes ☐ No ☐
Study Number allocated	……
Blood samples for Serum creatinine taken? Date: Time: Initials (A Nurse/Doctor who took the sample):	Yes ☐ No ☐ ……. /…… / …… …….. ……
Day 0: IV Gentamicin dosing and blood sampling	
IV Gentamicin dosing Dose administered: Date: Time: Outcome: Initials (A Nurse/Doctor who administered the drug):	 ……..mg ……. / ……../ …….. …….. Uneventful ☐ Eventful ☐ If eventful briefly state the observations: …….…… …….…… ……. …….....

Population pharmacokinetic sampling schedule following administration of the first dose of intravenous gentamicin for the estimation of clearance, volume of distribution and determination of peak serum gentamicin levels.

Participant Number	Sampling schedule (hours)															
	¼	½	1	2	3	5	6	8	10	12	14	16	18	20	23	24
01		x				x										
02					x										x	
03		x					x									
04		x		x												
05		x						x								
06			x											x		
07		x											x			
08		x							x							
09	x							x								
10										x			x			
11			x								x					
12		x										x				
13				x										x		
14					x										x	
15		x								x						
16		x						x								
17		x														x
18		x									x					
19		x										x				
20									x							x
21	x							x								
22							x									
23						x										
24	x															
25	x															

Detailed schedule for blood samples collection following administration of the first dose of intravenous gentamicin.

Participant Number	**Time (hrs) scheduled for collection of blood samples for estimation of PopPK parameters**									
	Date the first dose was administered	Time (hrs) the first dose was administered	Planned date for the collection of the first sample	Actual date the first sample was collected	Planned time (hrs) for the collection of the first sample	Actual time (hrs) the first sample was collected	Planned date for the collection of the second sample	Actual date the second sample was collected	Planned time (hrs) for the collection of the second sample	Actual time (hrs) the second sample was collected
01										
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25										

Day 1: IV Gentamicin dosing and blood sampling	
IV Gentamicin dosing	
Dose administered:	……..mg
Date:	……. / ……../ ……..
Time:	……..
Outcome:	Uneventful ☐ Eventful ☐ If eventful briefly state the observations: …….…… ……..…… …….
Initials (A Nurse/Doctor who administered the drug):	…….....

Blood sampling time (hrs) scheduled for the determination of trough serum concentration for each participant after administration of the second dose of intravenous gentamicin.

Participant Number	Time (hours) after administration of the second dose of intravenous gentamicin															
	¼	½	1	2	3	5	6	8	10	12	14	16	18	20	23	24
01														**x**		
02														**x**		
03														**x**		
04														**x**		
05														**x**		
06														**x**		
07														**x**		
08														**x**		
09														**x**		
10														**x**		
11														**x**		
12														**x**		
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14														**x**		
15														**x**		
16														**x**		
17														**x**		
18														**x**		
19														**x**		
20														**x**		
21														**x**		
22														**x**		
23														**x**		
24														**x**		
25														**x**		

Detailed schedule for blood samples collection following administration of the second dose of intravenous gentamicin.

Participants Number	Time (hrs) scheduled for collection of blood samples for determination of trough serum gentamicin levels for each participant					
	Date the second dose was administered	Time (hrs) the dose was administered	Planned date for the collection of the sample	Actual date the sample was collected	Planned time (hrs) for the collection of the sample	Actual time (hrs) the sample was collected
01						
02						
03						
04						
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Day 2: IV Gentamicin dosing

IV Gentamicin dosing	
Dose administered:	……..mg
Date:	……. / ……../ ……..
Time:	……..
Outcome:	Uneventful ☐ Eventful ☐ If eventful briefly state the observations: …….…… …….…… …….
Initials (A Nurse/Doctor who administered the drug):	…….....

Day 3: IV Gentamicin dosing

IV Gentamicin dosing	
Dose administered:	……..mg
Date:	……. / ……../ ……..
Time:	……..
Outcome:	Uneventful ☐ Eventful ☐ If eventful briefly state the observations: …….…… …….…… …….
Initials (A Nurse/Doctor who administered the drug):	…….....

Day 4:Admission outcome	
Discharged alive or died?	Alive ☐ Died ☐
Date of discharge:	……. / ……. / ……
Day 7:Serum creatinine blood sampling	
How is the baby doing?	Well ☐ Not well ☐ A brief detail of the observations: …… ……
Blood samples for Serum creatinine taken? Date: Time: Initials (A Nurse/Doctor who took the sample):	Yes ☐ No ☐ ……. /…… / …….. ……. ……..
Study completion	
Did the baby complete the study without violating the protocol?	Completed ☐ Died ☐

Appendix II: Data Extraction Tool
SECTION A: SOCIAL DEMOGRAPHIC FACTORS.

1. Patient identification number:
2. Patient initials:
3. Date of birth:
4. Sex:

SECTION B: CLINICAL RECORDS OF THE PATIENT.

5. What infections does the patient have?

   a. _____
   b. _____
   c. _____

6. What is the height (cm) of the patient: _____
7. What is the Apgar score of the patient: _____
8. What is the weight (kg) of the patient: _____
9. What IV gentamicin dose (mg) the patient is receiving? _____
10. For how long (days) the patient will be on the prescribed IV gentamicin dose?

    _____

SECTION C: OTHERS

11. What other medications the patient is taking?

   a. _____
   b. _____
   c. _____
   d. _____

12. What medications/herbs the patient's mother is taking?

   a. _____
   b. _____
   c. _____
   d. _____

SECTION D: LABORATORY RESULTS

13. Serum gentamicin concentration
14. Serum creatinine level
    a. At Baseline
    b. After 10 days
15. eGFR calculations
    a. At Baseline
    b. After 10 days

Appendix III: Laboratory investigation form

MUHAS Gentamicin in Neonates Study 2021 **Study area:** Mwananyamala Regional Referral Hospital	**Gentamicin in Neonates Laboratory Investigation Form** Patient Study Number: …….. I/O …… Postal/residential Address …….. Date of Birth …… Sex……..……..…… Religion ……..…. Clinic/ward…….……
Request to **Specify** ☐ Gentamicin Concentration ☐ Creatinine	

Request date	**Requested by, name and signature**	Firm	Head of firm, name
……/……./2021	……..	……	……

Clinical notes, relevant for the investigation requested

……

……

Appendix IV: Informed Parental Consent Form (English version)

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

SCHOOL OF MEDICINE

DEPARTMENT OF CLINICAL PHARMACOLOGY

Informed Parental Consent Form for Research Looking into the Safety of Intravenous Gentamicin among Neonates

Introduction: The study is being conducted by Masanyiwa E James who is a second year student pursuing Master of Science in Clinical Pharmacology at Muhimbili University of Health and Allied Sciences (MUHAS), School of Medicine, Department of Clinical Pharmacology, as part of his training in Clinical Pharmacology. So, we invite you and your child to take part in this study looking into the safety of intravenous gentamicin among neonates as your permission is being sought to have your child participate in this study. Please read the following information carefully before you decide whether or not to give your permission. The study and the rights and well-being of your child as a participant, are described below.

Description of the study: Your baby is sick and the doctor is suspecting that your baby may have an infection and has prescribed gentamicin to be given through the vein. Gentamicin given through the vein is one of the important medicines for the treatment of suspected bacterial infection in neonates. However, it is not without potential problems: if the amount of medicine reaching the blood is beyond a certain point, the medicine may affect the ability to hear and impair the function of the kidney in the baby. For this reason, it is recommended that when this medicine is used the amount reaching the blood needs to be monitored and the function of the kidney and the ability to hear should also be monitored before, during and after treatment. This will help to prevent these negative effects of treatment by adjusting the amount being given to the baby to prevent it from accumulating to a level that is associated with the

negative effects. Unfortunately and for various reasons, this monitoring is not usually done in many hospitals in our country, so we are doing this study to determine whether the amount of gentamicin reaching the blood is within the prescribed range and if is associated with hearing loss and impairment of the kidney function in the baby or not.

Since the study will involve assessing the kidney function of your baby, this process will require drawing small amount of blood sample (0.5mL), heelprick, from your baby and this will be done before your child is given treatment with gentamicin. After the first dose, another small volume of blood sample (0.5mL) will be drawn twice within 24 hours after administration of the first dose of intravenous gentamicin during the first day of treatment in the interval that is scheduled based on chance alone among neonates participating in the study. The drawing of a blood sample, will be repeated again on the second day of treatment with gentamicin where the same small volume of blood sample (0.5mL) will be collected after the second dose is administered but this time, blood sample will be collected only once within 24 hours. Blood samples collected following administration of the first and second dose of gentamicin will be taken to the laboratory to measure the amount of gentamicin present in the blood. The blood sample for monitoring kidney function will be taken to the laboratory to measure the level of a biochemical substance known as creatinine in the blood, which will help us to know the kidney function of your baby before treatment. This procedure will be repeated on the seventh day following cessation of treatment with gentamicin where another small volume of blood sample (0.5mL) from your baby will be collected to measure the serum creatinine levels for the purpose of assessing the kidney function of your child after cessation of treatment with gentamicin to see if there is any change in kidney function, which may have been brought about by treatment with gentamicin. You will also be informed of the results of the blood tests of your baby carried out for the purpose of this study and any other tests ordered by the doctor caring for your baby whenever they are available from the laboratory.

Risks and Benefits of participating into the study: There are no risks to your child's safety. Blood sampling procedures will be done in a way that your child is not contracting infections: the pricking site cleaned with 70% methylated spirits and the number of pricks will be limited

to three within 24 hours. The total blood volume that will be collected from your baby during the study will not be more than 2.5mL which is approximated to 1% of all the blood your child has. This blood volume is minor and cannot give any complication or risk to your child's health. Nevertheless, the ethics experts agree that the study procedures will not be harmful to the participating babies. Because this study will determine the amount of gentamicin reaching the blood and assess the kidney function of your child, your child will benefit from close monitoring and treatment whenever need arises. However, the indirect benefit of participating in this study is that the results will provide important information which will shed light on whether the current practice of gentamicin dosing without monitoring in neonates is safe or not. Recommendations made from the findings of this study will be for the benefit of all future patients.

Confidentiality: Blood samples drawn from your child will only be used for the purposes of research and will not be available to anyone for other uses aside from the purposes of this research. The laboratory results of your child will not be associated with his/her name. Rather, your child will be assigned a participation number during collection of blood samples. Also, the laboratory results will be kept confidential and will be available only to professional researchers and staff. If the results of this study are published, the data will be presented in group form and individual children will not be identified.

Freedom to Withdraw or Refuse Participation: You are free to agree or not to agree the participation of your child in this study and also you are free to withdraw (stop your baby's participation) at any time, without giving a reason and without penalty or loss. Your decision to withdraw will not affect your baby's future medical care. Should you choose to withdraw your baby from the study; the data already collected will only be kept with your permission.

Grievance Procedure: If you have any concerns or dissatisfied with any aspect of this study, you may report your grievances anonymously if desired to the ethics committee board of MUHAS at +255 -022-2152489and to the hospital authority of Mwananyamala regional referral hospital at +255714-203-868.

Questions: Please feel free to ask the investigator any questions before signing the consent form or at any time during or after the study.

Principal Investigator: Masanyiwa E James (second year student pursuing Master of Science in Clinical Pharmacology) at Muhimbili University of Health and Allied Sciences (MUHAS), School of Medicine, Department of Clinical Pharmacology.
If you have further questions concerning matters related to this research, please contact the principal investigator at +255755808953

Informed Consent Statement

I, _____, give permission for my child, _____ to participate in the research project entitled, "**Population Pharmacokinetics of Gentamicin among Neonates Admitted at Mwananyamala Regional Referral Hospital (Dar es salaam, Tanzania)**." The study has been explained to me and my questions answered to my satisfaction. I understand that my child's right to withdraw from participating or refuse to participate will be respected and that his/her results and identity will be kept confidential. I give this consent voluntarily.

Parent/Guardian Signature:

_____ _____

Signature *Date*

Investigator Signature:

_____ _____

Signature *Date*

Appendix V: Informed Parental Consent Form (Swahili version)

CHUO KIKUU CHA AFYA MUHIMBILI NA SAYANSI SHIRIKISHI

SKULI YA TIBA
IDARA YA PHARMACOLOJIA

Fomu ya idhini ya Wazazi inayowapa ruhusa watoto wao kushiriki kwenye Utafiti unaohusu Kuangalia Usalama wa dawa ya Gentamicini inayotolewa kwa njia ya mshipa kwa watoto wachanga.

Utangulizi: Utafiti huu unafanywa na Masanyiwa E James ambaye ni mwanafunzi wa mwaka wa pili akisomea masomo ya uzamili wa Sayansi katika Pharmacolojia katika Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili (MUHAS), Skuli ya Tiba, Idara ya Pharmacolojia, kama sehemu ya mafunzo yake katika masomo ya uzamili wa Sayansi katika Pharmacolojia. Kwa hiyo, tunakualika wewe na mtoto wako kushiriki katika utafiti huu unaoangalia usalama wa dawa ya gentamicini inayotolewa kwa njia ya mshipa kwa watoto wachanga waliolazwa katika kitengo cha watoto wachanga hospitali ya rufaa ya mkoa ya mwananyamala Dar es salaamkwani ruhusa yako inatakiwa ili mtoto wako ashiriki katika utafiti huu. Tafadhali soma taarifa zifuatayo kwa uangalifu kabla ya kuamua ikiwa utatoa idhini au la. Utafiti utakavyofanyika na haki na ustawi wa mtoto wako kama mshiriki, zimeelezewa hapa chini.

Maelezo jinsi utafiti utakavyofanyika: Mtoto wako anaumwa na daktari anashuku kuwa mtoto wako anaweza kuwa na maambukizi na ameamuru apewe dawa ya gentamicini itakayotolewa kupitia mshipa. Gentamicini inayotolewa kupitia mshipa ni moja wapo ya dawa muhimu kwa matibabu ya watoto wachanga wanaoshukiwa kuwa na maambukizo ya bakteria. Hata hivyo, dawa hii ya gentamicini huwa inasababisha matatizo ikiwa kiwango cha dawa kinachofika kwenye damu ni zaidi ya kiwango fulani kilichopangwa na kinachojulikana ulimwenguni kote. Dawa hii inaweza kuathiri uwezo wa kusikia na kudhoofisha utendaji kazi wa figo kwa mtoto. Kwa sababu hii, inashauriwa kwamba wakati dawa hii inatumiwa kiasi kinachofikia damu kinahitaji kufuatiliwa/kupimwa na utendaji kazi wa figo na uwezo wa kusikia pia unapaswa kufuatiliwa kabla, wakati na baada ya matibabu. Hii itasaidia kuzuia

athari hizi mbaya za matibabu kwa kurekebisha kiwango cha dawa anachopewa mtoto ili kuzuia dawa ikawa nyingi kwenye damu na kufikia kiwango ambacho kinahusishwa na athari mbaya. Kwa bahati mbaya na kwa sababu mbalimbali, ufuatiliaji huu haufanyiki katika hospitali nyingi katika nchi yetu, kwa hiyo tunafanya utafiti huu ili kubaini kama kiwango cha gentamicini kinachofika kwenye damu kipo katika kiwango kilichowekwa na pia kubaini endapo inahusishwa na kuathiri uwezo wa kusikia na kudhoofisha utendaji kazi wa figo kwa mtoto au la.Kwa kuwa utafiti huu utahusisha kutathmini utendaji kazi wa figo wa mtoto wako, mchakato huu utahitaji kutoa kiwango kidogo cha sampuli ya damu (0.5mL), kisiginoni, kutoka kwa mtoto wako na hii itafanyika kabla ya mtoto wako kupatiwa matibabu ya dawa ya gentamicini. Baada ya dozi ya kwanza ya gentamicini, kiasi kingine kidogo cha sampuli ya damu (0.5mL) itatolewa mara mbili ndani ya masaa 24 baada ya kutolewa kwa dozi ya kwanza ya gentamicini siku ya kwanza ya matibabu katika muda uliopangwa bila kufuata mpangilio rasimi kwa watoto wote wachanga wanaoshiriki utafiti huu. Utaratibu huu utarudiwa tena siku ya pili ya matibabu ambapo kiwango kile kile cha sampuli ya damu (0.5mL) kama kilichotolewa siku ya kwanza kitakusanywa baada ya dozi ya pili ya gentamicini kutolewa, lakini wakati huu sampuli ya damu itakusanywa mara moja tu ndani ya masaa 24. Kisha, sampuli za damu zilizokusanywa kufuatia kutolewa kwa dozi ya kwanza na ya pili ya gentamicini kitachukuliwa na kupelekwa maabara ili kupima kiwango cha gentamicini iliyopo kwenye damu. Sampuli ya damu ya ufuatiliaji wa utendaji kazi wa figo itachukuliwa na kupelekwa maabara kupima kiwango cha biokemikali inayojulikana kama creatinine katika damu, ambayo itatusaidia kujua utendaji kazi wa figo wa mtoto wako kabla ya matibabu. Utaratibu huu utarudiwa siku ya saba baada ya kumaliza matibabu na dawa ya gentamicini ambapo kiasi kidogo cha sampuli ya damu (0.5mL) kutoka kwa mtoto wako kitakusanywa kupima kiwango cha creatinine kwenye damu kuona kama kuna mabadiliko yoyote katika utendaji kazi wa figo, ambayo inaweza kuwa imeletwa na matibabu ya dawa ya gentamicini. Utaarifiwa pia kuhusu matokeo ya uchunguzi wa damu ya mtoto wako uliofanywa kwa kusudi la utafiti huu na vipimo vingine vyovyote vilivyoamriwa na daktari anaemuhudumia mtoto wako wakati wowote ambao majibu kutoka maabara yanapopatikana.

Hatari na Faida za utafiti huu: Hakuna hatari yoyote kwa usalama wa mtoto wako itakayotokana na utafiti huu. Taratibu za kuchukua sampuli ya damu zitafanywa kwa njia ambayo mtoto wako hatapata maambukizi: tundu ambalo litatumika kipindi damu ikitolewa litasafishwa kwa kutumia 70% methylated spiriti na matundu yatakayotumika kutolea damu yatapunguzwa na kuwa kati ya matatu ndani ya masaa 24. Kiasi cha damu kitakachokusanywa kutoka kwa mtoto wako wakati wa utafiti kitakuwa sio zaidi ya mililita 2.5 ambayo inakadiriwa kuwa asilimia 1 ya jumla ya ujazo wa damu aliyonayo mtoto wako. Kiasi hiki cha damu ni kidogo sana na hakiwezi kuleta shida yoyote au hatari yoyote kwa afya ya mtoto wako. Hata hivyo, wataalamu wa maadili wanakubali kwamba taratibu za utafiti huu utakavyofanyikahazitakuwa na madhara kwa watoto wanaoshiriki. Kwa sababu utafiti huu utaonyesha kiwango cha gentamicini kilichopo kwenye damu na kutathmini utendaji kazi wa figo wa mtoto wako, kwahiyo mtoto wako atafaidika na ufuatiliaji/kupimwa na kujua kiwango cha dawa ya gentamicini kilichopo kwenye damu yake na kupatiwa matibabu kwa ukaribu wakati wowote mahitaji yanapotokea.Hata hivyo, faida isiyokuwa ya moja kwa moja ya kushiriki katika utafiti huu ni kwamba matokeo ya utafiti huu yatatoa taarifa muhimu ambayo itatoa mwangaza kama utumiaji wa dawa ya gentamicini kwa watoto wachanga bila kupima kiwango kinachofika kwenye damu ni salama au la. Pia mapendekezo yatakayotolewa kutokana na matokeo ya utafiti huu yatakuwa na faida ya wagonjwa wote wa baadae.

Usiri wa utafiti huu: Sampuli za damu zilizotolewa kutoka kwa mtoto wako zitatumika tu kwa madhumuni ya utafiti huu na hazitapatikana kwa mtu yeyote kwa matumizi mengine kando na malengo ya utafiti. Matokeo ya maabara ya mtoto wako hayatahusishwa na jina lake. Badala yake, mtoto wako atapewa nambari ya ushiriki wakati wa ukusanyaji wa sampuli za damu. Pia, matokeo ya maabara yatahifadhiwa kwa siri na yatapatikana tu kwa watafiti na wafanyikazi watakaoshiriki kwenye utafiti huu. Ikiwa matokeo ya utafiti huu yatachapishwa, taarifa itawasilishwa katika mfumo wa kikundi na watoto mmoja mmoja hawatatambuliwa.

Uhuru wa Kujiondoa au Kukataa Kushiriki katika utafiti huu: Uko huru kukubali au kutokubali ushiriki wa mtoto wako katika utafiti huu na pia uko huru kujiondoa (kusimamisha ushiriki wa mtoto wako) wakati wowote, bila kutoa sababu na bila adhabu au hasara. Uamuzi wako wa kujiondoa hautaathiri huduma ya matibabu ya mtoto wako ya baadae. Iwapo

utachagua kumtoa mtoto wako kwenye utafiti; taarifa iliyokusanywa tayari itahifadhiwa tu kwa idhini yako.

Utaratibu wa kutoa malalamiko: Ikiwa una wasiwasi wowote au haujaridhika na hali yoyote ya utafiti huu, unaweza kuripoti malalamiko yako bila kujulikana kwa bodi ya kamati ya maadili ya Chuo Kikuu cha Afya Muhimbili na Sayansi Shirikishi kwa namba za simu: +255 -022-2152489 na kwa mamlaka ya hospitali ya hospitali ya rufaa ya mkoa wa Mwananyamala kwa namba za simu +255714-203-868.

Maswali: Tafadhali jisikie huru kumuuliza mtafiti maswali yoyote kabla ya kusaini fomu hii ya idhini au wakati wowote, wakati au baada ya utafiti.

Mtafiti Mkuu: Masanyiwa E James (mwanafunzi wa mwaka wa pili anaesomea masomo ya uzamili wa Sayansi katika Pharmacolojia) katika Chuo Kikuu cha Afya na Sayansi Shirikishi Muhimbili (MUHAS), Skuli ya Tiba, Idara ya Pharmacolojia.

Ikiwa una maswali zaidi juu ya maswala yanayohusiana na utafiti huu, tafadhali wasiliana na mtafiti mkuu kwa namba +255755808953

Taarifa ya idhini

Mimi, _____, ninampa ruhusa mtoto wangu, _____ kushiriki katika utafiti huu unaoitwa, "**Population Pharmacokinetics of Gentamicin among Neonates Admitted at Mwananyamala Regional Referral Hospital (Dar es salaam, Tanzania).**" Utafiti umeelezewa kwangu vizuri na maswali yangu yakajibiwa na kuridhika. Ninaelewa kuwa haki ya mtoto wangu kujiondoa kushiriki au kukataa kushiriki itaheshimiwa na kwamba matokeo yake na kitambulisho kitahifadhiwa kwa siri. Ninatoa idhini hii kwa hiari.

Saini ya Mzazi / Mlezi:

_____ _____

Saini *Tarehe*

Saini ya Mtafiti:

_____ _____

Saini *Tarehe*

Appendix VI: **Ethical clearance for a study**

UNITED REPUBLIC OF TANZANIA

MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY

MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES

OFFICE OF THE DIRECTOR - RESEARCH AND PUBLICATIONS

Ref. No.DA.282/298/01.C/ Date: 19/03/2021

MUHAS-REC-03-2021-522

Masanyiwa E James,
MSc. Clinical Pharmacology,
School of Medicine,
MUHAS

RE: APPROVAL FOR ETHICAL CLEARANCE FOR A STUDY TITLED: Population Pharmacokinetics of Gentamicin among Neonates Admitted at Mwananyamala Regional Referral Hospital (Dar es salaam, Tanzania).

Reference is made to the above heading.

I am pleased to inform you that the Chairman has on behalf of the University Senate, approved ethical clearance of the above-mentioned study, on recommendations of the Senate Research and Publications Committee meeting accordance with MUHAS research policy and Tanzania regulations governing human and animal subjects research.

APPROVAL DATE: 19/03/2021
EXPIRATION DATE OF APPROVAL: 18/03/2022

STUDY DESCRIPTION:
Purpose:
The purpose of this population based pharmacokinetic study is to determine the population pharmacokinetics (popPK) of gentamicin among neonates in Dar es salaam, Tanzania so as to see what kind of gentamicin serum levels are achieved following once daily dosing of intravenous gentamicin administration.

The approved protocol and procedures for this study is attached and stamped with this letter, and can be found in the link provided:
https://irb.muhas.ac.tz/storage/Certificates/Certificate%20-%20485.pdf and in the MUHAS archives.

The PI is required to:

1. Submit bi-annual progress reports and final report upon completion of the study.
2. Report to the IRB any unanticipated problem involving risks to subjects or others including adverse events where applicable.
3. Apply for renewal of approval of ethical clearance one (1) month prior its expiration if the study is not completed at the end of this ethical approval. You may not continue with any research activity beyond the expiration date without the approval of the IRB. Failure to receive approval for continuation before the expiration date will result in automatic termination of the approval for this study on the expiration date.
4. Obtain IRB amendment (s) approval for any changes to any aspect of this study before they can be implemented.
5. Data security is ultimately the responsibility of the investigator.
6. Apply for and obtain data transfer agreement (DTA) from NIMR if data will be transferred to a foreign country.
7. Apply for and obtain material transfer agreement (MTA) from NIMR, if research materials (samples) will be shipped to a foreign country,
8. Any researcher, who contravenes or fail to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III section 10 (2)
9. The PI is required to ensure that the findings of the study are disseminated to relevant stake holders.
10. PI is required to be versed with necessary laws and regulatory policies that govern research in Tanzania. Some guidance is available on our website https://drp.muhas.ac.tz/.

Dr. Bruno Sunguya
Chairman, MUHAS Research and Ethics Committee

Muhimbili University of Health and Allied Sciences
DIRECTOR
Research & Publications
Box 65001

Cc: Director of Postgraduate Studies

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/6; Ext. 1038; Direct Line:+255-22-2152489;Telefax:+255-22-2152489;E-mail:drp@muhas.ac.tz;Web:https://www.muhas.ac.tz

Appendix VII: **A permission letter to do a study at MRRH**

UNITED REPUBLIC OF TANZANIA
MINISTRY OF EDUCATION, SCIENCE AND TECHNOLOGY
MUHIMBILI UNIVERSITY OF HEALTH AND ALLIED SCIENCES
OFFICE OF THE DIRECTOR – POSTGRADUATE STUDIES

In reply quote;

Ref. No. HD/MUH/T.410/2019

23rd March, 2021

The Medical Officer in charge,
Mwananyamala Regional Referral Hospital,
P.O. Box 61665,
DAR ES SALAAM

Re: INTRODUCTION LETTER

The bearer of this letter is Masanyiwa E. James, a student at Muhimbili University of Health and Allied Sciences (MUHAS) pursuing MSc. Clinical Pharmacology.

As part of his studies he intends to do a study titled: "***Population Pharmacokinetics of Gentamicin Among Neonates Admitted at Mwananyamala Regional Referral Hospital (Dar es Salaam, Tana***"

The research has been approved by the Chairman of University Senate.

Kindly provide him the necessary assistance to facilitate the conduct of his research.

We thank you for your cooperation.

Ms. Victoria Mwanilwa
For: DIRECTOR, POSTGRADUATE STUDIES

cc: Dean, School of Pharmacy, MUHAS
cc: Masanyiwa E. James

9 United Nations Road; Upanga West; P.O. Box 65001, Dar Es Salaam: Tel. G/Line: +255-22-2150302/3; Ext. 1015; Direct Line:+255-22-2151378;Telefax:+255-22-2150465;E-mail.dpgs@muhas.ac.tz;Web:https://www.muhas.ac.tz

NMB
MAGOMENI BRANCH

DATE TIME TERMINAL ID
06/05/2021 12:09
2075272906810

AGENT ID: 20518792
TRAN NUM: 101AGCC211262572
REF NO: EC100902920052

BILL PAYMENT
GEPG PAYMENT SUCCESSFUL
Name: MASANYIWA E JAMES
Control No : 991320489472
Provider:GoTHoMIS -
Mwananyamala Regional Referral
Hospital
Bill Desc:Hospital Bill
Bill Paid(Principal):50,000.00
Total Amount Paid:Tsh 50,000.00

MASANYIWA E JAMES