FRACTIONAL EXCRETION OF SODIUM AS AN EARLY PREDICTOR OF ACUTE KIDNEY INJURY IN TERM NEONATES WITH PERINATAL ASPHYXIA AT KENYATTA NATIONAL HOSPITAL

A dissertation submitted in part fulfillment of the requirement of the University of Nairobi for the award of the degree of Master of Medicine in Paediatrics and Child Health.

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DECLARATION

This dissertation is my original work and has not been presented for the award of a degree in any other university

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ABBREVIATIONS

AKI - Acute kidney injury

AKIN - Acute Kidney Injury Network

ANC- Antenatal Clinic

APGAR - Appearance, Pulse, Grimace, Activity, Respiration

APH – Antepartum Hemorrhage

ARF – Acute Renal Failure

ATN – Acute Tubular Necrosis

ATP – Adenosine TriPhosphate

BP – Blood Pressure

BVM – Bag, valve and mask

Cl – Chloride

C/S – Caesarean Section

CM – Centimeter

DCT – Distal Convoluted Tubule

ECF - Extracellular Fluid

ERC – Ethics and Research Committee

FENa – Fractional Excretion of Sodium

FEUr – Fractional Excretion of Urea

GA – Gestational Age

GFR – Glomerular Filtration Rate

HIE – Hypoxic Ischemic Encephalopathy

KDHS – Kenya Demographics and Health Survey

KNH – Kenyatta National Hospital

MOD – Multi Organ Dysfunction

Na – Sodium

Na/K ATPase – Sodium/Potassium ATPase

NBU – New Born Unit

NGAL – Neutrophil gelatinase- associated lipocalin

NICU – Neonatal Intensive Care Unit

NPV – Negative Predictive Value

PCT – Proximal Convoluted Tubule

PCr – Plasma Creatinine

PNa – Plasma Sodium

PO – Pre renal oliguria

PPV – Positive Predictive Value

PRA – Plasma Renin Activity

pRIFLE – pediatric Risk, Injury, Failure, Loss, End stage renal disease

RBF – Renal Blood Flow

RDS – Respiratory Distress Syndrome

RFI – Renal Failure Index

RRT – Renal replacement therapy

sCr – Serum creatinine

SPSS – Statistical Products and Services Solutions

TAL – Thick Ascending Loop of Henle

UCr – Urine Creatinine

UNa – Urine Sodium

UO – Urine Output

USA – United State of America

V/E – Vacuum Extraction

WHO – World Health Organisation

DEFINITION OF TERMS

Term newborn: Infants born at or after 37 completed weeks of gestation using Ballard's score. [32]

Apgar Score: This is a simple method to do a quick assessment on the health of a newborn immediately after birth and at 5, 10, 15 and 30 minutes. The score is determined by evaluating the newborn baby based on five criteria at birth, on a scale from zero to two, then adding up the five values thus obtained. The resulting Apgar score ranges from zero to ten. These five criteria are summarized using words chosen to form the acronym (**A**ppearance, **P**ulse**, G**rimace, **A**ctivity, and **R**espiration). The score is shown in appendix IV.

Perinatal asphyxia: "Failure to initiate and sustain breathing at birth." [1] **plus** Apgar Score **plus** clinical evidence of hypoxic ischemic encephalopathy Sarnat and Sarnat stage 1, 2 or 3 as shown in appendix V. " [1,2]

Acute Kidney Injury: AKI is defined as serum creatinine level greater than 100 µmol/l at 72h of life.

Fractional excretion of sodium : the ratio of the sodium clearance to the creatinine clearance, expressed as a percent. It measures the percentage of the sodium filtered by the kidney which is excreted in the urine.

ABSTRACT

Introduction

World Health Organization (WHO) defines perinatal asphyxia as "Failure to initiate and sustain breathing at birth plus an Apgar score of less than 7 at 5minutes'' [1] . Newborns suffering from perinatal asphyxia present with multiorgan dysfunction, with studies demonstrating that the kidney is the most affected organ. AKI in asphyxia has been reported to have an incidence of 50 – 70%. Standard practice of diagnosis of AKI has been via measurement of serum creatinine and urine output. In neonates these have several shortcomings: it reflects maternal creatinine in the first 48 – 72 hrs of life, and it is a late marker of injury, and up to 80% of AKI is non oliguric. This study therefore sought to determine whether fractional excretion of sodium (FENa), which is deranged in acute tubular necrosis as is characteristic of AKI in asphyxia, could be used to identify neonates with birth asphyxia who eventually suffer AKI on the first day of life.

Objective

To determine the diagnostic utility (sensitivity, specificity, PPV and NPV) of fractional excretion of sodium (FENa) measured on day 1of life in the diagnosis of AKI on day three of life in neonates with perinatal asphyxia in Kenyatta National Hospital.

Study design: This was a hospital based cross sectional design study.

Study methods

Newborns who had a diagnosis of perinatal asphyxia using the Apgar scoring and Sarnat and Sarnat clinical hypoxic ischemic encephalopathy staging whose parents consented to the study were enrolled within 24 hours of birth. Urine sodium and creatinine and serum sodium and creatinine were measured on the first day of life, and used to calculate FENa. On the third day of life serum creatinine was measured and a diagnosis of AKI based on levels $\geq$100 μmol/l. Results were analyzed for associations between deranged FENa on day 1 of life and AKI at three days of life.

Results:

One hundred at eight neonates were admitted to KNH's NBU with perinatal asphyxia during the study period, 79 of whom survived to the third day of life and were thus recruited, 3% of whom had HIE stage I, 91% stage II and 6% stage III. The mean weight was 3289g (SD 478), and mean length was 47cm (SD 3). There were 41 males and 38 females. Most (79%) of the neonates had been delivered in KNH. Thirty out of seventy nine neonates met the criteria for diagnosis of AKI on day 1 of life ($\geq$100 μmol/l) giving a prevalence of 38%. FENa was deranged ($\geq$2.5%) in 63% of the neonates on day one of life. Twenty seven of the thirty neonates with AKI on the day three of life had positive FENa on the first day of life giving us a sensitivity of 90%. On the other hand, only 26/49 of the neonates without AKI had negative FENa on day 1 of life giving us a specificity of 53%. Among the 50 neonates with positive FENa on day one of life, 27 had AKI on day 3 of life giving us a PPV of 54%, while 26/29 neonates without AKI on the third day of life had negative FENa thus giving us a high NPV of 90%. FENa as an early predictor of AKI had a modest AUC of 0.715.

Conclusion :

Our study showed the prevalence of AKI to be high at 38% among neonates with perinatal asphyxia with those with severe HIE stage being affected the most. FENa is an easy to do and easily available test with a high sensitivity (90%) and a good positive likelihood ratio of 1.9 which makes it a good screening test. Our low sensitivity however means that it can falsely identify neonates not having AKI as having AKI, but the benefits of preventative measures outweigh the cost implication. Despite its modest PPV, it is a useful test since the benefit of early treatment of AKI would outweigh the cost of undertaking the test.

INTRODUCTION

Perinatal asphyxia and Acute Kidney Injury

World Health Organization (WHO) defines perinatal asphyxia as "Failure to initiate and sustain breathing at birth plus an APGAR score of less than 7 at 5minutes'' [1] According to the World Federation of Neurology Group, perinatal asphyxia has been defined as "a condition of impaired blood gas exchange leading, if it persists, to progressive hypoxemia and hypercapnia. Diagnosis requires a blood gas" [2] This however is impossible in most settings in Kenya as most hospitals do not have the equipment to do blood gas analysis.

Fetal hypoxia can have a variety of causes which can be classified into these groups: antepartum, intrapartum and postpartum. The antepartum risk factors include maternal-placental factors such as pre eclampsia, anaemia, post datism, diabetes mellitus with vasculopathy, and fetal factors such as intrauterine growth restriction. Intrapatum risk factors include uterine tetany, premature separation of the placenta, cord compression or knotting, inadequate maternal oxygenation from hypoventilation from anesthesia among others. Post partum risk factors include failure of oxygenation due to severe forms of respiratory diseases or severe congenital cyanotic heart disease, anaemia from severe hemorrhage or hemolytic disease and shock also resulting from severe hemorrhage or overwhelming sepsis.

According to the WHO, perinatal asphyxia is one of the biggest contributors of early death in neonates. accounting for an estimated 900 000 deaths each yearly [3]. Other studies have estimated perinatal asphyxia causing 840,000 or 23% of all neonatal deaths worldwide [4]. Kenyan statistics, according to the Kenya Demographics and Health Survey (KDHS 2014) [5], estimated neonatal mortality rate at 22 per 1,000 live births, which is a drop from 31 deaths per 1,000 live births in 2008. However, perinatal asphyxia still contributes to a significant proportion of morbidity and mortality. In a one year retrospective study done by Ayaya et al [6] in the Moi Teaching and Referral Hospital, perinatal morbidity was estimated at 667 per 1000 babies admitted, with the most common cause of morbidity stated to be perinatal asphyxia (39.2%).

A study done in Kenyatta National Hospital (KNH) showed that perinatal asphyxia accounted for 20% of the weekly admissions to the Newborn Unit and had a poor outcome with a mortality of 31.1% by day 7 [(7)]. Data from monthly mortality audits in KNH NBU report perinatal asphyxia to be amongst the leading three diagnosis for admission and death, the other two being prematurity and respiratory distress syndrome[(8)] .

Newborns with perinatal asphyxia are prone to multiorgan dysfunction, and studies have shown that the worst affected organ in an asphyxiated term infant is the kidney [(9)]. According to Acute Kidney Injury Network (AKIN), AKI is defined as "an abrupt (within 48 hours) reduction in kidney function, currently defined as an absolute increase in serum creatinine (SCr) of at least 0.3 mg/dl (≥26.4μmol/l), a percentage increase in SCr of at least 50% (1.5-fold from baseline), or a reduction in urine output (documented oliguria of less than 0.5 ml/kg per hour for more than 6 hours" [(10)].

There is however no global standard acceptable definition of AKI in neonates. Because of the different definitions, AKI incidence post neonatal asphyxia is reported in up to 50 – 72% [(11)] of cases. The increasing incidence of AKI correlates with the severity of perinatal asphyxia[(12)]. AKI can either be pre – renal or intrinsic renal failure. Among newborns admitted to NICU, approximately 6% to 8% have intrinsic ARF, with the most common cause cited as severe perinatal asphyxia [(11)].

The fractional excretion of sodium (FENa) is the ratio of the clearance of sodium to the clearance of creatinine, expressed as a percent. It measures, in percentage, how much sodium filtered by the kidney is excreted in the patient's urine. It is obtained by measuring plasma and urine sodium, rather than by urinary sodium concentration interpretation alone, as the sodium concentration in urine differs with water reabsorption.

In clinical use, the FENa is useful in the evaluation of acute kidney failure in order to deduce whether hypovolemia or reduced effective circulating plasma volume is contributing to the kidney failure (low FENa values). Loss of sodium due to ATN or other causes of intrinsic kidney failure can be suggested by higher FENa values.

It is obtained from the following formula:

$$FE_{Na} = 100 \times \frac{\text{sodium}_{\text{urinary}} \times \text{creatinine}_{\text{plasma}}}{\text{sodium}_{\text{plasma}} \times \text{creatinine}_{\text{urinary}}}$$

in which UNa is the urinary concentration of sodium (mEq/L), Ucr is the urinary concentration of creatinine (mg/dL), PNa is the plasma sodium concentration (mEq/L), and Pcr is the plasma creatinine concentration (mg/dL).

FENa is deranged as early as day one of life in newborns with asphyxia who develop AKI [12], and as such has the potential to be used for its diagnosis and thus early institution of appropriate management.

LITERATURE REVIEW

Newborns suffering from perinatal asphyxia are prone to multiple organ dysfunction due to a redistribution of cardiac output to sustain perfusion to critical organs e.g. adrenals, brain and heart, while potentially leading to renal ischemia. This leads to damage to other organs, most affected being the kidneys. [9]

Acute Kidney Injury in Perinatal Asphyxia

Prevalence of AKI in asphyxia

AKI incidence post neonatal asphyxia is reported in up to 50 – 72% in several studies [11]. This essentially underscores a huge burden of AKI in asphyxia. Studies have used different serum creatinine cut offs to define AKI. Most of the prevalence studies investigating newborn AKI report its existence to be between 11.7 and 70 % [12-19]

Shah et al [13] in 2004 did a retrospective cohort study to assess multi organ dysfunction in infants with post asphyxia HIE , renal dysfunction was the most prevalent with 91/130 (70%) involvement. In another retrospective study by Leila [14] et al to study multi organ dysfunction in neonates with HIE, multi organ dysfunction (MOD) was diagnosed in 74 % (74/100), with renal dysfunction being the most common, observed in 64% (47/74). In both these studies, renal dysfunction was defined as oliguria/anuria or serum creatinine >1mg/dl (88 μmol/l).

Studies that used creatinine values >1.5 mg/dL (133 μmol/l) included a retrospective study by Karlowicz [15] in the US, which was aimed at determining the prevalence of AKI in moderate and severe asphyxiated full-term neonates. Of the 33 neonates in the study, 20(61%) with severe asphyxia had AKI, with none of 33 with moderate asphyxia having AKI ($P<0.0001$).

Essajee et al [16] conducted a prospective cohort study in Kenya using NGAL as an early marker of AKI post asphyxia. AKI was found in 56% (60/108) of the study population. A prospective

cohort study done by Alaro et al [17] in KNH found the prevalence of AKI to be 11.7% amongst newborns with perinatal asphyxia.

Other studies defined AKI as serum creatinine >1mg/dl (88 μmol/l). This included Gupta et al [12] who carried out a prospective case controlled study in India to determine the incidence of AKI in perinatal asphyxia. The results showed that 33 of 70 (47.1%) had AKI.

Other studies used AKIN classification for AKI while others used pRIFLE classification. Kaur et al [18] did a prospective cohort study in newborns with perinatal asphyxia in India, and used AKIN classification for AKI. The total incidence of AKI was 41.7%, with 9% (1/11) of newborns with moderate asphyxia and 56% (12/25) with severe asphyxia developing AKI.

In Africa, a prospective cohort study done by Medani et al [19] in Sudan aimed to determine the pattern of AKI in asphyxiated neonates and its relation to the grade of hypoxic ischemic encephalopathy (HIE). AKI was defined by pRIFLE and a prevalence rate of 54.1% (46/85) was observed.

Pathophysiology of AKI

Perinatal asphyxia causes reduced blood flow to the kidneys secondary to hypotension and hypovolemia , which can lead to impairment in both functions of the tubules and glomerular filtration rate (GFR). AKI can either be pre – renal or intrinsic renal failure. Renal hypoperfusion due to systemic hypotension causes prerenal failure and subsequently there is failure of maintainance of renal blood flow due to loss of autoregulation. Severe or prolonged renal hypoperfusion leads to renal parenchymal damage, a situation which leads to the evolution of pre renal failure into intrinsic AKI.

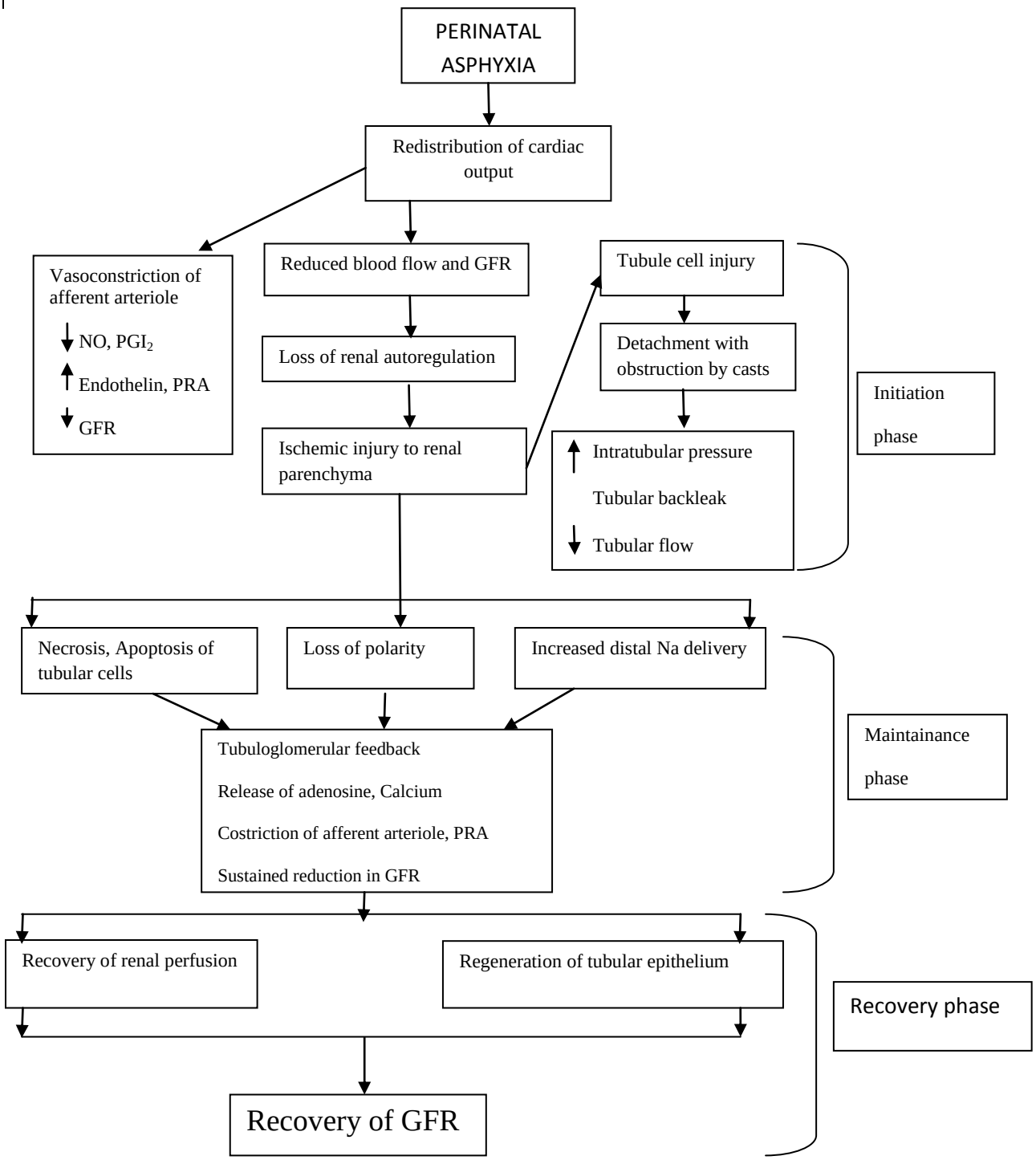
The evolution of intrinsic AKI has three phases: initiation, maintenance, and recovery (Figure 1 below), with differing severity ranging from mild tubular dysfunction to ATN (with or without oliguria and anuria), to infarction and corticomedullary necrosis with irreversible kidney damage.

The initial period of renal hypoperfusion is the *initiation phase* (hours to days) , during which there is evolving ischemic injury. The GFR declines because reduction in blood flow to the kidneys reduces the glomerular ultrafiltration pressure due to an increase in vasoactive mediators such as endothelin, plasma renin activity (PRA), adenosine, thromboxane and reduction in nitric oxide, prostacyclin and natriuretic peptides which are vasodilatory. This leads to vasoconstriction of afferent arterioles. Secondly, casts within the tubules composed of epithelial cells and necrotic debris obstruct the flow of glomerular filtrate within tubules[(20)]. Subsequent increase in pericapilarry hydrostatic pressure in the Bowman's space leads to a further decline in GFR. Finally, there is backleak of glomerular filtrate through injured tubular epithelium.

The last medullary segment of the proximal tubule (S3 segment, pars recta) has the most prominent ischemic injury, accompanied by the medullary portion of the TAL of the loop of Henle[(21)]. Tubule cells lose polarity with short periods of ischemia reperfusion and on prolonged or severe ischemia the epithelial cells are damaged irreversibly, which leads to impairement in re-absorption at the tubules resulting in increase in sodium excretion in the tubular fluid leading to imbalance in electrolytes[(22)] .

The ***maintenance phase*** *(typically*1 to 2 weeks) leads to injury of the renal cell. It entails stability of GFR albeit at low levels. During the maintenance phase of ATN, the main factors are nephronal and cellular. Programmed cell death (apoptosis) forms the main cellular injury, which is then is followed by changes in intracellular calcium metabolism, phospholipid breakdown, release of free oxygen radicals, altered cell polarity, loss of tight cell junctions between cells, disruption of the cytoskeleton ,loss of the cell brush border, and loss of major cellular functions, e.g., Na+/K+-ATPase, and cell swelling. All these processes are accompanied depletion of cellular ATP.

Figure 1 Pathophysiology of Ischemic/ Intrinsic AKI



Renal parenchymal cell repair and return of GFR towards preillness levels characterizes the ***recovery phase***.

AKI in perinatal asphyxia is not without its consequences. Mortality rates in asphyxiated newborns with AKI in various studies have been reported as 31.1% by day 7 [(7)], 71.4% by day 7[(19)] and 14.1% by day 7 [(12)]. In a study done by Gupta[(12)], those with perinatal asphyxia showed changes in 5 (6.6%) cases noted as changes in echotexture, increased kidney size, and loss of corticomedullary differentiation as assessed by renal sonography.

Diagnosis of Acute Kidney Injury

In spite of the available functional systems of classifying and diagnosing AKI (pRIFLE and AKIN criteria), diagnosis in neonates has proven to be a challenge since the classification systems are based on the patients level of serum creatinine (SCr) and urine output (UO). AKI in neonates and more so in perinatal asphyxia has been shown in several studies to be non oliguric.

In a retrospective study by Karlowicz [(15)], he defined non oliguric renal failure as urine output > 1ml/kg/hour after day one of life. Medani et al [(19)] carried out a prospective cohort study , in which oliguric renal failure was diagnosed if the patient's urine output was less than 1 ml/kg/hour, while Gupta et al [(12)] defined oliguria as urinary output of less than 0.5ml/kg/hr. The outcome was as depicted table 1.

Table 1: Oliguric vs. Non oliguric AKI

Study	Sample size	Title	Outcome
Medani [(19)], 2013 Prospective cohort Country - Sudan	85	Acute kidney injury in neonates with asphyxia admitted to a tertiary neonatal unit in Sudan	AKI in 54.1% (46/85). Non oliguric 65% (30/46)
Gupta [(12)], 2009 Prospective Case Control Study - India	98 Case vs control (70/28)	Renal Failure in Asphyxiated Neonates	47.1% with AKI, non-oliguric 78% cases and oliguric type 22% of cases.

Generally, non oliguric AKI can be attributed to the greater total body water in newborns compared to adults, especially in preterms with values as high as 80%, and, in addition, immature tubular development leading to greater urine output. In asphyxia, injury to the tubular cells leads to loss of the capacity to avidly reabsorb sodium thus leading to natriuresis and consequently diuresis.

Limitations of serum creatinine

Traditionally, SCr has been used to diagnose AKI. However, it has several shortcomings in the neonatal period which include:

- SCr in the first 48 – 72 hours of life is usually a reflection of maternal creatinine values, not the infants renal function
- Normal nephrogenesis begins from 8 weeks GA and is complete by 34weeks. Depending on maturity of the kidney, there is a steady improvement in GFR from 10-20mls/min/1.73m^2 by week 1 of life to 30 – 40mls/min/1.73m^2 by two weeks after delivery, in line with alterations in RBF after which there is gradual steady improvement over the first months of life. Due to this low GFR, normal serum creatinine levels are thus distributed over a wide range of values depending on maturity and postnatal age.
- Changes in SCr concentration may not be noted until there is loss of 25 to 50% of kidney function.
- Since SCr is secreted by tubular cells, even at low GFR SCr will still be low thus will overestimate renal function.
- Measurement of SCr does not differentiate between renal derangements that are hemodynamically mediated and those that are not e.g. pre renal vs. intrinsic or obstructive renal failure.

Novel biomarker

Incidence of AKI in asphyxia has been shown to be high through several studies, and outcomes have remained poor, thus there has evolved a need to identify new biomarkers which would be able to anticipate the diagnosis of AKI within hours or days prior to the reduction in the UO or

changes in SCr. Identification of a biomarker that can differentiate between the different causes of AKI may change the approach to AKI management and lead to the implementation of preventive interventions.

In the early acute setting, appropriate biomarkers for kidney injury should have several characteristics which include:

- Should be specific to the kidney, and be able to differentiate between pre-renal, intrinsic and post-renal causes of kidney damage.
- Should posses the ability for earlier detection of kidney injury.
- Should be able to specifically isolate the cause of kidney injury
- Should be able to pinpoint particular sites of damage in the kidneys and able to provide information on disease changes in the primary location of damage.
- Should be reliable, easily measurable, prompt and ideally non invasive.
- Should be stable in its matrix
- Lastly, should be an inexpensive, widely affordable and available marker to measure

New biomarkers e.g Serum cystatin C and NGAL have been shown by recent studies to be early non invasive biomarkers. However, most of these biomarkers are not readily available in Kenya.

FENa: A solution to the problem?

FENa is the ratio of the clearance of sodium to the clearance of creatinine, expressed as a percentage. FENa has historically been utilised to distinguish between pre renal kidney failure and ischemic renal failure. The measurement of FENa is by urinary and serum creatinine and sodium levels, and is independent of urine output. FENa is dependent on tubular function which depends on autoregulation of blood flow to the kidneys.

Na_, K_-ATPase localized at the basolateral membrane aids in active sodium reabsorption. Greater than 60% of the sodium that is filtered is reabsorbed in the proximal convoluted tubule (PCT), 20% in the thick ascending limb (TAL) of the loop of Henle, while the remaining 10% of

sodium (Na) and chloride (Cl) in the distal convoluted tubule (DCT). Absorption of sodium proximally increases during development by three- to four fold [23]. In the immature kidney, a larger fraction of filtered Na is delivered the distal nephrons. During development of the kidney, FENa decreases from as high as 13% in the fetus to about 3% in the premature neonates less than 30 weeks of gestation, to 2% in term neonates secondary to maturational process [24].

Due to the decreased ability to actively reabsorb sodium, FENa values in term newborns are higher than those in older children (less than 2.5% percent versus less than 1 percent). There is decreased ability to undergo anaerobic metabolism in the S3 segment during periods of decreased oxygen tension such as in perinatal asphyxia, which leads to impaired reabsorption of sodium thus higher values of FENa.

Table 2: Interpretation of FENa

FENa value	Type of AKI	Mechanism
<2%	Pre renal AKI	Almost all of the filtered sodium is reabsorbed, which is an appropriate response to hypovolemia.
>2.5%	Intrinsic AKI in the absence of diuretics	Impaired TAL and DCT absorption of Na

Validation studies

Several studies validate the importance of FENa in differentiating pre renal failure from intrinsic renal failure. An adult prospective cohort study done by Carvounis et al [25] analyzed the sensitivity and specificity of both FENa and FEUr in differentiating prerenal failure from ATN. Results showed low FENa (<1%) levels in patients with untreated plain prerenal failure, and values >1% in those with both prerenal disease treated by diuretics and those with ATN. The sensitivity and specificity of FENa was high at 91% and 82% respectively.

Another adult prospective cohort study by Pépin et al [26] recruited patients in a tertiary care centre, 99 patients in total, who developed AKI. In patients in whom diuretics was not administered and in those who received diuretics, the specificity and sensitivity of FENa were 75% and 78% and 81% and 58% respectively.

A study done by Yassin [27] looked at both FENa and FEUr in 40 adult patients with AKI who had circulatory shock. The patients were grouped into 26 patients who had prerenal (group-1) and 14 who were found to have renal azotemia (group-2). FENa cut off was at 1% pre renal and >1% intrinsic AKI. Group – 1 had significantly lower FENa (0.99 ± 0.66) as opposed to group – 2 (2.57 ± 1.73, P <0.05). The sensitivity and specificity of FENa were 71.4% and 69.4% respectively.

A pediatric study done by Hisayo et al [36] recruited 74 patients (42 boys, 32 girls) in Japan, with 102 episodes of AKI. The children's mean age was 7.1 years, with an age range from 0 to 18 years. The study's aim was 'To assess the utility of FEUr in the differential diagnosis of AKI in children'. FENa cut off was <1% for pre renal and >/=1% for ATN. The children were divided into ATN (n=33), pre renal AKI (n=37), and pre renal AKI with furosemide (n=32) . Sensitivity of FENa < 1% was53 % (17/32), 95 % (35/37) and 75 % (52/69) in all cases of prerenal AKI with furosemide , prerenal AKI without furosemide and prerenal AKI respectively, with the specificity shown to be 90.9% (30/33).

Table 3: Summary of validation studies

Study	Sample size	Title	Outcome
Carvounis et al [25] 2002, USA Prospective cohort study	102 adult renal cases. 50 pre renal, 27 pre renal treated with diuretics and 25 ATN.	'Significance of the fractional excretion of urea in the differential diagnosis of acute renal failure'.	FENa <1% pre renal AKI, >/=1% in cases treated with diuretics and ATN. Sensitivity 91% and specificity 82%.
Pépin et al [26] 2007, Canada Prospective cohort study	99 adult patients	'Diagnostic Performance of Fractional Excretion of Urea and Fractional Excretion of Sodium in the Evaluations of Patients With Acute Kidney Injury With or Without Diuretic Treatment'	Those without diuretics, sensitivity 78%, specificity 75% with PPV of 86% and NPV of 64% With diuretics: sensitivity 58%, specificity 81%
Yassin [27] 2011, Egypt. Prospective cohort study	40 adult patients	'Comparison between fractional excretion of sodium and fractional excretion of urea in differentiating prerenal from renal azotemia in circulatory shock'	FENA cut off point 1.1% with sensitivity 71.4% and specificity of 69.4%
Hisayo et al [36],Tokyo, Japan. Prospective cohort study.	74 children	'T o find out the utility of FEUr in the differential diagnosis of AKI in children'.	FENA cut off point <1% for pre renal and >/=1% for ATN, with sensitivity 95% for pre renal and specificity of 90.9%

			for ATN.

Studies using FENA in asphyxia

In a study done by Matthew et al [28], 42 neonates in NICU on treatment for RDS and or sepsis were recruited, all of whom had oliguria, which was defined as urine output of less than 1ml/kg/hour for 12 hours or longer. After receiving either mannitol at 1g/kg or fluid challenge at 20mls/kg of normal saline, those with improved urine output were defined as having pre renal oliguria (PO), while those with persistent oliguria and azotemia with no other evidence of renal failure were defined as having renal failure. Sixteen neonates (38%) met the criteria for RF all of whom had perinatal asphyxia. FENa based on creatinine was found to have a mean value of 0.95% in patients with PO as opposed to 4.25% in those who had renal failure ($p<0.01$). The conclusion of the study was that sharp demarcation of the two groups was possible only when a FENa >/= 2.5% was used and that FENa >/= 2.5% was seen to differentiate renal failure from functional oliguria in the study.

Gupta et al [12] did a prospective case controlled study to find out the incidence of AKI in perinatal asphyxia. Serum urea, creatinine and electrolytes and sodium and creatinine in urine were measured within 24 hours after delivery and on the third day of life. Neonates with asphyxia were further divided into two groups, A1 without AKI and A2 with AKI. The control groups were labeled as B. A statistically different mean FENa was 0.6 ±0.56% in-group A and 0.29 ± 0.27% in-group B was noted.

Kaur et al [18] did a prospective cohort study in newborns asphyxia admitted to NICU in India. Baseline serum electrolytes were done within 6 hours of birth, with daily repeats till four days of life. Urine was collected between 24 to 36 hours and 72 to 96 hours of age. Mean FENa values at 24 – 36 hours in 15 neonates who developed AKI within that period was 5.59% as opposed to FENa values of 1.18% in the 21 neonates without AKI during that period (P 0.007). At 72 to 96 hours, FENa values in 6 neonates with AKI had a mean of 9.42% as opposed to 1.26% FENa in 30 neonates without AKI at that point in time (P 0.004).

In a prospective case controlled study by Roberts et al [29] 21 babies of 34-41 weeks gestational age with moderate to severe birth asphyxia were recruited. AKI was diagnosed by urinary retinol

binding protein: creatinine ratio. Fractional excretion of sodium was measured, results were reported without administration of diuretics. AKI was diagnosed in 19% (4/21). Mean FENa values were 31. 9% in those with AKI. That in control infants was below 1%.

Table 4: Summary of FENa studies in AKI

Study	Sample size	Title	Outcome
Matthew et al, [26] 1980. Prospective cohort study Country - US	42	Neonatal Renal Failure: Usefulness of Diagnostic Indices FENa cut off <2.5% pre renal oliguria, >/= 2.5% renal failure	38% (16/42) had AKI and asphyxia, with mean value of FENa of 0.95% in patients with PO as opposed to 4.25% in patients with renal failure (p<0.01).
Gupta et al [12] 2005 Prospective case controlled study Country - India	98 Case vs. control (70/28)	Renal Failure in Asphyxiated Neonates	Mean FENa was 0.6 ±0.56% in-group A (AKI) and 0.29 ± 0.27% in-group B (no AKI) which is a highly significant difference.
Kaur [18], 2011 Prospective cohort study. Country - India	44	'Evaluation of glomerular and tubular renal function in neonates with birth asphyxia'	1st 24 – 36 hours, mean FENa value 5.59% in AKI vs 1.18% without AKI (P 0.007). At 72 – 96 hours of life, mean FENa 9.42% in AKI vs 1.26% without AKI (P 0.004)
Roberts et al [29] 1990 Case Control, London	21 cases, 50 controls	'Prediction of acute renal failure after birth asphyxia'	AKI was diagnosed in 19% (4/21). FENa mean was 31. 9%. That in control infants was below 1%.

STUDY JUSTIFICATION

The incidence of AKI in neonatal asphyxia is reported in up to 50 – 72% [11] of cases. A recent study done in KNH found an 11.7% prevalence rate of AKI among newborns with perinatal asphyxia [17], while another study by Essajee et al [16] in KNH and Pumwani noted a prevalence rate of 56%. With lack of an identifiable universally acceptable AKI definition in neonates and the fact that its diagnosis in neonates is currently problematic as it relies on changes in serum creatinine and oliguria, efforts are being made to evaluate better methods that can be used to detect AKI before damage has been done to the kidneys.

Studies have reported high mortality rates amongst newborns with perinatal asphyxia who subsequently develop AKI. Therefore there exists a need for identification of a biomarker that can be used to diagnose AKI prior to derangements in creatinine, in order to institute appropriate management strategies to prevent or treat AKI early in those affected.

FENa is an indicator of tubular damage in neonates with the test being historically used to differentiate pre renal from intrinsic renal failure. Abnormalities in FENa can be picked up from as early as 6 hours within birth; it is an easy to do and readily available test. Studies looking at the sensitivity and specificity of FENa in differentiating pre renal from intrinsic AKI have values as high as 91% and 82% respectively [25]. However these studies have mostly been done in adults and this study therefore sought to establish whether FENa can be used to predict AKI early in perinatal asphyxia, and determine its sensitivity and specificity in our set up.

RESEARCH QUESTION

Is the fractional excretion of sodium (FENa) measured on day 1 of life an early predictor of AKI in neonates with birth asphyxia at Kenyatta National Hospital's NBU?

OBJECTIVES

Primary Objective

To determine the diagnostic utility (sensitivity, specificity, PPV and NPV) of fractional excretion of sodium (FENa) measured on day 1 of life in the diagnosis of AKI in neonates with perinatal asphyxia in KNH. AKI will be diagnosed by serum creatinine level $\geq$100µmol/l on the third day of life.

Specific objectives

1. To determine the sensitivity of FENa as an early diagnostic marker for AKI in neonates with perinatal asphyxia.
2. To determine the specificity of FENa as an early diagnostic marker for AKI in neonates with perinatal asphyxia.
3. To determine the PPV of FENa as an early diagnostic marker for AKI in neonates with perinatal asphyxia.
4. To determine the NPV of FENa as an early diagnostic marker for AKI in neonates with perinatal asphyxia.

METHODOLOGY

Study design:
This was a hospital based cross sectional study.

Study area:
The study was carried out at the Newborn Unit at the Kenyatta National Hospital (KNH), the tertiary referral and teaching hospital for the College of Health Sciences, University of Nairobi. It is also the main inpatient hospital for the low and middle-income society in Nairobi and its environs. The newborn unit is a level III unit which admits all sick neonates born in KNH, and also handles transfers from other hospitals. The unit admits between 160 and 200 neonates each month, over 20% whom are term babies diagnosed with perinatal asphyxia [(7)].

Study population:
The study population was term newborns admitted in KNH NBU with a diagnosis of birth asphyxia within 24hours of birth using the Apgar scoring [(30)] and Sarnat and Sarnat clinical staging of hypoxic ischemic encephalopathy [(31)] outlined in appendices IV and V respectively.

Study Tool:
A standardized questionnaire was used for collecting data from the enrolled participants, which was administered after obtaining informed consent from the parent/guardian. The questionnaire was pre tested in the KNH New born unit among newborns with perinatal asphyxia. (Appendix 1)

Study period
The study ran for a period of four months (July 2016 to October 2016)

Study personnel:

1. The lead investigator was the supervisor in charge of the research team, whose role was to ensure proper documentation and perform standard procedures on enrolled participants. The lead investigator also ensured all the materials needed were available and that all data collected was entered in to the computer systems daily.

2. Research assistants: with the help of two research assistants (clinical officers), data was collected. They received training on standard ways of doing procedures for the study.

Inclusion criteria:

- Term newborn (≥ 37 completed weeks) assessed by modified Ballard's exam [32] as outlined in appendix III
- Age 0-24 hours at initial assessment
- "Failure to initiate and sustain breathing at birth." 1 **plus** Apgar Score less than or equal 7 at 5 minutes **plus** clinical evidence of hypoxic ischemic encephalopathy Sarnat and Sarnat stage 1, 2 or 3 as shown in appendix V. " The highest level of neurological impairment was used to place the infant in the appropriate stage.
- Consent by the parent or caregiver

Exclusion criteria:

- Newborns with major congenital anomalies
- Newborns who developed jaundice in the first three days of life.
- Those who die within three days of the study.

Sampling technique:

The sampling technique used was consecutive sampling.

Sample size

Sample size at the required absolute precision level for sensitivity and specificity was calculated by Buderer's formula [33]

$$\text{Sample size } (n) \text{ based on sensitivity} = \frac{Z^2_{1-\alpha/2} \times S_N \times (1-S_N)}{L^2 \times Prevalence}, \text{ and}$$

$$\text{sample size } (n) \text{ based on specificity} = \frac{Z^2_{1-\alpha/2} \times S_p \times (1-S_p)}{L^2 \times (1-Prevalence)},$$

where n = required sample size,

S_N = anticipated sensitivity, set at 0.98

S_P = anticipated specificity, set at 0.98

α = size of the critical region (1 − α is the confidence level), set at 95% confidence interval

$z_{1-\alpha/2}$ = standard normal deviate corresponding to the specified size of the critical region (α), and

L = absolute precision desired on either side (half-width of the confidence interval) of sensitivity or specificity. Level of precision (set at±5%)

Prevalence = set at 56% using results from Farida Essajee et al's [18] study

Using the above formula, sample size for a sensitivity of 98% was 54 neonates while a sample size based on specificity of 98% was 71 neonates. Thus, minimal sample sizes of 68 neonates were to be recruited to the study.

Recruitment procedure

Enrollment of participants

All term neonates aged 0-24 hours admitted at KNH NBU were assessed for perinatal asphyxia using the Apgar scoring [30] and Sarnat and Sarnat clinical staging of hypoxic ischemic encephalopathy [31] outlined in appendix II and III respectively. The most severe sign was used to categorize the severity of the perinatal asphyxia. The gestational age was ascertained by Ballard exam [32]

All term newborns that met the criteria were consecutively enrolled within the first 24 hours of life irrespective of day or night admission.

The parents or caregivers of the term neonates with perinatal asphyxia that satisfied the inclusion criteria were requested to participate in the study. Only after explaining of the reason of the study and its expected benefits and possible harms was informed consent obtained from the parent/caregiver.

Newborns with malformations and those that did not survive to the third day of life were excluded from the study.

Day 1: Determination of FENa

- A single urine sample was obtained within 24 hours of birth from each of the study subjects using in and out catheterization and for those in whom urine specimen wasn't obtained at first try, urine collecting bags were left in place.
- The urine collected was used to assay urine electrolytes i.e. urine sodium and creatinine.
- Serum sodium and creatinine levels were measured by drawing 0.5 to 1ml of blood sample into a microtainer on the first day by quick heel sampling.
- The specimens were transported to the laboratories immediately after they were collected.
- FENa was calculated by the following formula:

$$FE_{Na}=100 \times \frac{\text{sodium}_{\text{urinary}} \times \text{creatinine}_{\text{plasma}}}{\text{sodium}_{\text{plasma}} \times \text{creatinine}_{\text{urinary}}}$$

- The cut off for a positive FENa was $\geq$ 2.5% while a negative FENa was <2.5%

Day 3: Diagnosis of AKI

- Serum creatinine levels were measured by drawing 0.5 to 1ml of blood sample into a microtainer on the third day of life by quick heel sampling.
- Definition of AKI was set at a serum creatinine level $\geq$ 100μmol/l.

Diagnostic utility

- FENa results obtained on day 1 of life were correlated with serum creatinine values on day 3 of life to find out whether the neonates with a diagnosis of AKI on the third day of life also had positive FENa (>2.5%) on day 1 of life. This was used to determine whether FENa values on day 1 of life can be used as an early predictor of AKI in newborns with perinatal asphyxia.

Laboratory analysis

The Ion Selective Electrodes (ISE) module of the COBAS INTEGRA systems was used for the quantitative determination of sodium and creatinine in urine[(34)]. The specimens were automatically diluted 1:6 (l +5) by the instrument for readings on urine sodium levels while they were automatically diluted by the machine to1:25 (1+24) in order to get the creatinine values. The test principle for the creatinine estimation uses Buffered kinetic Jaffe reaction without deproteinization. Creatinine reacts in alkaline solution with picrate to form a yellow-red adduct. There is direct proportionality between the creatinine concentration in the specimen to the rate of dye formation (color intensity). It is determined by measuring the increase in absorbance at 512 nm. Urine sodium and creatinine levels results were documented. Within 2 hours the serum sample was centrifuged and analyzed using Cobas Integra machine using the compensated Jaffé method [(35)]. The Cobas Integra automatically calculated the analyte concentration of each sample. The Cobas Integra uses the Precinorm U or Precinorm U plus for reference range control, and the Precipath U or Precipath U plus for pathological range control. The control interval was 24 hours. The machine was calibrated every seven days using deionised water as zero calibrater according to the standard reference material guidelines. Serum and plasma

samples contain proteins which react non-specifically in the Jaffé method. For compensation of serum and plasma results, values were automatically corrected by -18µmol/l.

DATA MANAGEMENT AND ANALYSIS

Data was collected using a standardized questionnaire and entered into a password protected database. During entry, data collection forms were stored in a secured lockable cabinet to prevent unauthorized access.

Data analysis was done using the IBM® SPSS Statistics software version 21. Data cleanliness was ensured by comparing entered data with the hard copy forms after entry was complete.

On each day, neurologic exam was carried out and the infants categorized according to their HIE Stage.

Categorical data such as neurological scores and AKI scores were presented using frequency tables whereas continuous data for example length, birth weight, gestational age, head circumference were summarized using measures of central tendency and dispersion.

Factors associated with the accurate diagnosis of AKI using FENa were determined using chi-squared tests for categorical comparisons and t-tests for continuous comparisons. Independent factors associated with accurate diagnosis of AKI using FENa were determined using binary logistic regression methods.

FENa sensitivity was determined by calculating what proportion of neonates with AKI diagnosis on the third day of life were positively identified by FENa levels >2.5% on day 1 of life. Specificity was determined by calculating what proportion of neonates without a diagnosis of AKI on the third day of life had a negative FENa on the first day of life.

The PPV was determined by calculating what proportions of neonates with positive FENa on day 1 of life truly had AKI on day 3 of life based on serum creatinine > 100µmol/l. The NPV was determined by calculating what proportion of neonates with negative FENa on day 1 of life did not have a diagnosis of AKI on day 3 of life.

Analysis is as depicted by Table 5 below

Table 5: Diagnostic utility of FENa

	AKI by sCR day 3	No AKI on day 3	Total
FENA positive day 1	a	B	a + b
FENA negative d1	c	D	c + d
	a + c	b + d	N

Sensitivity = a/(a+c)

Specificity = d/(d+b)

PPV = a/(a+b)

NPV = d/(c+d)

ETHICAL CONSIDERATIONS

This protocol, together with the informed consent document and any further modifications was reviewed and approved by the KNH's Ethics, Research and Standards Committee, and a letter of approval from the committee was obtained prior to commencement of the study.

Parents/caregivers were given full information about the study and a written consent was obtained from them. No emergency/resuscitation measures were overlooked and they were given priority to other procedures. No beneficial treatment was withheld from the patients. Subject confidentiality was strictly upheld by the principal investigator, research assistants and other supporting parties, and no information concerning the study or data collected was released to any unauthorized person. Study details were given to the clinician taking care of the neonate.

RESULTS

General characteristics of all the mothers and neonates

During the study period, 108 neonates with perinatal asphyxia were admitted, but only seventy nine babies survived to the third day of life, 41 (52%) of whom were male.

STUDY FLOW DIAGRAM

The diagram below summarises the recruitment procedure up to obtainment of desired outcomes.

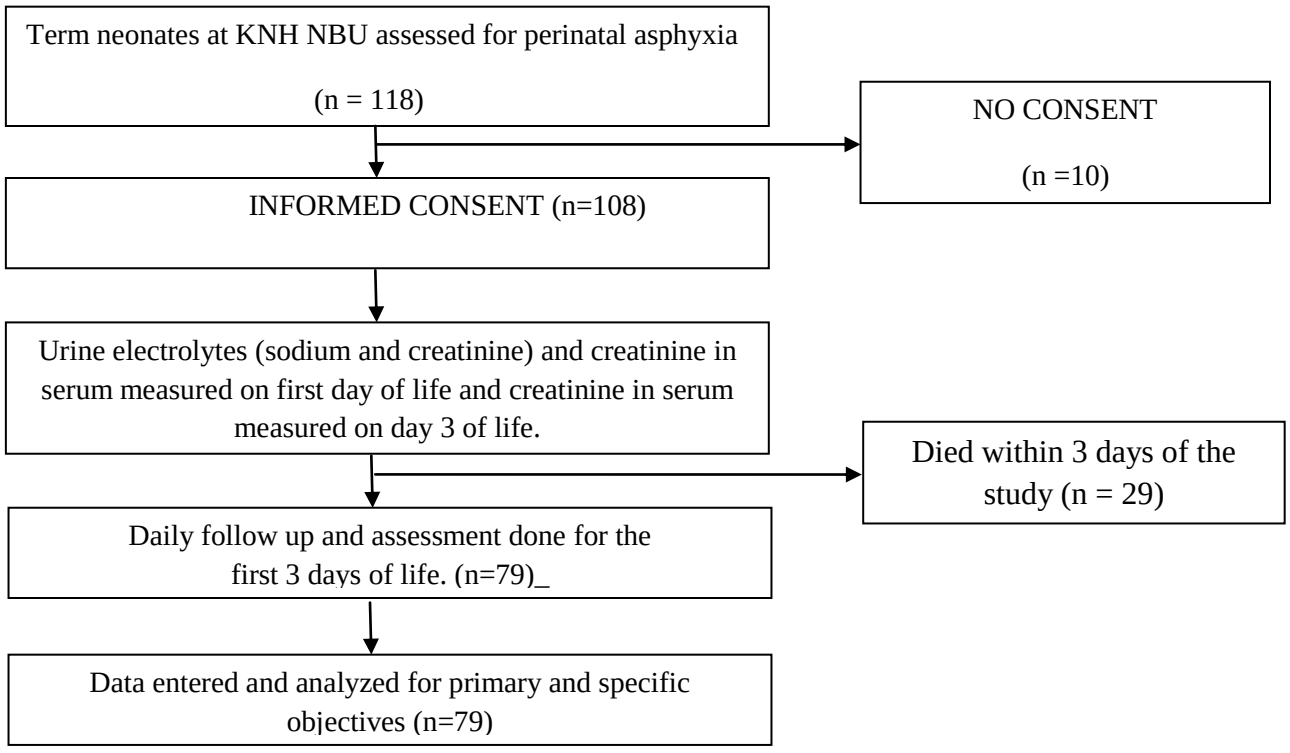


Table 6: Characteristics of the neonates on admission

Characteristics	**Frequency(%)/ Mean (SD)**
Gender	
Male	41 (52)
Female	38 (48)
Gestational age in weeks	39 (1)
Birth weight in grams	3289 (478)
Length in centimeters	47 (3)
Head circumference in centimeters	36 (2)
Place of delivery	
KNH	61(79)
Other health facility	16(21)
Mode of delivery	
Vertex vaginal	53(67)
C/S	26(33)
Apgar Score at 5 minutes	
Mean	6 (1)
Mild (6-7)	53 (67)
Moderate (4-5)	23(29)
Severe (0-3)	3(4)
Resuscitation with BVM	
Yes	31(39)
No	48(61)
Intubation+ mechanical ventilation	
Yes	20 (25)
No	59 (75)

The mean weight was 3289 g (SD=478) and the mean length was 47 cm (SD=3) .The neonates weight ranged from 2200 to 4600 g as depicted in table 6 above.

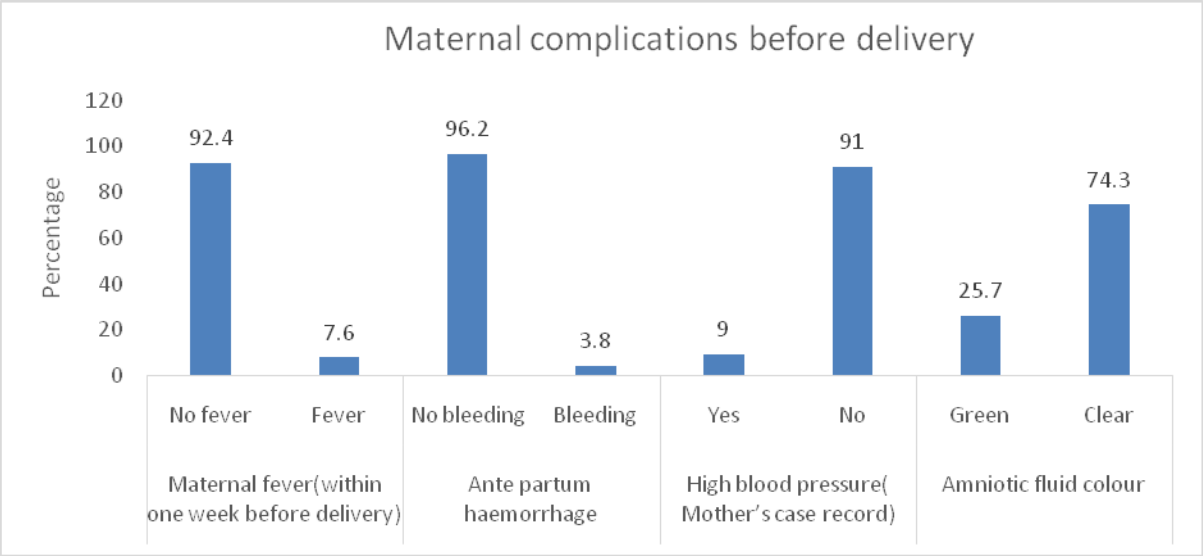
Majority of the deliveries took place in KNH (79%) by vertex vaginal mode (67%). Two neonates did not have documentation of place of delivery. Of the neonates recruited, 39% were resuscitated via bag valve and mask. The mean Apgar score was 6 (SD=1), with 68 % with mild, 29 % moderate and 3 % severe asphyxia on the day of admission as depicted in Table 6.

Table 7: Characteristics of the mothers

Variable	Frequency (%)
Marital status	
Single	7 (9)
Married	71 (90)
Widowed	1 (1)
Occupation	
Salaried/formal employment	6 (8)
Informal employment	2 (3)
Self employed	12 (15)
Casual worker	16(20)
Unemployed	43(54)
Number of ANC visits	
Twice	1(1)
More than twice	78 (99)
Parity	
Primigravida	6 (8)
Para 1	46 (58)
Para 2 and above	27 (34)
Education level	
Primary	5 (6)
Secondary not completed	26 (33)
Secondary completed	16 (20)
Tertiary and beyond	32 (41)

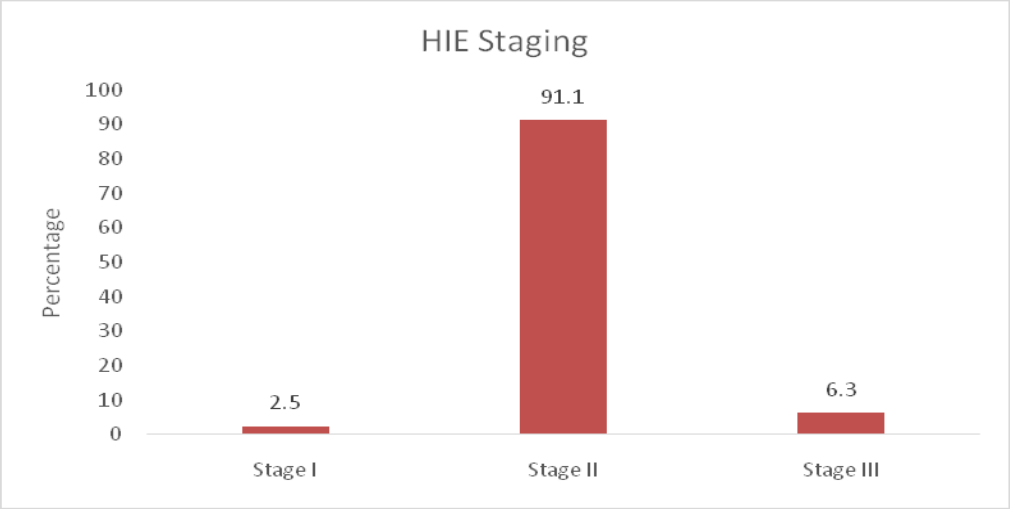
The mean age of the mothers was 24 (SD = 5) yrs, the youngest being 15 yrs and the oldest 38 years; 90% were married. Of the 79 mothers, 58 % were Para 1+0. The majority (54%) of the mothers were unemployed, with 53% and 40 % having attained secondary and tertiary education respectively. All mothers reported to have attended ANC, 98% reporting having attended more than twice.

Figure 2: Maternal complications



Among the mothers of the neonates recruited, 53% (n=43) did not have any of the complications listed in Figure 2 above. Six (8%) and seven (9%) of the mothers respectively reported to have had fever within one week of delivery and high blood pressure respectively, while only 4% (n=3) had ante partum hemorrhage. Majority (74%) of the mothers reported clear amniotic fluid during delivery, although 9 mothers' admission notes had no documentation of amniotic fluid colour.

Figure 3: HIE stage of the neonates on admission



The majority (91%) of the neonates admitted had HIE Stage II on the first day of admission.

Table 8: Prevalence of AKI using Creatinine and probable AKI by FENa

	Gender						
	Male=41		Female=38		Total		
	N	%	N	%	n	%	p-value
AKI(Serum Creatinine ≥100µmol/l	21	51.2	9	23.7	30	38.0	0.012
Probable AKI (FENa ≥2.5%)	27	65.9	23	60.5	50	63.3	0.624

Thirty out of seventy nine neonates had serum creatinine of ≥100µmol/l on day three of life which translated to an AKI prevalence of 38%. Of the neonates with AKI, 51% were male and 24% female, which was statistically significant (p = 0.012).

FENa on day 1 of life was positive (≥2.5%) in 50 neonates (63.3%) of which 27 (66%) were males and 23 (60%) females. There was no statistical difference between males and females (p = 0.624)

Table 9: Diagnostic Utility of FENa

	AKI by creatinine		
AKI by FeNA	**Serum Creatinine** ≥100µmol/l	**Serum Creatinine** <100µmol/l	Total
FeNA ≥2.5%	27	23	50
FeNA <2.5%	3	26	29
Total	30	49	79

AKI by creatinine (cut off 100 µmol/l)

Sensitivity 90% (95% CI 79% - 100%)

Specificity 53% (95% CI 39%- 68%)

Positive likelihood ratio 1.9

Negative likelihood ratio 0.19

Of the neonates recruited, 27/30 (90%) had a positive FENa (defined as FENa >/=2.5%) on day one of life and AKI diagnosed on day 3 of life, thus setting our sensitivity at 90%. Amongst the 49 neonates who did not have AKI on day 3 of life, 26 neonates had negative FENa on day 1 of life, thus setting the specificity of the test at 53%.

Of the 50 neonates who had positive FENa on day one, 27 of them had AKI diagnosed on day 3 of life thus setting the PPV at 54%. Conversely of the 29 neonates who had negative FENa on day 1 of life, 26 of them had no AKI based on serum creatinine, thus giving a NPV of 90%.

Fig 4: Graph of sensitivity vs specificity of FENa

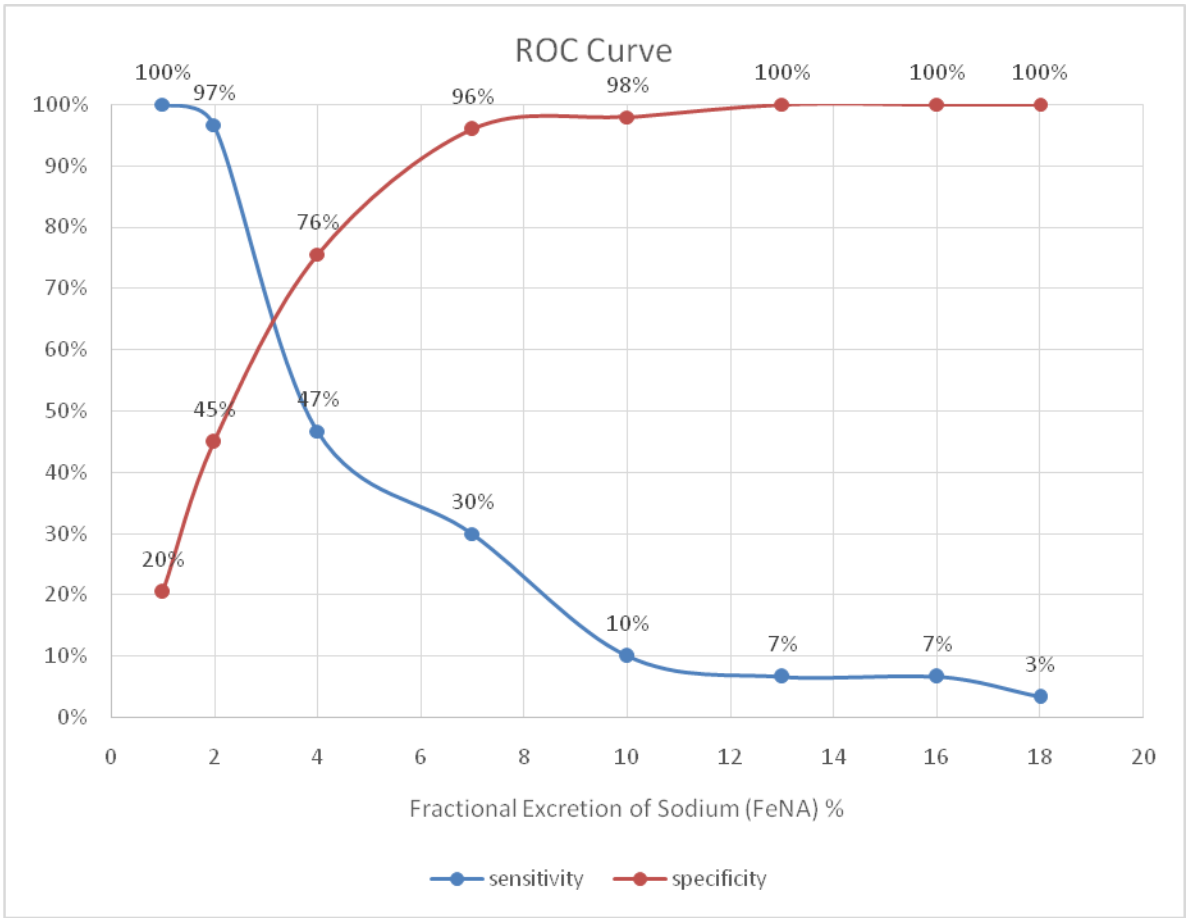


Figure 4 above plots the sensitivity and specificity of FENa at 90% vs 53% respectively at a chosen cut-of off >/= 2.5%.

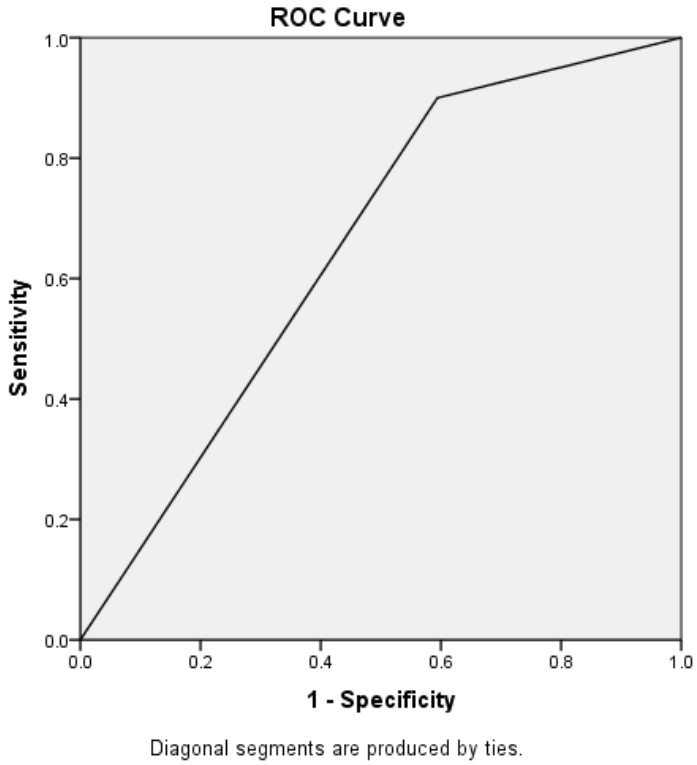


Figure 5: Receiver operating characteristic (ROC) curve for Fractional excretion of sodium (FENa) on day 1

Figure 5 above shows a moderate efficacy (AUC 0.715) in AKI prediction.

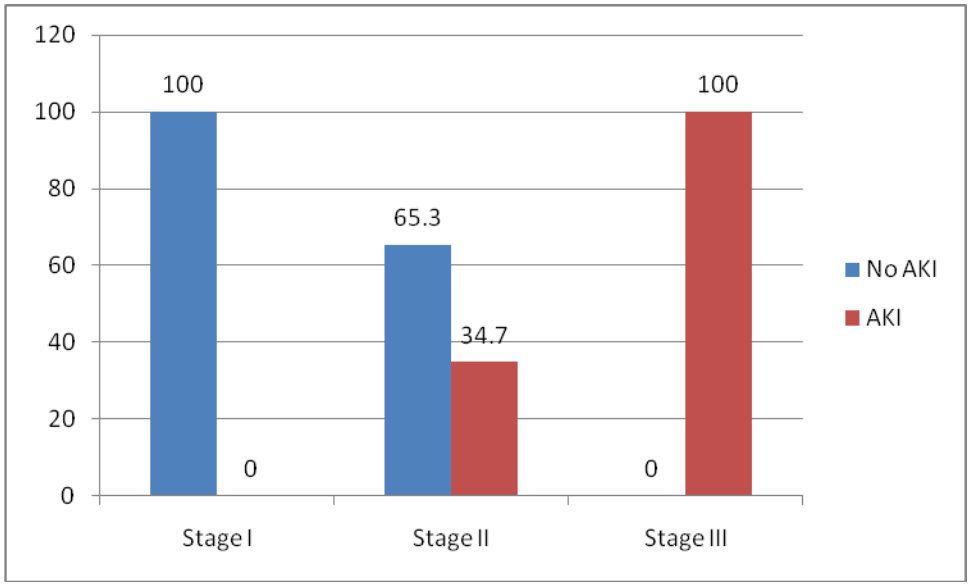
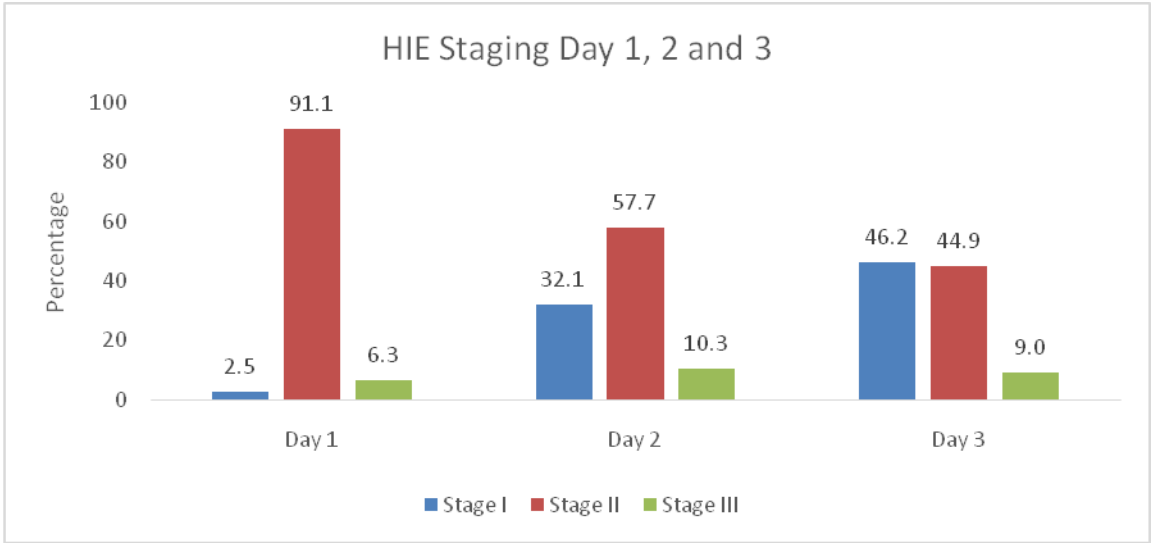


Figure 6: Correlation between HIE Stage and AKI

Figure 6 above shows that AKI was highest in those with HIE Stage III (100%), with no AKI documented in patients with HIE Stage I which is statistically significant (p value 0.008).

Figure 7: Progression of HIE Stage during first three days



Progressively, babies with HIE Stage II showed improvement by day three of life (as assesed by Sanart and Sanart HIE stage) thus steadily increasing the number of neonates in Stage I by the third day of life. Not much change was seen in neonates with Stage III HIE.

DISCUSSION

Most previous investigators used serum creatinine levels above133 µmol/ l[15, 17] to diagnose AKI. Studies by Shah et al [13], Leila [14] and Gupta [12] used levels of creatinine in serum of greater than 1 mg/dL (88 µmol/l) at 72 hrs of life to diagnose AKI. So as to increase diagnostic probability, serum creatinine level of 100 µmol / l at 72 hours of life was chosen.

The prevalance of AKI using serum creatinine levels of ≥100 µmol/l on the third day of life was found to be 38% (30/79). Studies by Shah et al [13], Leila [14] and Gupta [12] used serum creatinine of greater than 1 mg/dL (88 µmol/l) to diagnose AKI, which showed a prevalence of 70%, 64% and 47.1% respectively. Studies that used serum creatinine of more than 1.5 mg/dL (133 µmol/l) included those by Karlowicz [15] and Alaro et al [17] which had prevalence of 61% and 11.7 % respectively. The prevalence of AKI in our study lies within the range found in other studies (11.7% - 72%) which is reflective of the fact of AKI in asphyxia being a universal health problem.

Our study showed a higher prevalence of AKI in boys than girls, with 51% male and 24% females giving a male to female ratio of 2.33:1 which was statistically significant (p 0.012). This is similar to what has been reported in other studies for example Esajee et al [16] indicated a male-to-female ratio of 2.3:1. Documented increase in susceptibility of perinatal disorders in the male neonate could be an explanation for this finding [37].

We set out to assess the importance of FENa in the early diagnosis of AKI in neonates with perinatal asphyxia since AKI post asphyxia has been associated with poor outcomes [7, 12, 17]. FENa has been studied in mostly adult population to differentiate between pre renal AKI and ATN (which is the type of renal injury demonstrated in neonates with asphyxia) with sensitivities and specificities ranging between 71.4% - 95% and 69.4% - 90% respectively [25,26,27,36].

However, very few studies have been done using FENa as a biomarker for AKI in neonates with asphyxia.

In this study in Kenyan newborn infants, the specificity and sensitivity of detecting AKI as indicated by serum creatinine levels of ≥100 μmol/l was 53% (95% CI 39% - 68%) and 90% (95% CI 79% - 100%) respectively with a moderate efficacy (AUC 0.715) in prediction of AKI. Our sensitivity of 90% is comparable to both Carvounis et al [25] and Hisayo et al [36] at 91% and 95% respectively, which makes FENa a good screening test for AKI. This would essentially translate to the early diagnosis of AKI and thus earlier management strategies put in place in terms of fluid and electrolyte balance, avoidance of nephrotoxic drugs, fluid restriction to insensible loses and urine output in oliguric/anuric patients, and preparation for renal replacement therapy as studies have shown high mortality rates in neonates with perinatal asphyxia and AKI. [7,12,19]

Our modest specificity of 53% (95% CI 39% - 68%) means that the test, however, can also falsely identify neonates without AKI on the third day of life as having positive FENa on day 1 of life. This means that one can incur expenses of costing and undergo the procedure of obtaining the samples and end up having no AKI. However it should be put into consideration that the severity of AKI in asphyxia and the implication of delayed management on mortality should be weighed against the cost of obtaining false positive results, and that none of the interventions undertaken are harmful.

Our study showed a modest PPV of 54 % but a high NPV of 90%. The low PPV essentially would mean that slightly over half of the neonates who test positive for FENa will actually be diagnosed as having AKI using serum creatinine on day 3 of life. However, it should be noted that since creatinine only shows an estimate of renal function and not of injury, and since it is a late marker of injury, then this ideally means that the neonate could be having AKI but there is no change in serum creatinine until a substantial loss of kidney function occurs. On the other hand, FENa as a predictor of AKI has a high NPV, meaning that the test is also quite useful in

that of those neonates who tested negative with FENa on day 1 of life, chances are quite high that the neonates will not end up with AKI on the third day of life.

The positive and negative likelihood ratios for FENa ≥2.5% was 1.9 and 0.19 respectively. A neonate with positive FENa test will therefore be almost twice as likely to end up with AKI than one without abnormal FENa, the opposite holding true, i.e a neonate with negative FENa is indicative of absence of AKI.

There is increasing incidence of AKI with increase in severity of AKI [12, 38]. This can be attributed to a redistribution of cardiac output to vital organs potentially leading to severe renal ischemia. Our study showed AKI to be highest amongst neonates with HIE Stage III (100%) and 35% in neonates with HIE Stage II (p 0.008) with no AKI documented in patients with HIE Stage I. Gupta et al. [12] study showed significantly higher blood creatinine and urea levels in the neonates with asphyxia as compared to that in the control group (P<0.001) and (P <0.05). Kaur et al's [18] study showed prevalence of 9.1% and 56% respectively in neonates with moderate and sever asphyxia. Alaro et al's [17] study showed AKI to be highest in neonates with HIE Stage III (42.9%) and lowest in Stage I (4.8%). Our findings of 100% could be attributed to the small sample size in newborns with HIE Stage III ($n = 5$).

STUDY STRENGTHS

- The principal investigator and assistant recruited all the neonates and were able to follow them up daily in the NBU with no difficulties.
- The study received good support from the KNH laboratory staff with prompt analysis and dispatch of results.

STUDY LIMITATIONS

- Early perinatal death i.e. within three days of birth of the severely ill neonates gave us a small sample size for babies with HIE Stage III which might have skewed some results for example correlation between AKI and HIE Stage.
- True specificity and sensitivity of FENa would be hampered by the use of serum creatinine as the gold standard for the definition of AKI due to its intrinsic shortcomings.
- Unavailability of urine sample during catheterization necessitating separate collection times for urine and serum specimen from some study participants.
- The Apgar score is user dependent.

CONCLUSION

FENa is an easy to do and readily available test with a high sensitivity (90%) and a good PLR of 1.9 which makes it a good screening test. Despite its modest PPV and specificity of 54 and 53% respectively, it is a useful test since the benefit of early treatment of AKI would outweigh the cost of undertaking the test.

RECOMMENDATIONS

- FENa has shown to have high sensitivity and should thus be in cooperated into the initial laboratory evaluation in neonates with asphyxia on the first day of life.
- Further studies following up neonates for a longer follow up period to assess whether a significant number of the patients initially deemed false positive using FENa would actually fit the criteria for AKI using current creatinine values thus improving on our specificity.

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APPENDICES

Appendix 1: Questionnaire

1.Questionnaire Serial No.	**Patient's Hospital No.**	**Date (dd/mm/yy)**
2. 0 Personal details		
2.1 Gender	[_] Male	[_] Female
2.2 Date of birth (dd/mm/yy)		
2.3 Time of admission into NBU (24 hr clock)		
2.4 Gestational age in weeks		
2.5 Birth weight in grams		
2.6 Length in centimeters		
2.7 Head circumference in centimeters		
2.8 Apgar score at 5 minutes		
2.9. Resuscitation with BVM	[_] No [_] Yes	
2.9.1 *If yes for 2.9, what was the duration* in minutes?		
3.0 Intubation+ mechanical ventilation	[_] No [_] Yes	
4.0 Sarnat and Sarnat clinical staging of HIE		
4.1 Level of consciousness : [_] alert/hyper alert [_] lethargic [_]coma		
4.2 Muscle Tone : [_]normal [_] hypotonic [_]flaccid		
4.3 Suck reflex : [_]active [_]weak [_] absent		
4.4 Moro reflex : [_]exaggerated [_]incomplete [_]absent		
4.5 Grasp reflex : [_] normal/exaggerated [_]weak [_]absent		
4.6 HIE stage : [_] 1 Normal [_] 11 Abnormal [_] III Abnormal		
5.0 Mother's Data		
5.1 Date of birth : (dd/mm/yy)		

5.2 Relationship to the newborn. If not mother		[_]Father [_]Aunt	[_]Sibling [_]Other relative [_]Non relative	[_]Uncle [_]Grand parent
5.3 Parity				
5.4 Marital status	[_]Single [_]Married [_]separated [_]Widowed [_]Don't know			
5.5 Occupation	[_]Salaried/formal employment [_]Informal employment [_]Self employed [_]Casual worker [_]Unemployed			
5.6 Level of education	[_]none [_]Not completed primary level [_]Primary level completed [_]Not completed secondary level [_]Secondary level completed [_]Tertiary and beyond			
5.7 Antenatal clinic visits	[_]No	[_] Yes		
5.7.1	*If yes for 5.7 how many times?*	[_] Once [_]Twice [_]more than twice		
5.9 Place of delivery	[_]Home [_]KNH [_]Other health facility [_]en route to health facility			
6.0 Mode of delivery	[_]Vertex vaginal [_]Breech vaginal [_]C/S [_]V/E			
6.1 Maternal fever(within one week before delivery)	[_]No fever [_] Fever			
6.2 Ante partum haemorrhage	[_]No bleeding [_]Bleeding			

6.3 High blood pressure(Mother's case record)	[_] NO [_] YES
6.4 Convulsion during pregnancy	[_] NO [_] YES
6.5 Other chronic diseases	[_]NO [_] YES
6.5.1	*If yes for 6.5 what disease*
6.6 Duration of labour	
6.7 Duration of rupture of membranes	
6.8 Amniotic fluid colour	[_] Green [_] Clear
6.0 DAILY CLINICAL	

Time after initial assessment	Day 1 Date: Time:	Day 2 Date: Time:	Day 3 Date: Time:	

6.10 Level of consciousness [_]alert[_] lethargic[_]coma
6.11 Muscle tone[_]normal [_]hypotonic [_]flaccid
6.12 Seizures [_]absent[_]focal/multifocal[_]generalized
6.13 Suck reflex [_]active[_]weak[_]absent
6.14 Moro reflex [_]normal/exaggerated[_]weak[_]absent
6.15 Grasp reflex: [_]normal[_]weak[_]absent
6.16 HIE Stage: [_]Normal [_] IIAbnormal [_]IIIAbnormal
6.20 Intubation+ mechanical ventilation [_]No [_]Yes
6.30 Mode of feeding in 24 hours [_]IV Fluids[_]Breastmilk[_]Formula[_]Mixed feeding
6.31 Amount of feed in 24 hours [_]Less[_]Adequate[_]More 6.40 Serum creatinine in μmol/l on day 1 and 3
6.50 Serum sodium in mmol/l on day1 6.60 Urine sodium in mmol/l on day 1 6.70 Urine creatinine in μmol/l on day 1
6.80 Medications administered 6.90 FENa [_]<2% [_]2-2.5% [_]>2.5% 7.00 [_]With AKI [_]Without AKI

Appendix II: pRIFLE Classification for AKI

pRIFLE Classification

Category	Estimated Creatinine Clearance*	Urine Output
Risk (R)	Decrease by 25%	< 0.5 mL/kg/hr for 8 h
Injury (I)	Decrease by 50%	< 0.5 mL/kg/hr for 16 h
Failure (F)	Decrease by 75% or < 35 mL/min/1.73 m^2	< 0.3 mL/kg/hr for 24 hr or anuric for 12 h
Loss (L)	Loss of renal function > 4 weeks	
End-Stage (E)	End Stage Renal Disease	

*Calculated with Schwartz equation: Length (cm) × K (constant) / serum creatinine

Appendix III: Ballard's maturational rating

MATURATIONAL ASSESSMENT OF GESTATIONAL AGE (New Ballard Score)

NAME _____ SEX _____
HOSPITAL NO. _____ BIRTH WEIGHT _____
RACE _____ LENGTH _____
DATE/TIME OF BIRTH _____ HEAD CIRC. _____
DATE/TIME OF EXAM _____ EXAMINER _____
AGE WHEN EXAMINED _____
APGAR SCORE: 1 MINUTE _____ 5 MINUTES _____ 10 MINUTES _____

NEOMUSCULAR MATURITY

NEUROMUSCULAR MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
POSTURE								
SQUARE WINDOW (Wrist)		>90°	90°	60°	45°	30°	0°	
ARM RECOIL			180°	140–180°	110–140°	90–110°	<90°	
POPLITEAL ANGLE	180°	160°	140°	120°	100°	90°	<90°	
SCARF SIGN								
HEEL TO EAR								
						TOTAL NEUROMUSCULAR MATURITY SCORE		

PHYSICAL MATURITY

PHYSICAL MATURITY SIGN	SCORE -1	0	1	2	3	4	5	RECORD SCORE HERE
SKIN	sticky friable transparent	gelatinous red translucent	smooth pink visible veins	superficial peeling &/or rash, few veins	cracking pale areas rare veins	parchment deep cracking no vessels	leathery cracked wrinkled	
LANUGO	none	sparse	abundant	thinning	bald areas	mostly bald		
PLANTAR SURFACE	heel-toe 40–50 mm: -1 < 40 mm: -2	>50 mm no crease	faint red marks	anterior transverse crease only	creases ant. 2/3	creases over entire sole		
BREAST	imperceptible	barely perceptible	flat areola no bud	stippled areola 1–2 mm bud	raised areola 3–4 mm bud	full areola 5–10 mm bud		
EYE / EAR	lids fused loosely: -1 tightly: -2	lids open pinna flat stays folded	sl. curved pinna; soft; slow recoil	well-curved pinna; soft but ready recoil	formed & firm instant recoil	thick cartilage ear stiff		
GENITALS (Male)	scrotum flat, smooth	scrotum empty faint rugae	testes in upper canal rare rugae	testes descending few rugae	testes down good rugae	testes pendulous deep rugae		
GENITALS (Female)	clitoris prominent & labia flat	prominent clitoris & small labia minora	prominent clitoris & enlarging minora	majora & minora equally prominent	majora large minora small	majora cover clitoris & minora		
							TOTAL PHYSICAL MATURITY SCORE	

Reference
Ballard JL, Khoury JC, Wedig K, et al: New Ballard Score, expanded to include extremely premature infants. J Pediatr 1991; 119(3):417–423. Reprinted by permission of Dr Ballard and Mosby—Year Book, Inc.

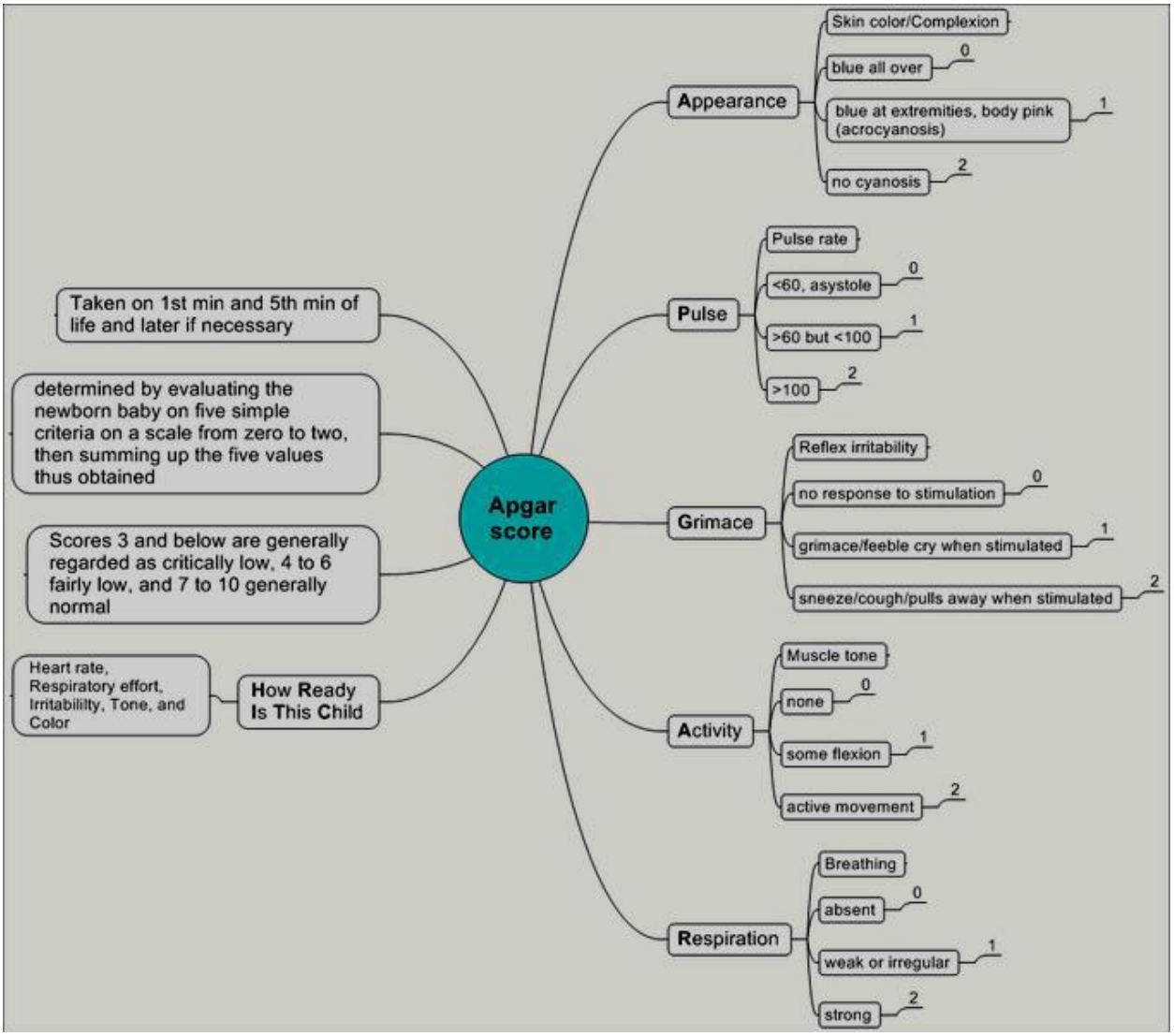
SCORE
Neuromuscular _____
Physical _____
Total _____

MATURITY RATING

SCORE	WEEKS
-10	20
-5	22
0	24
5	26
10	28
15	30
20	32
25	34
30	36
35	38
40	40
45	42
50	44

GESTATIONAL AGE (weeks)
By dates _____
By ultrasound _____
By exam _____

Appendix IV: Apgar score



Appendix V: Definition and staging of perinatal asphyxia.

"Failure to initiate and sustain breathing at birth."[1] PLUS clinical evidence of hypoxic ischemic encephalopathy Sarnat and Sarnat stage 1, 2 or 3.

Sarnat and Sarnat Clinical Staging of Hypoxic Ischemic Encephalopathy

Variable	**Stage 1**	**Stage 2**	**Stage 3**
Level of consciousness	Alert/Hyperalert	Lethargy	Coma
Muscle tone	Normal	Hypotonia	Flaccidity
Seizures	Absent	Focal or Multifocal	Generalised
Reflexes			
Suck	Active	Weak	Absent
Moro	Exaggerated	incomplete	Absent
Grasp	Normal/ Exaggerated	Weak	Absent

Appendix VI: Standard operating procedures for the measurement of weight, length and head circumference

Weight: Babies was weighed in the NBU nude in a warm environment using a basin scale with high sides to ensure baby's safety. Three weight readings was taken and an average taken to the nearest 0.1grams (gm). The scale was checked against a standard weight of two kilograms (kgs) at the beginning of each day and calibrated to zero.

Length: The length was measured with the help of an assistant using a stadiometer. Three supine measurements was taken and the average recorded to the nearest 0.1 centimeter (cm).

Head circumference: The head circumference was measured using a tape measure. Three occipitofrontal circumference measurements was taken and the average recorded to the nearest 0.1cm.

Fluid regimens given, infusions, transfusions, and medications given were recorded.

Appendix VII: Study budget

Category	Remarks	Units	Unit cost (Kshs)	Total (Kshs)
Proposal development	Computer services	70hrs	1	5,000
	Printing drafts	4500pgs	25	11,250
	Proposal copies	600pgs	15	9,000
	Binding	10	500	5,000
Data collection	Training day	1 day		10,000
	2 research assistants	40 days	1000	80,000
Study materials	Study questionnaire	500pgs	10	5,000
	Other stationery	20	50	1000
Study equipment	Urinary catheters	70	300	21,000
	Urine collecting bags	70	200	14,000
Laboratory tests	FENa	70	2400	168,000
	Creatinine serum	70	600	42,000
Data analysis	Statistician			30,000
Thesis write up	Comp services	50hrs		5,000
	Printing thesis	200pgs	25	5,000
	Binding	10	500	5,000
Contingency				50,000
Total				**466,250**

Appendix VIII: Consent form for parents / guardians of participants

TITLE: FRACTIONAL EXCRETION OF SODIUM AS AN EARLY PREDICTOR OF AKUTE KIDNEY INJURY IN TERM NEONATES WITH PERINATAL ASPHYXIA AT KENYATTA NATIONAL HOSPITAL

SCOPE: This informed consent is for enrolled participants in the study, and was read to them by a qualified research assistant before answering the questionnaire.

SERIAL NO: DATE: SITE:

Investigator: Dr. Esther M. Njiru,
Tel 0721-363535
Email: magaks8@gmail.com
Address- 87822 Mombasa, Kenya

Supervisor: Dr. Bashir Admani,
Tel 0721967818
Email: pedbashir@yahoo.com

Part 1: Information Sheet

Introduction : you are being invited to take part in this study to assess fractional excretion of sodium as an early predictor of acute kidney injury in term neonates with perinatal asphyxia at the Kenyatta National Hospital's Newborn Unit.

Perinatal asphyxia is a condition resulting from your new born baby failing to breathe immediately after birth. This problem may be associated with injury to the kidney and its early detection will help early initiation of appropriate care.

If you do not understand any words, kindly stop the research assistant as you go through the information and they will take time to explain. In case of later queries, the investigator is available on phone any time.

Purpose of the study: This study primarily aims to determine whether the amount of sodium that your baby passes in urine can be used to find out early whether your baby is likely to develop kidney injury as a result of being unable to breathe on their own immediately after birth. This information will help us in making certain recommendations regarding service provision so as to improve health care.

Procedures**:** You were provided with a questionnaire. The questionnaire can be answered individually, or the questions read out to you as you provide the interviewer with the appropriate response. You may skip any question you wish not to answer. The information is confidential, no name will be written on the forms, only an identification number and only the research investigators have access to your details.

Sampling**:** A urine sample will be collected from your baby by sterile insertion of a catheter which will then be removed after the sample is obtained. A blood sample will be collected from

your baby as well within 24 hours of birth and again at the third day of life to measure the functions of the kidney.

Risks**:** The specimens will be collected with utmost care to ensure that your child does not develop infection in the bladder. The procedure to obtain the blood specimen will be slightly uncomfortable for your baby. If possible the parent/ guardian or nurse will massage and talk to the baby to distract from pain. If the child is able to breastfeed this was encouraged, or child placed at skin to skin contact with the parent.

Benefits: Your baby will benefit from improved quality of services provided at the newborn unit as a result of this study. You will not cater for the costs of the laboratory tests done.

Voluntariness: The study is fully voluntary. There is no financial rewards to your child for participating in this study. One is free to participate or withdraw from the study at any point. Refusal to participate will not affect the care given to your child.

Confidentiality: All information collected will be kept in strict confidence. No information regarding you, your child or your family will be released to any person without your written permission. Information will be shared by clinical teams taking care of your child, so that your child can benefit from the possible interventions available.

Sharing results: We shall share the information that we get from this research with you. Results will be published at completion of the study so that other interested people may benefit from the research. However, we will not reveal the identity of your child during the publication of results.

Who to contact: If you have any questions about the study or the results, you can contact the principal investigator, **Dr. Esther M. Njiru on 0721363535**

Any question on your rights as a research participant can be addressed by contacting **Kenyatta National Hospital Ethics and Research Committee by calling (254-020) 2726300 Ext 44355**

Part II: Certificate of consent.

I, _____ guardian of _____(name of child) has had the information explained to me.

I AGREE/DISAGREE (cross out as appropriate) to participate/for my child to take part in the study.

I understand that our participation is fully voluntary and that I can withdraw /withdraw my child from the study at any point and this will not affect me/ my child's care in any way. I have been

given adequate opportunity to ask questions and seek clarification on the study and these have been addressed satisfactorily.

Participant/Guardian's signature: _____ Date: _____

I, _____ declare that I have adequately explained to the above participant/guardian the study procedure and risks and given him/her time to ask questions and seek clarification regarding the study. I have answered the questions raised to the best of my ability.

Investigator's signature: _____ Date: _____

Only necessary if the parent/guardian cannot read:

I attest that the information concerning this research was accurately explained to and understood by the caregiver and that informed consent was freely given by the parent/guardian.

Witness' signature: _____ Date: _____

Witness' name: _____ Time:_____

FOMU YA KUPATA KIBALI CHA WAZAZI / WALEZI WA WASHIRIKI

Kifunguo: Hii fomu ya kupata idhini ni kwa ajili ya watoto waliolazwa katika Hospitali ya Taifa ya Kenyatta, ambao tunuwakaribisha kushiriki katika utafiti. Jina la mradi wa utafiti wetu ni "Upimaji wa sehemu ya sodiamu kama ishara ya mapema ya kuumia papo hapo figo katika watoto wanaozaliwa na ukosefu wa hewa"

Mimi ni Dk. Esther Njiru, mwanafunzi katika Chuo Kikuu cha Nairobi kutafuta masomo ya utaalamu katika afya ya watoto. Mimi ninafanya utafiti juu ya ugonjwa wa kupumua kwa watoto ambao wamekishazaliwa kwa muda usiozidi siku moja ambayo ni kawaida sana katika nchi hii. Nitakupa taarifa na kukukaribisha kwa utafiti huu.

Kunaweza kuwa na baadhi ya maneno ambayo huelewi. Tafadhali uliza na mimi nitachukua muda kueleza. Kama una maswali baadaye, unaweza bado kuniuliza

Sababu ya utafiti: Ugonjwa wa kupumua unaweza kusababisha magojwa mengine kwa kila kiungo kwa watoto ambao wamekishazaliwa. Utafiti huu utazingatia ugonjwa wa figo kwa hawa watoto ambao wamekishazaliwa na shida ya kupumua. Ugonjwa huu husababaisha maafa mengi kwa watoto wachanga ndiposa umuhimu wa kuuangaza mapema na kuutibu mapema.

Maandalizi ya utafiti: Utafiti huu utahusu kupima damu na makojoo kwa mtoto wako ili kuweza kupata ugonjwa wa figo mapema na kuitibu mapema. Utafiti utafanyiwa kwa njia ya kupitia mahojiano ya moja kwa moja.

Kushiriki katika utafiti huu ni wa hiari kabisa. Bado utapokea huduma zote katika hospitali hii hata usipochagua kushiriki. Unaweza kujiondoa katika utafiti huu wakati wowote.

Utafiti utafanyika katika kipindi cha siku tatu za kwanza za maisha ya mtoto wako. Wakati huo, tutathmini afya ya mtoto wako kila siku.

Maadhara: Tutahakikisha usafi ili mtoto wako asipate maambukizi kwenye mkojo na tutajaribu iwezekanavyo kuzuia motto asihisi uchungu.

Habari kukuhusu ambayo tutakusanya kutoka mradi wa utafiti huu utakuwa siri.

Mawasiliano: Maswali yoyote yanaweza ulizwa hivi sasa au baadaye, hata pia baada ya utafiti imeanza. Baadaye ukitaka kuuliza maswali, wasiliana nami Esther M. Njiru kupitia nambari hizi: 0721363535.

Nimesoma/ Nimesomewa maelezo haya na nimepewa nafasi ya kuuliza maswali kuhusu hayo maelezo. Nimeidhini kushiriki katika utafiti huu kwa hiari.

Jina lako_____

Sahihi ya Mshiriki _____

Tarehe_____

Nina uhakika kuwa nimemsomea mwakilishi fomu hii, nilihakikisha kwamba mshiriki ameelewa.

Nilithibitisha kuwa mshiriki alipewa nafasi ya kuuliza maswali kuhusu utafiti nakuyajibu vema kwa kadri ya uwezo wangu. Mimi nathibitisha kwamba mwakilishi hakulazimishwa kutoa kibali

Jina la Mtafiti / Mtu kuchukua kibali_____