PLASMA AND URINE NEUTROPHIL GELATINASE ASSOCIATED LIPOCALIN LEVELS AS MARKERS OF ACUTE KIDNEY INJURY IN CRITICALLY ILL CHILDREN AGED 1-12 YEARS AT KENYATTA NATIONAL HOSPITAL (NAIROBI).

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H58/90010/2016

A dissertation submitted to the University of Nairobi, Department of Human Pathology for examination in partial fulfillment for the award of Master of Science degree in Clinical Chemistry.
ACKNOWLEDGEMENTS

Our appreciations go to the management of Kenyatta National Hospital (KNH) and the department of research and programs for facilitating and funding this study. I thank my supervisors, Prof. Angela Amayo, Dr. Francis Ndiang’ui, Dr. Zakayo Thaimuta and Dr. Christine Gichuhi for their guidance and support throughout the study period. I wish to acknowledge Prof. Christine Kigondu for the immense support in conceptualizing this study. I acknowledge the staffs at the KNH Pediatrics and Laboratory Medicine departments especially biochemistry laboratory led by Mr. Leonard Bosco Otieno and renal laboratory led by Mr. Wilfred Gatua, not forgetting Audrey and Mr. Henry Barasa of KPCC laboratory for their support. I acknowledge Mr. James Kiambuthi of Lued E.A and my classmates’ Ignatius Wanyonyi, Lewis Mutinda, Annette Irari and Joyce Maghanga for their support and encouragement.

God bless you.
DEDICATION

To all that have natured me to grow both physically and academically, my wife Christine Osale and our lovely son Ayden Dylan Kipkorir
DECLARATION

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OPERATIONAL DEFINITIONS

**Biomarker:** a measurable substance in an organism whose presence is indicative of some phenomenon such as disease, infection, or environmental exposure.

**Nephrotoxic:** damaging or destructive to the kidneys

**Acute:** an illness that is of short duration, rapidly progressive, and in need of urgent care.

**Pathophysiology:** the functional changes that accompany a syndrome or disease.

**Creatinine:** a chemical waste molecule that is generated from muscle metabolism

**Neutrophil:** a type of white blood cell, a granulocyte that is filled with microscopic granules, little sacs containing enzymes that digest microorganisms

**Lipocalins:** a family of proteins which transport small hydrophobic molecules such as steroids, bilins, retinoid, and lipids.

**Pediatric:** a branch of medicine dealing with the development, care, and diseases of infants, children, and adolescents.

**Critically ill:** patients whose conditions are life-threatening and who require comprehensive care and constant monitoring.
LIST OF ABBREVIATION

ADQI - Acute Dialysis Quality Initiative
AKI - Acute Kidney Injury
ATN - Acute Tubular Necrosis
BUN - Blood Urea Nitrogen
CV_A – Coefficient of variation analytical
CV_I - Coefficient of variation intra individual
eGFR - Estimated Glomerular Filtration Rate
GFR - glomerular filtration rate
KDIGO – Kidney disease improving global outcomes
KNH - Kenyatta National Hospital
LOQ – Lower limit of quantitation
NGAL- Neutrophil gelatinase associated lipocalin
uNGAL- urine Neutrophil gelatinase associated lipocalin
PICU - Pediatric Intensive Care Unit
RIFLE - Risk-Injury-Failure-Loss-End stage kidney disease
sCr - Serum Creatinine
U.O – Urine output
RRT - Renal Replacement Therapy
# TABLE OF CONTENT

**Contents**

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>3</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>4</td>
</tr>
<tr>
<td>OPERATIONAL DEFINITIONS</td>
<td>5</td>
</tr>
<tr>
<td>LIST OF ABBREVIATION</td>
<td>6</td>
</tr>
<tr>
<td>ABSTRACT                                     ..................................................................</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>12</td>
</tr>
<tr>
<td>1.0 Background</td>
<td>12</td>
</tr>
<tr>
<td>1.1 Problem Statement</td>
<td>12</td>
</tr>
<tr>
<td>1.2 Justification</td>
<td>13</td>
</tr>
<tr>
<td>1.3 Study Question</td>
<td>13</td>
</tr>
<tr>
<td>1.4 Study Objectives</td>
<td>13</td>
</tr>
<tr>
<td>1.4.1 Broad Objective</td>
<td>13</td>
</tr>
<tr>
<td>1.4.2 Specific Objectives:</td>
<td>13</td>
</tr>
<tr>
<td>1.4.3 Secondary objectives:</td>
<td>13</td>
</tr>
<tr>
<td>1.5 CONCEPTUAL FRAMEWORK</td>
<td>14</td>
</tr>
<tr>
<td>1.5.1 Study variables</td>
<td>14</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>15</td>
</tr>
<tr>
<td>2.0 LITERATURE REVIEW</td>
<td>15</td>
</tr>
<tr>
<td>2.1 Introduction</td>
<td>15</td>
</tr>
<tr>
<td>2.2 Terminology and definitions</td>
<td>15</td>
</tr>
<tr>
<td>2.3 Pathophysiology of AKI</td>
<td>17</td>
</tr>
<tr>
<td>2.4 Epidemiology of AKI</td>
<td>18</td>
</tr>
<tr>
<td>2.5 Diagnosis of AKI</td>
<td>19</td>
</tr>
<tr>
<td>2.6 Creatinine as a marker kidney function</td>
<td>19</td>
</tr>
<tr>
<td>2.7 AKI biomarkers</td>
<td>21</td>
</tr>
<tr>
<td>2.7.1 Neutrophil gelatinase-associated lipocalin (NGAL)</td>
<td>21</td>
</tr>
<tr>
<td>2.7.2 Molecular characteristic and Cellular Sources of NGAL in Humans</td>
<td>21</td>
</tr>
<tr>
<td>2.7.3 NGAL in Acute Kidney Injury</td>
<td>22</td>
</tr>
<tr>
<td>CHAPTER THREE</td>
<td>24</td>
</tr>
<tr>
<td>3.0 MATERIALS AND METHODS</td>
<td>24</td>
</tr>
<tr>
<td>3.1.1 Study Design</td>
<td>24</td>
</tr>
<tr>
<td>3.1.2 Study area</td>
<td>24</td>
</tr>
<tr>
<td>3.1.3 Study population</td>
<td>24</td>
</tr>
<tr>
<td>3.1.4 Inclusion criteria</td>
<td>24</td>
</tr>
</tbody>
</table>
LIST OF TABLES AND FIGURES

Table 1: KDIGO definition and classification .............................................................16
Flow chart 1: Admission/0 hr. blood sample collection and processing .................. 25
Flow chart 2: Admission/0 hr. urine sample collection and processing ................. 26
Flow chart 3: 48-hour post admission blood sample collection and processing .........26
Table 2: Demographic Characteristics of study subjects ...........................................30
Table 3: Location and diagnosis of study subjects ....................................................30
Table 4: Validation results for NGAL ......................................................................31
Table 5: Laboratory parameters findings .................................................................32
Table 6: Prevalence of AKI .....................................................................................33
Table 7: Urine NGAL sensitivity and specificity .......................................................33
Table 8: Urine NGAL sensitivity and specificity .......................................................34
Table 9: Prevalence regression statistic ..................................................................34
Figure 1: Correlation plot between Plasma creatinine and Plasma NGAL ............35
Figure 2: Correlation plot between Plasma creatinine and Urine NGAL ...............36
Figure 3: Correlation Plot between Urine and Plasma NGAL ...............................36
Table 10: Correlation analysis ...............................................................................39
Figure 4: correlation between absolute neutrophil counts with plasma NGAL .......38
Table 11: Correlation between plasma NGAL and absolute neutrophil counts .......39
Table 11: NGAL Regression Analysis .....................................................................40
ABSTRACT

Background

Acute kidney injury (AKI) refers to a form of kidney dysfunction that rapidly develops within 7 days or less, the resulting complications being metabolic, systemic and death in the extreme. Kidney damage in AKI is due to ischemia, exposure to toxic substances, inflammatory processes or obstruction of the urinary tract. Critically ill children are susceptible to AKI, incidences range from 1 to 31%. Serum creatinine levels and/or urine output are required to diagnose AKI. In critically ill patient’s population serum creatinine and urine values are considered insensitive and additional tools are required to diagnose AKI. Several AKI biomarkers have been fronted with Neutrophil Gelatinase Associated Lipocalin (NGAL) receiving more attention, the goal is to maximize the opportunity for early identification of AKI and intervention which translates to favorable outcomes.

Objective

To evaluate the potential of plasma NGAL and urine NGAL as a marker of AKI in critically ill children aged 1 - 12 years at KNH.

Methodology

This was a prospective cross-sectional study carried out in a population of critically ill children aged 1 -12 years admitted at Kenyatta National Hospital, pediatric unit. Forty-eight study participants were enrolled, urine and blood samples were obtained from study subjects on admission and another blood sample was collected 48 hours post admission. Admission and 48hour post admission plasma creatinine was estimated using Dirui reagents (Dirui industrial company Shenzhen, China) Admission plasma and urine NGAL were analyzed using an NGAL kit (Bio Porto Diagnostics A/S, Denmark). All analyses were done on Biolis 50i (Tokyo Boeki Japan) chemistry analyzer. Data was analyzed using STATA statistical package and included both descriptive and inferential analysis.
Results

A total of 40 subjects completed the study. Majority of the participants (75%) were aged between 1 and 3 years, most (56%) were females. Seventy-one percent (n=34) of all the admissions had respiratory tract infection as the underlying pathology. Nine participants met the criteria for AKI giving a prevalence of AKI among critically ill children at KNH to be 28.5%, 95%CI [12.8, 41.8] based on plasma creatinine, as well as plasma and urine NGAL. A strong positive and significant correlation between Plasma and Urine NGAL (r = 0.869) was evident. Further statistical analysis showed that Plasma ($p = 0.029$) and Urine NGAL ($p = 0.000$) are statistically significant markers of AKI in critically ill children aged 1-12 years.

Conclusion

Plasma NGAL and Urine NGAL can detect AKI and can predict up to 34.7% of the AKI in critically children.
CHAPTER ONE

1.0 Background
Acute kidney injury (AKI) refers to a form of kidney dysfunction that rapidly develops within 7 days often resulting in metabolic and or systemic complications and in worse cases, death.\(^1\) Future risk of chronic kidney disease (CKD) is higher in patients who suffer any form of AKI. Damage to the kidney tissue is attributed to ischemia, exposure to nephrotoxic substances, inflammatory processes or an obstruction of the urinary tract.\(^8\) AKI diagnosis in current practice relies on serum creatinine levels and/or urine output parameters.\(^6\)

Serum creatinine and urine values could be considered insensitive in certain clinical context and as a result additional tools are required to diagnose AKI; this is relevant in critically ill patient’s population. Advances in the field of proteomics and genomics have been utilized in the identification of several novel AKI biomarkers. These markers vary in origin (anatomically), physiological function, and time of release after a renal insult. Some of these biomarkers have the potential to shed light on the underlying etiology and staging of the pathophysiological processes in AKI. NGAL is one of the most studied biomarkers for early detection of AKI, it maximizes on the opportunity for early intervention translating to favorable outcomes.

1.1 Problem Statement
Acute kidney injury (AKI) is common; it is present in 5% of hospital admissions and up to 60% in critical care patients.\(^9,12,13\) Reports indicate that the incidence is on the increase globally, it is associated with poor short-term clinical outcomes including longer hospitalizations, and adverse long-term outcomes, i.e. chronic kidney disease (CKD) with its attendant complications. Early diagnosis and appropriate intervention significantly reduce the risk of complications. This is however difficult using clinical and current diagnostic markers, Newer biomarkers have potential for early detection of AKI which would lead to timely intervention and better outcomes.\(^9,12,13\).
1.2 Justification

A study conducted in our setting revealed that urine NGAL is a useful early marker of AKI in neonates. There are no local reports on the utility of plasma or urine NGAL in children above 1 month of age. This study intention was to explore effectiveness of urine and plasma NGAL for early detection of AKI in severely ill children admitted at KNH. It is expected that findings from this study will aid in the establishment of new diagnostic algorithms for AKI to improve outcomes in children.

1.3 Study Question

Does plasma and urine NGAL aid in early detection of AKI?

1.4 Study Objectives

1.4.1 Broad Objective

To evaluate the potential of plasma and urine NGAL to detect acute kidney injury in critically ill children aged between 1 and 12 years at KNH

1.4.2 Specific Objectives:

1. To determine plasma creatinine at admission and 48 hours post admission.
2. To determine plasma NGAL and urine NGAL at admission.
3. To determine the prevalence of AKI based on plasma creatinine, plasma NGAL and urine NGAL,
4. To correlate the prevalence of AKI based on plasma creatinine, plasma NGAL and urine NGAL.

1.4.3 Secondary objectives:

1. To examine the absolute neutrophil count results on admission.
2. To correlate absolute neutrophil counts with plasma NGAL.
1.5 CONCEPTUAL FRAMEWORK

1.5.1 Study variables

a) Independent variables

Demographic data (age, gender), clinical diagnosis, admission location.

b) Dependent variables

Laboratory parameters (plasma creatinine, plasma NGAL, urine NGAL, and absolute neutrophil count).

1.5.2 Sample size determination

Cochran’s formula for a representative sample for proportions was used to estimate the sample size:\(^46\):

\[
n = z^2 \frac{p(1-p)}{e^2}
\]

Where: \(n\) is the sample size, \(Z^2\) is the desired confidence level (95%), \(e\) is the margin of error, \(p\) is the estimated prevalence.

Using \(z=1.96\), \(p=85.5\)\(^{17}\) and \(e=10\)%\(^{45}\)\)

The sample size was estimated to be 48 children.

1.5.3 Sampling method

The recruitment method was simple random sampling of children aged between 1 and 12 years admitted in critical condition at pediatric intensive care unit (PICU) and pediatric ward acute rooms. The principle investigator assessed the medical records of the potential study participants to establish eligibility. For the eligible study participant consent was sought from the parent or guardian, once consent was given urine and samples were collected appropriately. Blood sample was also obtained from the participants 48 hours post admission.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Introduction

AKI is a clinical syndrome characterized by an abrupt decline in kidney function, it accounts for both injury and impairment of the kidney functions. AKI is a syndrome of mixed etiology sepsis, ischemia and nephrotoxicity often co-exist complicating its diagnosis and management.

AKI is categorized as; pre-renal, intrinsic and post-renal. The ‘intrinsic’ AKI represents a true kidney dysfunction, whereas the other two AKI categories are outcomes of secondary renal pathologies that results to a fall in GFR. Unresolved pre- and post-renal conditions, mostly transform to ‘intrinsic’ AKI.¹

2.2 Terminology and definitions

William McNider was the first person to coin the term Acute Kidney Injury (AKI) in 1918. AKI became the adopted term in 2004 when acute renal failure (ARF) was redefined.¹

Formal agreement on the definition and diagnostic criteria for AKI has been achieved through three consensus groups. The ADQI group, in 2002 adopted the RIFLE criteria ², thereafter succeeded by the AKIN classification published 2007 ⁴

Substantial number of publications have demonstrated that RIFLE and AKIN classifications are widely accepted but their utilization is diverse in the definition and classification of AKI.⁵ To enhance uniformity the KDIGO work group merged RIFLE and AKIN classifications to achieve a single classification (Table 1) for clinical, research and public health practice.⁶
Table 1

KDIGO definition and classification of AKI

*Diagnostic criteria for AKI:*

AKI is defined by fulfilling one of the following:

- Rise in serum creatinine levels by $\geq 26.5$ μmol/l within 48 hours; or
- Rise in serum creatinine levels to $\geq 1.5$ times the baseline (known or presumed to have occurred within the preceding 7 days); or
- Urine volume of $<0.5$ ml/kg/h for at least 6 hours.

*AKI staging:*

<table>
<thead>
<tr>
<th>STAGE</th>
<th>Creatinine criteria</th>
<th>Urine output criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Elevation of serum creatinine by $\geq 26.4$ μmol/L</td>
<td>Urine output of $&lt;0.5$ ml/kg/h for a period of 6–12 hours</td>
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<td></td>
<td>Or</td>
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<tr>
<td></td>
<td>rise to 1.5–1.9 times from baseline</td>
<td></td>
</tr>
<tr>
<td>Stage II</td>
<td>A rise of serum creatinine to 2.0–2.9 times from baseline</td>
<td>Urine output of $&lt;0.5$ ml/kg/h for $\geq 12$ hours</td>
</tr>
<tr>
<td>Stage III</td>
<td>A rise of serum creatinine to $\geq 3.0$ times from baseline</td>
<td>Urine output of $&lt;0.3$ ml/kg/h for $\geq 24$ h</td>
</tr>
<tr>
<td></td>
<td>Or</td>
<td>Or</td>
</tr>
<tr>
<td></td>
<td>serum creatinine levels of $\geq 354$ μmol/L</td>
<td>anuria for $\geq 12$ hours</td>
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AKI acute kidney injury, KDIGO Kidney Disease Improving Global Outcomes, (Adopted from the KDIGO practice guidelines)
2.3 Pathophysiology of AKI

AKI refers to a clinical syndrome that arises when renal function is acutely decreased. Consequently, accumulation of uremic waste products occurs, and the body becomes incompetent in maintaining electrolyte, water and acid-base balance.\textsuperscript{7}

The pathophysiology of AKI is multifactorial and complex. Ischemia is atypical cause of AKI and is due to several etiologies. In response to ischemia the physiological adaptations of the kidney, can compensate up to a certain degree, if oxygen and substrates required for cellular metabolism are inadequate, cellular injury occurs leading to organ dysfunction.

The kidney as an organ is predisposed to cellular injury related to ischemia basically due to anatomic and structural association of the renal tubules and blood vessels in the medulla of the kidney.\textsuperscript{8} For optimum kidney function adequate kidney perfusion is mandatory and any alteration will lead, to a certain degree of cell injury and in severe cases it can lead to cell death.

An ischemic episode has direct effect to the entire nephron, especially the proximal tubular cells that are susceptible to injury. Intrinsic functions of the nephron are affected during an ischemic episode resulting in accumulation of toxic substances that lead to cell injury and death. AKI is also common in sepsis.
2.4 Epidemiology of AKI

Absence of standard definition of AKI has impacted negatively on incidence reporting. Reported incidence of AKI in most cases is dependent on the context of the epidemiological study i.e. definition criteria, population under study and/or geographical area of study.9

The incidence and the causes of AKI in developing and developed countries differ. Lameire et al in their review demonstrated the similarities and discrepancies in the reported incidence, etiology, pathophysiology, and its implications to public health across the globe.10

In developing countries, the main causes can either be hospital acquired i.e. renal ischemia, sepsis and nephrotoxic drugs in urban settings or community acquired i.e. diarrhea, dehydration and infectious diseases in the rural settings. AKI under-reporting still remain as one of the major impediment in understanding it true impact across the world.11

Although the etiology of AKI in the developed countries is not exclusively described, it occurs in approximately 15% in hospital admissions; the prevalence in critical care patients is estimated to be as high as 60%.9,12,13

Pediatric AKI is still inadequately described, epidemiological studies focusing in this population is still lacking, most studies conducted have focused on single centers and special groups in this population and therefore unlikely to give the true reflection of AKI pediatrics.14,15

A study carried out in the US, indicated that AKI occurred in 3.9 / 1000 of hospitalized children.15 Kaddourah et al reported a pooled prevalence of 26.9% in critically ill children and young adults.16 This study was earmarked as a valid benchmark for other studies based on the sample size used and its multinational composition.

Muithya et al found the prevalence rate of AKI to be 85.5% in their study that enrolled 117 critically ill children at Kenyatta National Hospital 17
2.5 Diagnosis of AKI

AKI diagnostic workup varies depending mostly on availability of diagnostic tools, clinical context, severity and suspected time span of AKI.\(^{18}\)

AKI is considered present if there is an increase of serum creatinine levels by $\geq 26.5$ $\mu$mol/l within 48 hours or a rise of at least 1.5 times from baseline observed in a period of 7 days,\(^{6}\) staging relies on variation in creatinine levels or urine output.

The use of creatinine and urine parameters is challenging and unreliable, mainly because these are markers of excretory function of the kidney. They are not kidney specific and their interpretation should always be in view of the clinical context, occasionally AKI definition is fulfilled but AKI is not evident clinically.\(^{18}\)

2.6 Creatinine as a marker kidney function

Creatinine, a metabolite of creatine is a by-product of muscle metabolic processes. Creatine is synthesized \textit{de novo} in the liver and kidneys from glycine and arginine and stored in the skeletal muscle. In the skeletal muscle creatine, is converted to phosphocreatine, a mobilizable energy reserve. Another source of creatine is dietary meat intake.\(^{19}\)

Gross creatinine generation from creatine is dependent on muscular activity, dietary meat intake and \textit{de novo} generation. Physiologically creatinine production is constant, thus serum creatinine concentration marks equilibrium between production and excretion.\(^{19}\)

The glomerulus filters creatinine freely without reabsorption or metabolism, tubular secretion also occurs. Based on this it is utilized as an indicator of kidney function.\(^{19}\)

Laboratory measurement of serum creatine is relatively cheap and is one of the routinely measured analytes in clinical chemistry across the world. The analytical measurement of creatinine is subject to several errors, interferences and imprecision.\(^{19, 20}\)

The limitations of creatinine measurement can be broadly divided into physiologic and analytical limitation.

Physiologic limitations of creatinine measurement include but not limited to the following: serum creatinine levels are dependent on glomerular filtration rate (GFR), muscular
metabolic processes, secretion by renal tubules and protein rich diet such as cooked red meat. Drugs e.g. trimethoprim and cimetidine also interfere with tubular secretion of creatinine.\textsuperscript{19}

The analytical limitations of creatinine measurement involve the analytical method, which can either be enzymatic or Jaffe methods.

In Jaffe methods, the colorimetric quantification is made from creatinine and picrate reaction products. This reaction has low specificity since picrate can also react with other pseudo-chromogens present in the serum matrix.\textsuperscript{19, 20, 21} The Jaffe assays are open to non-specific interactions with bilirubin and some drugs.\textsuperscript{22} These challenges have prompted several technical improvements to increase the precision of the Jaffe assays\textsuperscript{19}

Enzymatic assays are based on enzymatic reactions.\textsuperscript{20} Enzymatic assays have better analytical specificity, the sensitivity, especially at low creatinine concentrations as often seen in pediatric population.\textsuperscript{22} Improved precision of enzymatic assays, at lower creatinine concentrations makes it a suitable method for the pediatrics, and when Jaffe assays is deemed subject to interferences by pseudo chromogens. The performance of both assays at high creatinine ranges is comparable.\textsuperscript{19}

Despite recommendations by the creatinine standardization program for manufacturers to standardize their creatinine assays to a traceable standard measurement procedure to minimize systematic error inherent to lack of calibration, independent studies have demonstrated that the precision is still not very good especially at lower creatinine concentrations.\textsuperscript{23} The other challenge in creatinine measurement is random error or imprecision, random error is expressed by the $CV_{A}$. Enzymatic assays have lower estimated random error of 2\% than jaffe assays 5.5\%.\textsuperscript{19, 22} The best way to reduce the $CV_{A}$ for a given assay is to use the mean of duplicate or triplicate runs which is impractical and expensive to perform.\textsuperscript{19} Biological variation expressed as intra-individual CV ($CV_I$) should be considered when interpreting the magnitude of the change in creatinine results changes between two consecutive results. For creatinine the westgard blog estimates the $CV_I$ of creatinine to be 5.95\%.\textsuperscript{19}
2.7 AKI biomarkers

It is appreciated that in some clinical scenarios additional tools are required to diagnose and manage AKI more specifically in situations where the use of creatinine and urine values is challenging or difficult to interpret. The search and validation of new AKI biomarkers with the goal of replacing or complementing creatinine as a marker of kidney injury has been progressive and promising. Several biomarkers have been identified so far, they are diverse in terms of anatomical origin, physiological role, and time of release after insult, kinetics, and distribution.

2.7.1 Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL is a novel 21-kD protein of the lipocalin superfamily. Lipocalins are proteins with eight β-strands forming β-barrel calyces. These calyces’ main function is to bind and transport molecules of low molecular weight.

NGAL is part innate immunity having antimicrobial properties; above its antimicrobial activity NGAL seems to have other intricate biologic activities. The expression of NGAL has been shown to rise to 1000-fold in humans and rodents as a result of renal tubular injury. This rise can be demonstrated in urine and serum samples and can be utilized as marker of kidney injury. NGAL has also been shown to have growth factor effects that regulates some cellular responses, like proliferation, apoptosis, and differentiation.

2.7.2 Molecular characteristic and Cellular Sources of NGAL in Humans

Two independent Scandinavian groups were the pioneers in the quest to purify human NGAL also known as lipocalin 2 from neutrophils granules. Further molecular characterization revealed that human NGAL molecule exists as a monomer (25-kDa), homodimer (45-kDa) heterodimer (135-kDa).

NGAL is synthesized in the bone marrow during myelopoiesis and reserved in the neutrophil granules. NGAL mRNA is also expressed in the colon, trachea, lungs and kidney epithelium in low concentrations. Interleukin-1β has shown the ability to induce NGAL synthesis in cell lines in vitro. Up regulation of NGAL is also observed in pathologic or
stressful conditions.\textsuperscript{29} Furthermore, several studies have expressed the ability of oxidative stress, cytokines, and other stimuli to up regulate NGAL expression in human cell lines.\textsuperscript{29}

The complex molecular structure of NGAL can be attributed to its cellular origin. Experiments have shown that the homodimeric form is predominantly released by activated neutrophils.\textsuperscript{27,29} Contrary, stressed kidney epithelial cells release monomeric NGAL which is structurally different from those produced by neutrophils and they are not capable of forming dimers.\textsuperscript{29}

\textbf{2.7.3 NGAL in Acute Kidney Injury}

Initially NGAL was under investigation as marker of undifferentiated systemic inflammation but focused shifted towards the kidney when genomic analyses showed dramatic up regulation of NGAL genes after ischemic AKI in animal models.\textsuperscript{30} Further analyses confirmed NGAL production in the kidneys after an ischemic and nephrotoxic insult to the kidney, urinary concentration rose several times shortly after the insult.\textsuperscript{27,29} Subsequently, consistent outcomes across several studies on association between levels plasma and urine NGAL with severity of established AKI in critically ill patients has been witnessed.\textsuperscript{27}

Among many potential biomarkers of AKI, NGAL has shown more potential through extensive experimental and clinical studies and seems to be the commonly investigated and favorable early marker of AKI.\textsuperscript{32-45}

NGAL investigation has been conducted across different clinical settings where patients are at risk of developing AKI\textsuperscript{31}, these studies have been conducted in both adult\textsuperscript{34,36} and pediatric populations\textsuperscript{32,35,37,38}.

NGAL concentration in plasma and urine quantification is achieved using “research-based” assays kits and standardized clinical laboratory platforms.\textsuperscript{31} Studies on NGAL have yielded contradictory findings on its robustness as a biomarker. To confront these concerns Hasse \textit{M} et \textit{al} \textsuperscript{31} carried out a meta-analysis to evaluate the accuracy of NGAL and identifying factors that influence its utility in AKI.

Some important points of discussion exist in literature, studies evaluating the predictive value of NGAL, show significant variations in AKI definition, AKI clinical setting, and timing of NGAL measurement, these factors have generated significant modifiers that cloud
its utility as a marker of AKI. Most importantly a standardized cutoff NGAL concentration that defines AKI has not been published.  

Hasse M et al\textsuperscript{31} observed that NGAL level can serve as valuable early markers of AKI, in different clinical settings. Though it is important to note that in the studies included AKI diagnosis was based on serum creatinine concentration despite its shortcomings as a marker.

The performance of NGAL levels in serum, plasma and urine samples are comparable, they exhibit improved performance on standardized clinical laboratory platforms at $> 150$ ng/mL cutoff. However, $> 150$ ng/mL cut off is not validated and it might be necessary for each center to establish reference intervals and cutoff values to be used.

In a prospective study carried out at Pumwani Maternity Hospital and KNH, showed that uNGAL employing a cut-off value of 250ng/ml demonstrated the ability to predict AKI (AUC 0.724). u NGAL values were higher in AKI positive neonates.\textsuperscript{45}

NGAL predictive value for AKI in adults is low when compared to pediatric population. This could be due to the influence of co morbidities, sources of NGAL, and pathophysiology of AKI.\textsuperscript{31}

NGAL is a valuable tool in AKI diagnosis and prognosis, in order to have a more robust laboratory implementation more studies employing standardized assays are required with the expectations that diagnostic performance and utility of NGAL will improve soon.\textsuperscript{31}
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Methodology

3.1.1 Study Design
This was a prospective cross-sectional study carried out in a population of children aged between 1 and 12 years admitted at KNH pediatric unit in critical condition.

3.1.2 Study area
This study was carried out at KNH from the month of March 2018 through to July 2018. The clinical phase of the study was carried out at the pediatric department, the study sites were pediatric wards acute rooms and pediatric intensive care unit (PICU). The laboratory analytical phase was conducted at the biochemistry and renal unit laboratories.

3.1.3 Study population
Participants in the study were children aged between 1 and 12 years who were assessed by the clinician and admitted in critical condition to pediatric intensive care unit (PICU) and pediatric ward acute rooms during the study period.

3.1.4 Inclusion criteria
All children aged between 1 and 12 years, admitted as critically ill by the medical practitioners and whose parent or guardian gave and filled informed consent form (Appendix I), were included in the study.

3.1.5 Exclusion criteria
Children with established kidney impairment at the time of admission and all post-surgical cases were excluded from the study.

3.1.6 Case definition
Critical illness was defined according to WHO definition as any severe condition affecting the airway, breathing or circulation, or a rapid deterioration of conscious state in a child.
3.2 LABORATORY METHODS

3.2.1 Specimen Collection for laboratory analysis

Flow chart 1: Admission/0 hr. blood sample collection and processing

2ml of venus blood sample was drawn and transferred to lithium heparin tubes and labeled appropriately.

The samples were delivered to the laboratory, spun at 3000 rpm for 3 minutes, plasma was aspirated and transferred into 2 plasma vials.

Plasma creatinine was analysed from one of the vials and the results recorded.

NGAL was analysed on the second stored vial and results were recorded.

The second plasma vial was stored at -20 °C awaiting NGAL analysis.
Flow chart 2: Admission/0 hr. urine sample collection and processing

Catheterised participants

<table>
<thead>
<tr>
<th>5mls of urine sample was drawn from the catheter and transferred into a sterile urine container and labelled appropriately</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upon delivery to the laboratory 1 ml of urine sample was transferred into microvials, labelled and stored at -20°C awaiting NGAL analysis</td>
</tr>
</tbody>
</table>

Non catheterised participants

<table>
<thead>
<tr>
<th>Pediatric urine collecting bags was used to collect urine sample, the samples were then transferred into sterile urine containers and labelled appropriately</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upon delivery to the laboratory 1 ml of urine sample was transferred into microvials, labelled and stored at -20°C awaiting NGAL analysis</td>
</tr>
</tbody>
</table>

Flow chart 3: 48-hour post admission blood sample collection, processing and analysis

<table>
<thead>
<tr>
<th>1 ml of venous blood was collected, transferred into lithium heparin tubes and labelled appropriately</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upon delivery to the laboratory the samples were spun at 3000rpm for 3 minutes, the plasma was transferred into sample cups and labeled appropriately</td>
</tr>
<tr>
<td>The samples were analysed for creatinine concentration and the results recorded</td>
</tr>
</tbody>
</table>
3.3 Laboratory analysis

Creatinine analysis

Plasma creatinine levels for both admission and 48-hour samples were analyzed using Dirui reagents based on Jaffe principle (Annex 2), on the Biolis 50i (Tokyo Boeki Japan) automated chemistry analyzer located at the biochemistry laboratory, Kenyatta national hospital.

NGAL method verification experiment

NGAL was subjected to method verification experiments, the parameters verified were accuracy, precision, measurement range and lower limit of quantification.

- **Precision and Accuracy**

After a successful calibration of NGAL test on the Biolis 50i equipment, twenty runs on the low and high levels of controls were run and the data recorded. The data was analyzed to obtain the mean and coefficient of variation. Two levels of internal quality control (IQC) low and high were analyzed for the next four consecutive days to ascertain on the accuracy

- **Limit of quantification/Measurement range**

Dilution of the standards were made near the ranges stated by the manufacturer; twenty runs of the dilutions were analyzed to establish the range at which the test would give results with repeatability at both the low and high measurement range.

NGAL analysis

Batch analysis for Plasma and urine samples for NGAL levels was done after attaining a batch of the estimated sample size. The samples were thawed at room temperature for 30 minutes, after thawing was complete 200μl of sample (plasma/urine) was transferred into sample cups and labeled appropriately.

NGAL level estimation was done on the Biolis 50i (Tokyo Boeki Japan) automated chemistry analyzer located at Renal department laboratory Kenyatta national hospital using reagents from Bio Porto Diagnostics A/S, Denmark (Annex 3) based on particle enhance turbidimetric immunoassay.
3.4 Quality assurance

3.4.1 Creatinine
To ensure analytical quality was achieved two levels of Internal Quality Control (IQC) was conducted and met the acceptable criteria based on the westgard rules before the sample were analyzed. Quality assurance was enhanced further through the external quality assessment which the biochemistry laboratory participated and the performance for creatinine at the time of study was acceptable.

3.4.2 NGAL
Method verification was successfully conducted to confirm the manufactures’ claims. Two levels of internal quality control (IQC) were analyzed for 4 consecutive days and met the acceptable criteria based on westgard rules prior to the batch analysis of the samples.

3.4.3 Data
To minimize transcription errors, equipment print outs were used to transfer the data to the data collection form, the two were cross checked when transferring the data to Microsoft excel spread sheets before analysis.

3.5 Ethical Compliance
Ethical assent was granted by institutions ethics committee, KNH-UoN ERC (Annex 1)

3.6 Data management
Data was collected using data collection tool (Appendix 2) which was later transferred to Microsoft excel spread sheet. Quantitative data was summarized as a median (IQR). The nonparametric data was summarized in table form and interpretation made accordingly. Linear regression analysis was used to examine the correlation between the study variables.

The following criteria were used for identifying AKI:

1. A rise in creatinine levels by ≥26.5 μmol/l within 48 hours of admission. \(^1\)
2. A cut-off value for serum NGAL of 117.5 ng/mL was applied. \(^76\)
3. Urine NGAL criteria applied cut-off value of 250 ng/ml. \(^77\)

The prevalence of AKI was calculated using the following formula:

\[
\text{Prevalence} = \frac{\text{Total number with AKI}}{\text{Total number studied}} \times 100
\]
CHAPTER FOUR

4.0 Results

The study included 48 participants where 40 participants successfully completed the study indicating 83.6% completion rate. Demographic data and baseline blood samples were obtained for all the 48 study subjects. Unfortunately, four (4) study subjects died before the 48-hour blood sample was collected so only 44 subjects had both baseline and 48-hour creatinine results as required. Urine NGAL results on admission were available for only 40 subjects because 2 study participants were anuric at admission and remained anuric for 12 hours. Six (6) study subjects urine samples were contaminated by fecal matter during the process of sample collection and were unsuitable for NGAL analysis.

4.1 Characteristics of study participants

4.1.1 Demographic Characteristics

Among all the participants recruited (N=48) for the study, most of participants were female 56% (n = 27), than the male gender 44 % (n = 21), On the age distribution the majority of the participants were aged between 1 and 3 years with 72.9% (n = 35), the age group between 4 and 6 years were 12.5% (n = 6), age group between 7 and 9 years were 8.3% (n = 4) while age group between 10 and 12 years 6.3% (n=3). These are summarized in Table 2 below.
Table 2: Demographic characteristics of study participants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>N=48</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21</td>
<td>(44)</td>
</tr>
<tr>
<td>Female</td>
<td>27</td>
<td>(56)</td>
</tr>
<tr>
<td><strong>Age (years)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-3</td>
<td>35</td>
<td>(72.9)</td>
</tr>
<tr>
<td>4-6</td>
<td>6</td>
<td>(12.5)</td>
</tr>
<tr>
<td>7-9</td>
<td>4</td>
<td>(8.3)</td>
</tr>
<tr>
<td>10-12</td>
<td>3</td>
<td>(6.3)</td>
</tr>
</tbody>
</table>

Table 3: Location and Clinical diagnosis

<table>
<thead>
<tr>
<th>Location by admission</th>
<th>Pediatric ward acute room</th>
<th>36 (75%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pediatric intensive care unit</td>
<td>12 (25%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clinical diagnosis N=48</th>
<th>Respiratory tract infection</th>
<th>19 (40)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sepsis/Septic shock</td>
<td>5 (10)</td>
</tr>
<tr>
<td></td>
<td>Convulsive disorders</td>
<td>3 (6)</td>
</tr>
<tr>
<td></td>
<td>Respiratory tract infection+ Other</td>
<td>15 (31)</td>
</tr>
<tr>
<td></td>
<td>Others</td>
<td>6 (13)</td>
</tr>
</tbody>
</table>

4.1.2 Location and Clinical diagnosis

As shown in Table 3, majority of the patients 75% (n=36) were admitted in the pediatric acute room while twenty five percent (n= 12) of the participants were admitted in PICU.

Respiratory tract infection was the commonest underlying pathology accounting for 70% of the acute care admissions.
4.2 Laboratory parameters

4.2.1 NGAL method verification

Table 3: NGAL method verification results

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Manufacturer claim on Hitachi platform</th>
<th>Results from Biolis 50i platform</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIGH QC</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>503 ng/ml</td>
<td>531.6 ng/ml</td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>22.9</td>
</tr>
<tr>
<td>CV</td>
<td>3.3</td>
<td>4.3</td>
</tr>
<tr>
<td>LOW QC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>202 ng/ml</td>
<td>221.1 ng/ml</td>
</tr>
<tr>
<td>SD</td>
<td>-</td>
<td>23.5</td>
</tr>
<tr>
<td>CV</td>
<td>3.3</td>
<td>10.6</td>
</tr>
<tr>
<td>Measurement range</td>
<td>25 -3000 ng/ml</td>
<td>75 - 2750 ng/ml</td>
</tr>
<tr>
<td>LOQ</td>
<td>&lt;25 ng/ml</td>
<td>&lt;75 ng/ml</td>
</tr>
</tbody>
</table>

Table 4 summarizes the results for NGAL method verification done to ascertain manufactures claim of the assay performance. The results indicate that the analytical performance of NGAL using the Biolis 50i equipment is comparable to the manufacturer’s parameters. The precision of NGAL at high concentration (CV 4.3) is better than its performance at low concentration (CV 10.6)
4.2.2 Results from analysis of participants samples.

Analysis of creatinine and NGAL were done on baseline (Time 0) of admission. Repeat blood creatinine was done on plasma samples collected after 48 hours post admission. Absolute neutrophil count values were examined at admission. The results were analyzed and presented in table 4 below.

Table 5: Laboratory findings data

<table>
<thead>
<tr>
<th>Laboratory parameter</th>
<th>Median (IQR)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hr. Creatinine, Median (IQR) N=48, Mean (SD)</td>
<td>59(25.8)</td>
<td>62.5(17.0)</td>
</tr>
<tr>
<td>48hr Creatinine Median (IQR) N=44, Mean (SD)</td>
<td>78(25.3)</td>
<td>87.7(35.0)</td>
</tr>
<tr>
<td>Plasma NGAL (0hr) Median (IQR) N=48, Mean (SD)</td>
<td>304.7(276.7)</td>
<td>365.2(253.5)</td>
</tr>
<tr>
<td>Urine NGAL (0hr) Median (IQR) N=40, Mean (SD)</td>
<td>525.6(627.0)</td>
<td>701(585.6)</td>
</tr>
<tr>
<td>Absolute neutrophil count Median (IQR) N=48, Mean (SD)</td>
<td>10.4(5.8)</td>
<td>10.8(5.6)</td>
</tr>
</tbody>
</table>

0 hour and 48hour creatinine laboratory values were normally distribution unlike urine and plasma NGAL which had a rather skewed distribution. The admission absolute neutrophil count values also had a skewed distribution.
4.3 Prevalence of AKI

Table 6. Prevalence of AKI

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>KDIGO Criteria (N=44) Creatinine increase of ≥26.5µmol/l within 48hrs</td>
<td>13/44 (29.5%)</td>
</tr>
<tr>
<td>Plasma NGAL cut off 117ng/ml N=48</td>
<td>41/48 (85.4%)</td>
</tr>
<tr>
<td>Urine NGAL Cut off value 250ng/ml N=40</td>
<td>36/40(90.0%)</td>
</tr>
</tbody>
</table>

The prevalence of AKI based on the three individual markers urine NGAL showed the highest (90.0%) Plasma NGAL (85.4%) the lowest was Creatinine with 29.5%.

Table 7. Urine NGAL sensitivity/specificity

<table>
<thead>
<tr>
<th>AKI based on urine NGAL</th>
<th>Test outcome positive</th>
<th>True positive (TP) = 10</th>
<th>False positive (FP) = 25</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test outcome negative</td>
<td>False negative (FN) = 0</td>
<td>True negative (TN) = 5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>= TP / (TP + FN)</td>
<td>= TN / (FP + TN)</td>
</tr>
<tr>
<td>= 10 / (10 + 0)</td>
<td>= 5 / (25+ 5)</td>
</tr>
<tr>
<td>≈ 100%</td>
<td>= 16%</td>
</tr>
</tbody>
</table>
Data from the study as summarized in the table 7 above indicate that urine NGAL had a sensitivity of 100% and a specificity of 16% using creatinine as a gold standard.

Table 8. Plasma NGAL sensitivity/specificity

<table>
<thead>
<tr>
<th>AKI BASED ON PLASMA NGAL</th>
<th>Test outcome positive</th>
<th>True positive (TP) = 12</th>
<th>False positive (FP) = 26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test outcome negative</td>
<td>False negative (FN) =2</td>
<td>True negative (TN) = 4</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity = $\frac{TP}{TP + FN}$
= $\frac{12}{12 + 2}$
≈ 85.7%

Specificity = $\frac{TN}{FP + TN}$
= $\frac{4}{26 + 4}$
= 13.3%

Data from the study as summarized in the table 8 above indicate that plasma NGAL had a sensitivity of 85.7% and a specificity of 13.3% using creatinine as a gold standard.
Table 9: Regression statistics of AKI based on plasma creatinine, plasma NGAL and urine NGAL.

<table>
<thead>
<tr>
<th>Regression Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple R</td>
</tr>
<tr>
<td>R Square</td>
</tr>
<tr>
<td>Adjusted R Square</td>
</tr>
<tr>
<td>Standard Error</td>
</tr>
<tr>
<td>Observations</td>
</tr>
</tbody>
</table>

Out of the total number of study subjects 13 met the KDIGO definition of AKI, four were excluded because they lacked urine NGAL results due to anuria for 12 hours (n=2) and contamination (n=2) therefore 9 study data were subjected to regression analysis which gives the prevalence of AKI to be 28.5%, 95% CI [12.8- 41.8] based on plasma creatinine, as well as plasma and urine NGAL.
4.6 Correlations

Figure 1: Correlation plot between Plasma Creatinine and Plasma NGAL

There is a weak positive association between the change in creatinine and plasma NGAL.
Figure 2: Correlation plot between Plasma Creatinine and Urine NGAL

The data is widely distributed showing very minimal association between the change in creatinine and Urine NGAL.

Figure 3: Correlation Plot between Urine and Plasma NGAL

Figure 3: There is a positive relationship between urine and plasma NGAL. The data occurs closely indicating an association between the two variables.
### Table 10: Correlation analysis

<table>
<thead>
<tr>
<th>Correlations</th>
<th>Change in creatinine</th>
<th>Plasma NGAL</th>
<th>Urine NGAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change in creatinine</td>
<td>Pearson Correlation</td>
<td>1</td>
<td>.442</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.234</td>
<td>.140</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Plasma NGAL</td>
<td>Pearson Correlation</td>
<td>.442</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.234</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Urine NGAL</td>
<td>Pearson Correlation</td>
<td>.532</td>
<td>.869**</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td>.140</td>
<td>.002</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>9</td>
<td>9</td>
</tr>
</tbody>
</table>

**. Correlation is significant at the 0.01 level (2-tailed).

The findings summarized in table 10 above show a positive moderate relationship between creatinine and plasma NGAL ($r = 0.442$). There is a positive significant relationship between plasma creatinine and urine NGAL ($r = 0.532$). The analysis also reveals a strong positive significant correlation between plasma and urine NGAL ($r = 0.869$).
Figure 4: The correlation between absolute neutrophil counts with plasma NGAL.

There is a linear and strong relationship between Plasma NGAL and neutrophil count.
Table 11: Correlation between plasma NGAL and absolute neutrophil counts

<table>
<thead>
<tr>
<th></th>
<th>Plasma NGAL</th>
<th>Absolute neutrophil count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma NGAL</td>
<td>Pearson Correlation</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>40</td>
</tr>
<tr>
<td>Absolute Neutrophil count</td>
<td>Pearson Correlation</td>
<td>.005</td>
</tr>
<tr>
<td></td>
<td>Sig. (2-tailed)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>40</td>
</tr>
</tbody>
</table>

The analysis in table 11 shows that there is a positive correlation between plasma NGAL and neutrophil counts (r = 0.005) and p>0.05.
4.7 Regression Analysis.

Table 11: Evaluation of the ability of plasma and urine NGAL to detect AKI.

<table>
<thead>
<tr>
<th>Source</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Model</td>
<td>11598.9503</td>
<td>2</td>
<td>5799.47513</td>
</tr>
<tr>
<td>Residual</td>
<td>21827.0497</td>
<td>37</td>
<td>589.920263</td>
</tr>
<tr>
<td>Total</td>
<td>33426</td>
<td>39</td>
<td>857.076923</td>
</tr>
</tbody>
</table>

The analysis in table 12 show that Plasma ($p = 0.029$) and Urine NGAL ($p = 0.000$) are statistically significant predictors of AKI in critically ill children. Thus, the model is significant at ($F(2, 37) = 9.83$, $p = 0.0004$). Plasma NGAL and Urine NGAL in this study were able to predict 34.7% of the AKI in critically ill children.
CHAPTER FIVE

5.0 Discussion

This study was conducted at Kenyatta national hospital, department of pediatrics to evaluate the ability of plasma and urine NGAL, a new biomarker to improve on early diagnosis of AKI in critically ill children admitted in the pediatric ward acute rooms and pediatric intensive care unit.

The gender distribution for the study participants was females (56%) and male (44%) there was no statistical difference in the gender distribution this compares with to Muithya et al 2012 18 54.7 % female study participants, Arikan et al 3 also observed a distribution of 45% male participants. Majority of the study participants were aged between 1 and 3 years (72.9%), this finding is comparable to other studies 17,37,38,40 conducted in population of critically ill children of which they observed the mean age of the participants to be within this age group. This points out the significance of this age group as a target group for further investigations to try to understand the contributing factors towards an estimated higher morbidity and hospital admissions.

Respiratory tract infection was the major underlying pathology that led to admission as it was present in 71% of the entire study participant; this is consistent with other studies 4,16,17. Pneumonia is the most prevalent respiratory pathology that affects children as shown by our study and the other studies4,16,17 which also observed similar findings.

The prevalence of AKI based on creatinine was 29.5% this was based on an increase of ≥ 26.5 in creatinine values within 48hours of admission this is in contrast with a previous study17 in the same setting with similar study population that found a prevalence of 85.5%, we could not establish the main reason for the huge disparity in the two outcomes but it we noted that the definition for AKI were different and may have contributed to the disparity between the two studies, when related to a larger study 16 the findings are comparable with their findings that indicated a prevalence of 26.9% in critically ill children. The findings point out that prevalence of AKI in critically ill children is relatively high and care providers should be more vigilant in managing these patients.
Prevalence of AKI based on NGAL indicated higher prevalence than that of creatinine as a marker, urine NGAL showed a prevalence of 90.9%, and plasma NGAL 85.4%. This finding demonstrates a huge disparity between NGAL and creatinine and this can be attributed to the sensitivity and specificity of each individual marker in diagnosing AKI. Using creatinine as a gold standard the sensitivity of urine NGAL at a cutoff value of 250 ng/mL in this study was 100% while the sensitivity of plasma NGAL at a cutoff value of 117.5 ng/mL was 85.7% respectively, the sensitivity observed in our study are in agreement with other studies 31, 40, 43, 45, 46 that have found similar sensitivity for NGAL as 85%, 90%, 95% 88% and 90% respectively.

Our study observed low specificity for NGAL, urine NGAL had a specificity of 16% while plasma NGAL had a specificity of 13.3%, this was in contrast with previous studies31, 40 - 43 that observed better specificities for NGAL of 83%, 68%, 95%, 56% and 99.5% respectively. This discordant observation in specificity can be attributed to the composition of the study groups. Our study was composed of a heterogeneous group of critically ill children while the previous studies recording better specificity were conducted on specific patient population, this further affirms observations made by Hasse M et al 31 that the performance of NGAL improves when applied in specific patient population.

Interestingly Nicholas et al 46 in their study observed an impressive specificity of 99.5% in a heterogeneous patient population, this could be partly explained by the technique used to analyze NGAL, in their study they employed immunoblotting technique while in this study we used a turbidimetric technique for analysis, this might serve a pointer to the effect of the analytical technique and diagnostic performance of NGAL.

Regression analysis using all the three markers the prevalence of AKI in critically ill children at Kenyatta national hospital was found to be 28.5% .95% CI [12.8- 41.8] based on those subjects whose findings were consistent with the three criteria (KDIGO, uNGAL and pNGAL) used to define AKI in this study. This is almost similar the prevalence when creatinine is used as marker, this could be attributed to the fact than an increase in creatinine values (KDIGO criteria) was employed for the purpose of defining AKI in the study.

This study demonstrated a positive relationship between plasma creatinine levels and Plasma NGAL levels ($r = 0.442$), and urine NGAL($r = 0.532$) this signifies that a rise in creatinine level relates to a rise in both plasma and urine NGAL values though the statistical significance is weak, through literature search we did not come across published work that investigated
relationship between these parameters. There is a strong positive and significant correlation between Plasma and Urine NGAL ($r = 0.869$) based on this study, this implies that Plasma and Urine NGAL have a strong relationship, this imply that either urine or plasma samples can be used as the sample of choice in situations where the process of sample collection is considered to be noninvasive.

The study found that a linear relationship between plasma NGAL and absolute neutrophil count exists, but it is statistically insignificant ($r = 0.005$) and $p>0.05$ this could imply that though NGAL is stored in neutrophil granules, absolute neutrophil count in an individual does not bear any significant effect on the concentration plasma NGAL concentration.

This study finds Plasma ($p = 0.029$) and Urine NGAL ($p = 0.000$) to be statistically significant markers of acute kidney injury in critically ill children. These findings are in agreement with previously conducted studies$^{31, 40, 43, 45}$ that drew similar conclusion that NGAL has the ability to predict AKI across different clinical setting, these findings are encouraging and prompts further studies to evaluate the diagnostic performance of NGAL to aid in the translation from a research to a diagnostic tool.
5.1 Limitation of the study

Data for baseline kidney function for the study participants was not available to assess the kidney function at the point of admission and AKI diagnosis relied entirely on an increase in creatinine levels post admission. The other limitation in our study is that creatinine was used as a gold standard to define AKI despite its shortcomings, and this might have influenced the sensitivity and specificity of NGAL.

5.2 Conclusion /Recommendation

- This study finds plasma and urine NGAL to be statistically significant markers capable of detecting acute kidney injury in critically ill children.
- Based on this study findings the prevalence of AKI was 28.5 %, this indicates that AKI syndrome is prevalent among the critically ill children.
- There is a strong positive significant correlation between plasma and urine NGAL levels, implying that one of the two can be used as a sample of choice for estimating NGAL levels.
- There is a positive but statistically insignificant correlation between plasma NGAL and absolute neutrophil count indicating that absolute neutrophil count does not affect the levels of plasma NGAL.
- We recommend a larger prospective study be conducted to affirm these findings and examine the diagnostic performance of NGAL in AKI settings.
References.


47. Kasiulevičius.v, V. Šapoka, R. Filipavičiūtė, Sample size calculation in epidemiological studies, Gerontologija 2006; 7(4): 225–231