KIDNEY MORPHOLOGY IN PAEDIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT THE KENYATTA NATIONAL TEACHING AND REFERRAL HOSPITAL

A dissertation submitted in partial fulfillment of the requirements for the Degree of Master of Medicine in Human Pathology of the University of Nairobi

PRINCIPAL INVESTIGATOR:

DR. JOHN CHEGE NJUGUNA (MBChB)

H58/70149/2013

University of Nairobi.

Kenya.

DECLARATION

I hereby declare that this study is my original work and has not been presented for dissertation at any other university.

Dr. John Chege Njuguna, MBChB.

Registrar, Department of Human Pathology,

University of Nairobi.

Signature: _____ Date: _____

SUPERVISOR'S DECLARATION:

This dissertation has been developed under our guidance and approval as University supervisors;

SUPERVISOR(S):

1. DR. JOSEPH.R. NDUNGU.

   MBChB, MMed (Path), FCPath ECSA
   Lecturer/ Consultant Pathologist
   Anatomic Pathology Unit,
   Department of Human Pathology,
   School of Medicine,
   University of Nairobi.

   Signature: _____ Date: _____

2. DR. EDWIN. O. WALONG.

   MBChB, MMed (Path), FCPath ECSA
   Lecturer/ Consultant Pathologist
   Anatomic Pathology Unit,
   Department of Human Pathology,
   School of Medicine,
   University of Nairobi.

   Signature: _____ Date: _____

ACKNOWLEGMENTS

My sincere gratitude to Dr. Edwin Walong and Dr. Joseph Ndungu for the supervision, guidance and encouragement throughout the study period.

To Dr. Phillip Ayieko who provided statistical expertise during analysis of data.

To the University of Nairobi histopathology laboratory team, Mr Willis Ochuk and Mr John Kairu for technical assistance and support during processing of specimen material.

DEDICATION

I dedicate this dissertation to my entire family who offered much needed support, encouragement and resources during this study.

LIST OF ABBREVIATIONS AND ACRONYMS

AKI	Acute Kidney Injury
ATN	Acute Tubular Necrosis
CDC	Centre of Disease and Control
DIC	Disseminated Intravascular Coagulation
ERC	Ethical Regulatory Committee
HIV	Human Immunodeficiency Virus
IL-1	Interleukin 1
IL-6	Interleukin 6
IL-8	Interleukin 8
KEMRI	Kenya Medical Research Institute
KNH	Kenyatta National Hospital
KDHS	Kenya Demographic Health Survey
MCD	Minimal Change Disease.
MGN	Membranous Glomerulonephritis
MPGN	Membranoproliferative Glomerulonephritis
NSAID	Non-Steroidal Anti-Inflammatory Drugs
PCR	Polymerase Chain Reaction
PRESS	Pediatric Respiratory Surveillance Study
RPGN	Rapidly Progressive Glomerulonephritis
SARI	Severe Acute Respiratory Illness
STATA	StataCorp College Station, Texas USA

TB	Tuberculosis
TIN	Tubulointerstitial Nephritis
TNF	Tumor Necrosis Factor
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS:

DEFINITIONS

PRESS – A Pediatric Respiratory Disease Surveillance Autopsy Study which was conducted by the Kenya Medical Research Institute (KEMRI) and Centers for Disease Control (CDC) at Kenyatta National Hospital between August 2014 to December 2015 to determine the aetiology of Severe Acute Respiratory Illness(SARI) in children admitted to the hospital.

WHO Case definition of SARI Associated Mortality - Death associated with an acute respiratory infection with; history of fever or measured fever of ≥ 38 C°; and cough; with onset within 14 days.

Histopathologic Patterns:

Diffuse proliferative glomerulonephritis (DPGN). A term used to describe a distinct histologic form of glomerulonephritis in which more than 50% of the glomeruli (diffuse) show an increase in mesangial, epithelial, endothelial (proliferative), and inflammatory cells (i.e., glomerulonephritis).

Focal Segmental Glomerulosclerosis (FSGS). A characteristic, discrete and segmental solidification of the glomerular tuft that usually occurs in the perihilar region in continuity with the vascular pole. This may involve any segment of the glomerulus as the lesion evolves globally.

Rapidly progressive glomerulonephritis (**RPGN**). A syndrome of the kidney that is characterized by a rapid loss of renal function, (usually a 50% decline in the glomerular filtration rate (GFR) within 3 months) with glomerular crescent formation seen in at least 50% or 75% of glomeruli seen on kidney biopsies.

Minimal Change Disease (MCD). A common form of nephrotic syndrome in children, characterized by minimal histologic changes in the kidney on light microscopy.

Membranoproliferative glomerulonephritis (MPGN). Glomerular injury characterised by proliferation of mesangial and endothelial cells and expansion of the mesangial matrix cause by deposition of immune complexes within the mesangial space.

Membranous Glomerulonephritis (MGN). Glomerular injury characterised by subepithelial immune deposits and formation of perpendicular projections of material similar to the glomerular basement membrane.

Tubulointerstitial Nephritis (TIN). Inflammation of the renal interstitium characterised by recruitment of various inflammatory cells between the renal tubules.

Acute Tubular Necrosis. Injury to renal tubular epithelial cells, which results in cell death or detachment from basement membrane and is characterised by attempts at re generation of the damaged epithelium.

ABSTRACT

Background: Acute kidney disease in African children has been directly attributed to the high burden of infectious disease in the region. In severe infections, injury to the kidneys may arise through direct spread of causative microorganisms or indirectly through immunologic mechanisms. Acute respiratory illnesses contribute significantly to morbidity and mortality in children under the age of 5 years in Africa but accurate data on renal complications associated with these infections is extremely limited. Development of acute renal complications in children hospitalised for management of a respiratory illness may worsen the clinical course of the disease and contribute to morbidity or lead to mortality. This study described pathologic changes to the kidneys in children who died while undergoing treatment for Severe Acute Respiratory Illness (SARI) at the Kenyatta National teaching and referral Hospital (KNH).

Objectives: To describe the gross and histopathologic changes to the kidneys in fatal paediatric SARI cases at KNH and corelate these changes to aetiologic agent, age, and gender.

Design: A laboratory based cross-sectional descriptive study carried out from May 2017 to March 2018.

Setting: The Kenyatta National Hospital and University of Nairobi histopathology laboratories.

Materials and Methods: Sixty-four (64) preserved paraffin embedded tissue blocks collected from the kidneys in a previously conducted Paediatric Respiratory Surveillance Study (PRESS) at KNH were retrieved. Histologic sections from these blocks were stained using routine Haematoxylin and Eosin and special staining techniques (PAS, PASM and Masson's Trichrome) to describe histopathologic patterns of renal pathology. The gross descriptions of the kidneys recorded at autopsy and histomorphologic features identified through conventional light microscopy were utilised together to describe a final diagnostic pattern of kidney pathology for each case. These findings were correlated with SARI aetiologic agent, age, and gender for each case.

Results: All sixty-four (64) preserved kidney tissue blocks from the PRESS study were selected for review. Three (3) cases were excluded due to poor preservation and deaths

attributed to non-infective SARI causes, resulting in a sample size of 61 cases. The mean age was 10.7 months (SD ± 10), with an age range of 1 to 48 months, and a male to female ratio of 1:1.2. At autopsy, 19.7% of cases had abnormal morphologic appearance of the kidneys. The mean combined unfixed kidney weights in these cases were 33.5 ± 1.9gms for the right kidney and 33.1 ± 1.9gms for the left kidney. On light microscopic examination of the renal parenchyma, 98.4% of cases demonstrated glomerular pathology (DPGN 64.1%, FSGS 18%, Membranoproliferative/Membranous GN 13.1% and Crescents in 3.2%). Tubulointerstitial pathology was demonstrated in 60.6% of cases (ATN in 34.4% and focal TIN in 26.2%) while renal microvascular pathology was demonstrated in 21.1% of cases (Arteriolar arteriolosclerosis 11.5%, vascular congestion 6.3%, and thrombosis in 3.3%). There was no co relation between all histopathologic changes observed under microscopy with SARI aetiologic agent, age and gender ($p = > 0.05$ for bacterial, viral, fungal and protozoal infections).

Conclusions: There are significant acute pathologic changes to the kidneys in fatal paediatric SARI cases in KNH. These changes are however not directly attributable to the SARI aetiologic agent and are not age or gender specific. This therefore suggests that acute kidney injury in fatal paediatric SARI results from renal involvement in systemic broad spectrum immunologic assault due to overwhelming infection in these critically ill children.

1. INTRODUCTION

Majority of renal disease in Africa is widely accepted to be directly attributable to infectious diseases. This is due to the high prevalence of bacterial, fungal, protozoal and viral infections (1–3). African children are particularly prone to infections during the first five years of life with the higher prevalence of infectious diseases causing significant morbidity and mortality in this age group. The World Health Organization estimates that 46% of the world's under 5 mortality occurs in Sub –Saharan Africa with respiratory tract illnesses accounting for a significant proportion of these deaths (4,5). Locally, Kenya reported 188,928 deaths in the under-five age group in 2010 with respiratory tract infections accounting for 16% of these deaths (6). In the KDHS 2014, the under 5 mortality rate was reported as 52 per 1000 with 9% of children in this age group reporting illness with cough, rapid breathing, and symptoms of Acute Respiratory Illness within 2 weeks of the survey. 66% of these children were taken to a health facility or provider for advice or treatment and 53% had infections severe enough to receive treatment with antibiotics (7).

Respiratory infections have been associated with numerous well established and studied sequelae amongst which are renal complications (8–11). The kidneys may be involved through direct spread of microorganisms from infective foci in the respiratory tract or indirectly via immune mechanisms that damage the renal parenchymal structures and vasculature. Overwhelming infections may also lead to coagulopathy which may result in deposition of microthrombi in renal vasculature leading to hypoxic renal parenchymal damage. The final common result of these mechanisms presents as a syndrome of acute kidney injury characterised by haematuria, azotemia, decline in glomerular filtration rate (GFR), retention of urea and other nitrogenous waste products, and dysregulation of extracellular volume and electrolytes. Acute kidney injury has been independently associated with increased mortality in children (1,12,13). AKI is therefore likely to contribute to worsening of the initial respiratory illness, change the pharmacokinetics of medication administered during treatment and likely lead to higher morbidity and contribute to mortality in children admitted to hospital for management of a severe acute respiratory illness.

Accurate data on the incidence and causes of acute kidney injury in children is extremely limited due to the significant global variation in the definition of what constitutes acute renal injury and management of children diagnosed with acute renal injury. Furthermore, the

causes of acute renal disease vary widely across various geographic locations. Recent studies however indicate that the incidence of acute renal injury particularly in hospitalised children is on the rise (14). Moreover, during hospitalization due to infectious diseases, management is primarily focused on treatment of the primary cause of infection while any renal complications arising during treatment may be overlooked or severely underreported (3).

This study evaluated and characterised the morphology of the kidneys from tissues obtained at autopsy in children who died while undergoing treatment for SARI at KNH and described the specific kidney pathology observed.

2. LITERATURE REVIEW

Pathophysiology of kidney injury in respiratory infections.

The kidneys have wide variety of essential functions that include filtration and excretion of metabolic waste products, regulation of necessary electrolytes, fluid, and acid-base balance and stimulation of red blood cell production. They also serve to regulate blood pressure via the renin-angiotensin-aldosterone system, control the reabsorption of water and maintain intravascular volume. The kidneys also reabsorb glucose and amino acids and have hormonal functions via erythropoietin, calcitriol, and vitamin D activation. Anatomically, the renal parenchyma consists of the cortex and medulla all enclosed within a smooth layer of connective tissue which forms the renal capsule. The renal cortex consists of specialized functional units i.e. The glomerulus, tubular network, interstitium and renal vasculature. Each unit serves a specific function in the overall physiologic functions of the kidney. Various forms of injury to one or more of these specialized units may occur during severe infections (16).

Morphologically, the renal parenchyma responds in a limited number of histopathological patterns to a wide variety of injurious agents. During severe respiratory illness, direct spread of microbes, immunological mechanisms or vascular and haemostatic complications of these infections may result in injury to renal parenchyma.

The glomerulus responds to direct injury or to immune mediated insults by exhibiting various degrees of hyper cellularity, mesangial expansion, glomerular basement membrane changes, sclerosis or contraction of the Bowman's space. These changes collectively appear and are described as diffuse, focal, segmental or global patterns. The renal interstitium responds to acute injury by exhibiting leukocyte infiltration and displaying various degrees of interstitial oedema. The renal tubular system may show granular casts, dilatation, necrosis or inclusions when acutely assaulted while the renal vasculature responds by exhibiting signs of acute inflammation, thrombosis or hyalinization if injury persists. Specific patterns of injury may be common to two or more aetiologic agents.

Currently there are limited autopsy studies specific to renal pathology in children. While there have been descriptive necropsy studies on children in Africa dying from respiratory illness (15) and others separately describing morphologic patterns of renal injury during

infections, (16,17) studies specifically describing SARI associated renal pathology have not yet been carried out in the region.

Acute Kidney Injury in bacterial respiratory illness.

Prospective microbiology-based studies have found the leading bacterial cause of childhood pneumonia as *Streptococcus pneumonia* (pneumococcus), identified in 30–50% of cases. The second most common organism isolated in most studies is *Haemophilus influenzae* type b (Hib; 10–30% of cases), followed by *Staphylococcus aureus* and *Klebsiella pneumoniae*. In addition, lung aspirate studies have identified a significant fraction of acute pneumonia cases to be due to *Mycobacterium tuberculosis* (5,18). *Mycoplasma pneumonia* and *Chlamydia trachomatis* have also been implicated in community acquired 'atypical pneumonia' in hospitalized children (18).

Bacterial respiratory tract infections have been studied for renal complications with immune mediated acute post infectious glomerulonephritis following streptococcal infections being most widely reported (19–21). Immune mediated nephritis has also been reported in association with Klebsiella pneumonia (22). Mycoplasma pneumonia has also been recently implicated as an emerging cause of immune mediated post infectious nephritis (23–27).

Acute post infectious glomerulonephritis:

Acute post infectious glomerulonephritis caused by *Streptococcus pneumonia* infection of the upper respiratory tract is prototypic and the most widely reported cause of renal injury following acute bacterial respiratory illness. It presents as a syndrome of acute nephritis characterised clinically by oedema, haematuria, proteinuria, and hypertension as a result of injury to the glomerular basement membrane that allows leakage of fluids and plasma proteins thought the glomerulus and eventually into urine. The mechanism of renal impairment in this syndrome is via immune mediated injury to the glomerulus. Immune complexes formed due to an infection of the respiratory tract deposit on the glomerular basement membrane resulting in activation of complement and activation of pro inflammatory cytokines and recruitment of activated immune cells (21,24,28,29).Under conventional light microscopy, the morphologic changes in this type of immune mediated nephritis are represented by various degrees of mesangial hyper cellularity, endocapillary proliferation, capillary remodeling and accentuation of the glomerular tufts. There may also be infiltration of the mesangium by lymphocytes.

Mycoplasma pneumonia infections in children have also been implicated in causing immune mediated membranoproliferative glomerulonephritis(MPGN), proliferative endocapillary glomerulonephritis and minimal change disease(MCD) a result of immune mediated glomerular injury (23).

Genitourinary Tuberculosis:

Children hospitalized for management of SARI are more likely to be diagnosed or be on treatment for pulmonary tuberculosis (30). Genitourinary disseminated Tuberculosis is the most common form of haematogenously spread extra pulmonary tuberculosis, accounting for 27% of cases in adults in the developed world. At least 3-4% of these cases have a primary focus in the lung. Haematogenous dissemination from an active TB infection site may result in the formation of metastatic tubercles in the kidney. Although rare in the pediatric population, cases of genitourinary tuberculosis have been reported (31,32). In these cases, classical epithelioid granulomas with lymphocytic infiltration within the kidney parenchyma were reported on histology.

Kidney injury in viral respiratory illness.

Viral respiratory infections cause renal parenchymal damage mainly via the action of T cell mediated cytotoxicity, mononuclear cell activation and the action of toll like receptors that trigger an innate immunologic response against viral proteins and virus infected cells. The resulting action of pro inflammatory cytokines (IL-6, IL-8 and TNF) produced by these activated immune cells result in damage to the renal parenchyma (33). Kidney cells often are infected during viral illnesses but appear to be unusually resistant to injury compared to other organs and tissues however, viruria is commonly measurable during viral infections (34).The renal tubular network and its lining epithelial cells are more sensitive to viral cytopathic effects and immune mediated injury.

Viral respiratory illnesses have been reported to cause significant morbidity amongst Kenyan children. A prospective study has shown the prevalence of Respiratory Syncytial Virus (RSV) infections amongst both urban and rural children in Kenya as 12.5% of those studied in rural areas and 11.7% amongst those studied in urban areas. In this study RSV was commonly associated with SARI especially in the under one-year old population (35). In another study on SARI in an urban slum in Nairobi, RSV and influenza A and B viruses were estimated to account for 16.2% and 6.7% of SARI cases respectively and when taken

together, parainfluenza (PIV), adenovirus, and human metapneumovirus (hMPV) accounted for >20% additional cases (36).

Respiratory Syncytial Virus:

Cases of acute nephritis associated with RSV infections are rarely in literature. In many of the cases there is antecedent immunosuppression due to renal transplant. However, RSV has been linked with kidney injury that is indistinguishable from that caused by noninfectious aetiology in epidemiologic reviews (34). A case report in France has described acute renal failure in a child with concurrent RSV and adenovirus infection (37). In this case kidney biopsy and histology showed tubulointerstitial damage with a mild lymphocytic infiltrate and no necrosis. There were no virus inclusion bodies seen, no interstitial haemorrhage, glomerular or vascular injury.

Influenza A:

Although rarely reported, Influenza A has been associated with AKI in children. Shenouda et al had presented a series of four patients with various degrees of severe AKI associated with influenza infection (38). During this early period of reported studies, the underlying mechanisms of Kidney Injury associated with Influenza infections had not been established. Subsequently, Watanabe et al reported Acute Tubulointerstitial Nephritis (TIN), myoglobin pigment deposition and intravascular coagulation in histology from kidneys of patients with influenza type A infection (11). In that study, the disease severity was significantly higher amongst children. In a follow up series involving only hospitalized children infected with Influenza A, Watanabe reported Acute Kidney Injury in 25% of 45 cases studied (39).

Adenovirus Infections:

Severe disseminated Adenovirus infections have been associated with fatalities especially in the background of immunosuppression (40). Masafumi reported an autopsy series of 10 patients with necrotizing tubulointerstitial nephritis associated with adenovirus infections (8). In all ten cases there was reported immunosuppression form cytotoxic agents administered for chemotherapy of malignancies or from anti-rejection treatment after organ transplant. In that study viral inclusion bodies were demonstrated in renal tubular cells examined through histopathology. Subsequently, demonstration of related viral antigens through immunofluorescence confirmed the presence of adenovirus in the kidneys involving renal tubular cells in all ten cases. He proposed adenovirus as an important cause of necrotizing

tubulointerstitial nephritis in the immunocompromised patient. This characteristic necrotizing tubulointerstitial nephritis associated with adenovirus infections may be present in African children where the main causes of immunosuppression are HIV infection and malnutrition.

Other causes of acute nephritis in respiratory Illness.

Concurrent HIV infection.

The link between SARI and HIV infections amongst hospitalised has been well established. A South African study reported 74% of older children and adults hospitalised for treatment of SARI as having concurrent HIV infection (30). H.I.V is associated with a myriad of opportunistic infections of the lung. In an autopsy study on 36 HIV infected children to investigate lung morphology, 75% were identified to have concurrent infectious lung disease. Bacterial pneumonia was identified as the second most common aetiology after *Pneumocystis jirovenci.* Rhinovirus, adenovirus and influenza infections have also been commonly detected in children who have concurrent HIV and a concurrent SARI (41).

HIV has also been studied extensively due to its effect on the kidney and direct cytopathic effects on renal tissue. In a prospective South African study in which renal biopsies from HIV infected children were carried out, focal segmental glomerulosclerosis (FSGS) was present 65.3% children and 26.5% had collapsing glomerulopathy. Significant drug induced renal changes due to Anti-Retroviral agents was also reported in this study (42). In another prospective study focal glomerulosclerosis, mesangial hyperplasia, segmental necrotizing glomerulonephritis and minimal change disease were reported in kidney biopsies from children infected with HIV. In addition, tubulointerstitial infiltrates and glomerular dense deposits were seen in some cases (43).

Drug induced nephrotoxicity:

Drugs administered to hospitalised children on treatment for SARI may induce renal parenchymal injury. Commonly utilized agents such as penicillins, cephalosporins, aminoglycosides and NSAIDS may induce renal parenchymal damage through various mechanisms. NSAIDS, aminoglycosides and certain cephalosporins may induce acute tubular necrosis and interstitial nephritis via reduction in renal blood flow, while penicillins have been associated with hypersensitivity reactions leading to vasculitis in small renal blood vessels and resultant acute renal injury (44).

3. STUDY RATIONALE AND JUSTIFICATION

Paediatric renal disease remains an important cause of morbidity and mortality in Africa. The definitions and management of acute kidney injury vary widely in different geographic locations; however, it is widely accepted that majority of acute paediatric renal disease in Africa primarily occurs because of infectious diseases or as a complication of infectious disease. Severe Acute Respiratory Tract Illnesses caused by various infectious agents are a significant cause of morbidity and mortality in children under five years in Sub-Saharan Africa. Renal complications of these infections may be common but have not been adequately studied. Moreover, SARI associated renal pathology is currently unknown in our setting.

Investigation of renal disease during hospitalization for infectious disease may be extremely complex and costly. Biochemical investigations on urine and blood samples are routinely used to provide a partial diagnosis whenever renal compromise is clinically suspected as they easy to perform, have a quick turnaround time and are relatively inexpensive. However, the renal biopsy remains the gold standard in identification, characterization and classification of renal disease. Renal biopsies are not routinely carried out in our setting and may not be feasible in each case where renal pathology is suspected. An examination of the kidneys at autopsy provides a unique opportunity to retrospectively study renal pathology in children hospitalized for management of SARI and provide information that may influence guidelines on management of these children especially where complex renal investigations may not be feasible.

The care and management of children hospitalised for treatment of infectious diseases may be complex when renal complications occur, necessitating inclusion of a multi-disciplinary teams and specialized services including dialysis in treating these complications. These interventions are extremely recourse intensive. This study aims to sensitize the primary care physicians on possible renal pathology in children hospitalized for severe acute respiratory illnesses and provide early and timely positive influence on diagnostic modalities and therapeutic interventions in the care of these children before onset of major life-threatening complications.

Ultimately this study aims to aid in prevention of renal complications in children admitted to hospital with SARI, reduce hospital stays and mortality and result in reduction in cost of managing paediatric acute respiratory illness at KNH.

4. OBJECTIVES

4.1 Research Question:

What are the pathologic changes to the kidney in paediatric mortalities associated with severe acute respiratory illness at Kenyatta National Hospital?

4.2 Broad Objective.

To describe the pathologic changes to the kidneys in paediatric patients who succumbed to severe acute respiratory illness at Kenyatta National Hospital.

4.3 Specific Objectives.

1. To describe the gross pathologic changes to kidneys seen in fatal paediatric SARI.

2. To describe the histopathologic patterns of kidney pathology seen in fatal pediatric SARI.
3. To correlate morphologic patterns of kidney pathology in fatal pediatric SARI with the infectious agent, age and gender.

5. STUDY DESIGN MATERIALS AND METHODS

5.1 Study Design

This study is a laboratory based cross sectional descriptive study.

5.2 Study Setting

The study was conducted at Kenyatta National teaching and referral Hospital (KNH) and University of Nairobi histopathology laboratories both located within KNH. The hospital is the national referral hospital in Kenya and largest in the East and Central African region. It also serves as the primary teaching hospital for the University of Nairobi. It contains four paediatric wards each with approximately 60 beds. The hospital admits 14,000 paediatric patients annually. It also has an Intensive Care Unit (ICU) and a high dependency unit (HDU) where patients of all ages including all children requiring critical care are admitted. It also has a modern mortuary serving the hospital with a capacity of one hundred and thirty-six bodies where all autopsies are conducted.

5.3 Study Population:

WHO Case definition: SARI Associated Mortality - Death associated with an acute respiratory infection with; history of fever or measured fever of ≥ 38 C°; and cough; with onset within 14 days. (45)

This study was conducted on preserved and archived kidney tissue from paediatric mortality cases associated with Severe Acute Respiratory Illness from ages 1 month to 59 months. These tissues were obtained as part of an autopsy based Pediatric Respiratory Disease Surveillance Study (PRESS) conducted between august 2014 and December 2015.

5.4 Sample size Calculation:

Sample size was calculated using the Duncan's formula for a finite population.

$$n = \frac{NZ^2P(1-P)}{d^2(N-1) + Z^2P(1-P)}$$

Where:

n = Sample size

N = Total estimated accessible population = 100

Z = Standard normal value for 95% Confidence Interval = 1.96

P = Estimated proportion of renal disease in hospitalised children based on a study by Ladapo et al in Nigeria where 3594 paediatric admissions were evaluated over a four-year period. In this study kidney disease was reported in 8.9% (0.089) of all hospitalised children.

D = Precision error will be 5% (0.05)

Substituting;

$$n = \frac{100x1.96^2x0.089(1-0.089)}{0.05^2(100-1) + 1.96^2x0.089(1-0.089)} = 55.722$$

The minimum required sample size is 56 samples

A sample size of 64 was used to enable review of all renal tissue collected during the PRESS study.

5.5 Sampling Method

Consecutive and purposive sampling of preserved kidney tissue obtained at autopsy as part of the Pediatric Respiratory disease Surveillance Study (PRESS) conducted between August 2014 and December 2015 was used.

5.5.1 Inclusion Criteria

- Preserved kidney tissue blocks from paediatric mortality cases associated with Severe Acute Respiratory Illness cases aged 1-59 months collected during the PRESS study.
- All well preserved renal tissue.

5.5.2 Exclusion Criteria

- All pediatric mortality cases with known history of kidney disease prior to admission who were included in the PRESS study.
- Final cause of death not attributed to infectious aetiology.
- All poorly preserved kidney tissue.

5.6 Materials and Methods:

Clinical and demographic data from all paediatric autopsies carried out at KNH between august 2014 and December 2015 as part of the PRESS study to determine the aetiology of SARI was reviewed. During this preceding study, autopsies were performed within 24 hours of death following immediate refrigeration and sterile preservation of the bodies. The autopsies were conducted using the Letulle technique. Sterile techniques were used to obtain both trans-thoracic percutaneous and open biopsy samples of respiratory tract tissue from both lungs at multiple levels for analysis and identification of aetiologic agents of respiratory illness for each case. Identification of these agents was performed through light microscopic evaluation, immunohistochemistry and PCR on lung tissues samples obtained. Aetiologic agents causing the primary respiratory illnesses were identified and included in the final cause of death report for each case studied.

As part of the performing standardized autopsies during the PRESS study, gross descriptions of the kidneys and unfixed kidney weights were recorded for each case. Renal tissue samples were also taken from the upper, middle and lower pole of each kidney. Additional sampling was carried out from areas with any observable gross pathology. These renal tissue samples were immediately fixed in neutral buffered formalin before being processed and preserved in paraffin embedded tissue blocks.

All demographic and clinical data, gross description of kidney findings during autopsy and aetiologic agent(s) of SARI identified at autopsy for each case were catalogued and entered into a designed data collection proforma (Appendix I).

Formalin fixed paraffin embedded renal tissue blocks from the KNH archives for each PRESS corresponding autopsy case were retrieved. Histological sections were made from the tissue blocks at a thickness of 2 - 3 microns for 10 to 20 consecutive levels. Serial sections were then mounted on glass slides and staining performed using routine and special stains (Appendix III). Staining of the sections was performed; alternating over the consecutive sections, Haematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS), Periodic Acid Silver Methenamine (PASM) and Masson's Trichrome stains (Appendix IV-VII). Each special stain utilised enabled specific review of different anatomical structures within the renal parenchyma.

Conventional light microscopic examination was performed on all sections and kidney morphology recorded for each case. Systematic histopathologic descriptions were made on morphologic patterns in the glomerulus, the interstitium, the renal tubular system, and the renal vasculature. All light microscopic observations and descriptions were conducted using the Renal Pathology Society guidelines on standardization of histopathologic reporting of non - neoplastic kidney biopsies and tissues (62).

The glomerulus was assessed on size and cellularity, character of the glomerular basement membrane, mesangial expansion and Bowman's space, global or focal pathologic changes, glomerular capillary vessel changes or deposits and leukocyte extravasation. The renal tubular system was assessed on presence of casts, dilatation, cellular inclusions, or necrosis while the interstitium assessed for presence of oedema, inflammation or fibrosis. The renal vasculature was assessed for presence of hyalinosis, inflammation, thrombosis, necrosis, elastic changes or intimal thickening.

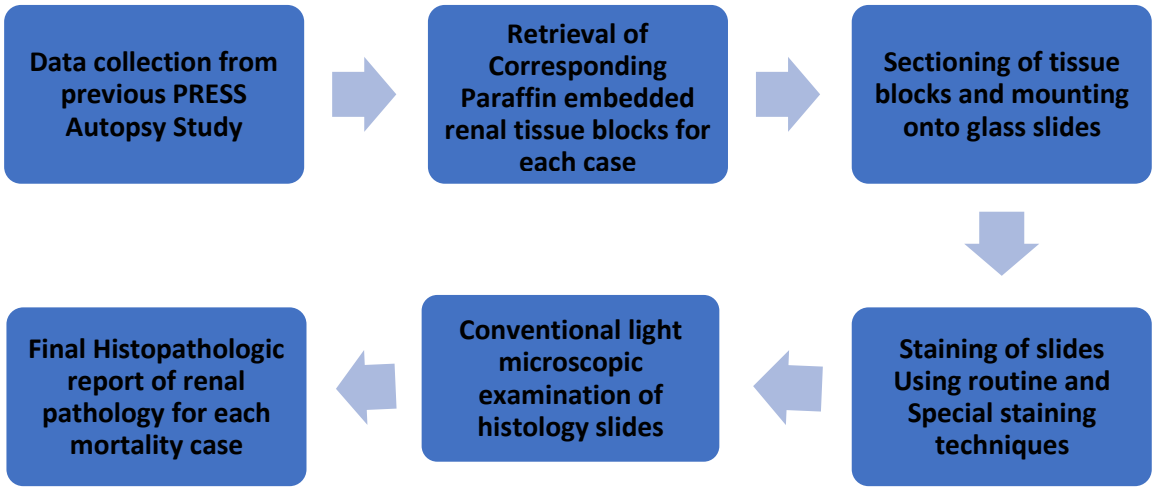
The Renal Pathology Society guidelines establish a minimal reporting standard for kidney tissue that ensures that all specific histopathologic elements and parameters required for formulation of a diagnosis are captured in the final histopathology report (Appendix II).

All clinical data from the preceding PRESS study and microscopic slides was analysed and data obtained reported in the following format.

- The gross (macroscopic) appearance and weights of the kidneys at autopsy from the previous PRESS study. The aetiologic agent of respiratory illness and biochemical renal function tests were also recorded from the findings of this previous study (Appendix I).
- The microscopic appearance of the processed kidney tissue.
- The adequacy of sampling of kidney tissue, inclusion of the cortex medulla and calyceal mucosa was assessed and reported.
- The status of the glomeruli was assessed and reported for number, hyper cellularity, sclerosis, leucocyte extravasation, mesangium and glomerular basement membrane changes.
- The interstitium was assessed for presence and pattern of inflammation, sclerosis and tubular atrophy.
- The presence of any infection directly observed within the kidney parenchyma.

- A concluding statement on the final patterns of pathologic change observed.
- The names of the two reporting pathologists and principal investigator signed for every case assessed. (Appendix II)

Figure 1: Study Procedure.



6. DATA MANAGEMENT AND STASTISTICAL ANALYSIS

All cases were assigned a unique study number and data collected using a structured data collection proforma for each.

Case data was entered into a well-designed Microsoft Excel database and data cleaned before analysis. All analysis was performed using Stata version 12.1(StataCorp, College Station, Texas USA)

Descriptive statistical analysis was performed using counts and respective percentages for categorical variables. Means with respective standard deviations or medians with respective interquartile ranges be used to describe the continuous data.

Bivariate comparisons were made between socio- demographic factors, and laboratory factors and their relation to the gross and microscopic findings (outcomes) using chi-square tests, fisher's exact tests and t-tests as appropriate while reporting respective p values. All statistical tests were evaluated at the 5% level of significance. Subsequently, descriptive and inferal statistics were presented as frequency, proportions and percentages in forms of narratives, tables, bar charts, pie charts and graphs. Microphotographs were used to present renal pathology observed on conventional light microscopy.

7. QUALITY ASSURANCE

All retrieved renal tissue blocks were well labeled and related data entered in well-designed data collection proformas (Appendix I).

A trained laboratory histotechnologist processed all tissue blocks.

All tissue blocks were processed adhering to the KNH/UON standard operating procedures (Appendix III-VII). All stains utilised were freshly prepared from properly stored reagents. All reagents were used in accordance with instructions from the manufacturer.

All renal histopathology findings were reported by the principal investigator and two blinded pathologists. A third experienced pathologist was used as a tiebreaker in case of lack of consensus.

Every tenth slide was examined by a third pathologist for review as a measure of quality control.

8. ETHICAL CONSIDERATIONS

Approval for the study was sought from the formal approval from the KNH/UON Ethics and Research Committee (ERC) and KNH administration. The study only commenced after ethical approval was obtained.

This study utilised data tissues collected during the preceding PRESS study. The principal investigator from the PRESS study had obtained ethical approval from the KEMRI Ethical and Research Committee to conduct his study (Appendix IX). The Approval included informed consent to perform clinical autopsies and to utilise tissues obtained during autopsy for future research (Appendix VIII).

Permission to utilise data and tissues collected by the Principal Investigator of the PRESS study was sought and granted (Appendix X).

Confidentiality of all data was maintained. All data collected during the study was kept with the principal investigator under supervision. Passwords were used to protect all digital data. No names were used instead a unique study number was used for each case

Once the study was completed all data was permanently deleted from all computes and all paperwork shredded.

All tissue blocks retrieved during the study were returned to their corresponding archives after processing of histology slides.

Upon completion of the study the findings were presented to the supervisors and the department of Human Pathology of the University of Nairobi. The findings will in future be published in peer reviewed journals and will be presented in seminars and medical conferences. Publishing or presentation of findings from this study will only be done after notification and approval to publish from the KNH-UoN ERC and KNH administration.

9. RESULTS

This study was conducted at the KNH/UON histopathology laboratories and involved analysis of preserved kidney tissues obtained in a preceding PRESS autopsy study in which 64 cases of SARI associated deaths in children aged between 1 month and 59 months were investigated for causative respiratory microbial agent. A total of 61 out of 64 cases were included in this study to identify the gross and histopathologic changes in the kidneys of these children. Three cases were excluded for the following reasons.

1. Poorly preserved kidney tissue in one case.
2. Death not associated with infective SARI aetiology in two cases. These fatalities were concluded to have been caused by organophosphate poisoning and aspiration pneumonitis respectively. No organisms were identified within lung tissue in these two cases.

Demographic characteristics

A total of 61 paediatric patients who died due to severe acute respiratory illness (SARI) were included in the analysis. Table 1 presents the demographic characteristics of the cases of fatal paediatric SARI. The mean age of the cases was 10.7 months (SD ± 10), and the age range from 1 to 48 months. Most of the patients who died from SARI were infants aged 1-11 months 44 (72.1%). There were 33 females accounting for 54.1% of the fatal cases and yielding a female to male ratio of 1.2: 1. The mean age of males (10 months) did not differ significantly (p = 0.599) from that of females (11.3 months).

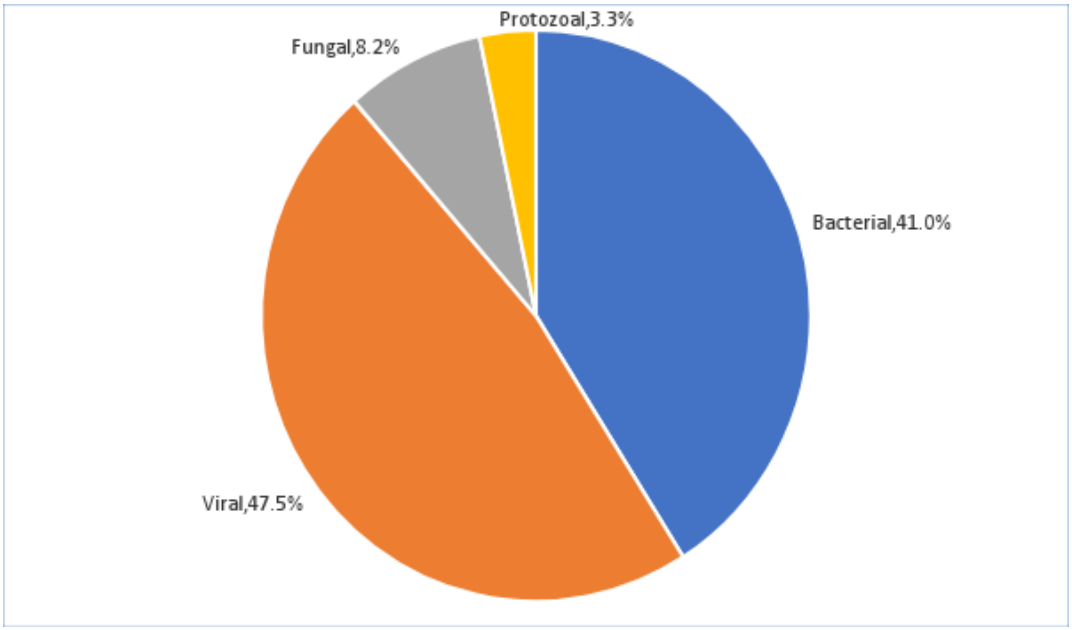
Table 1: Demographic characteristics of fatal paediatric SARI cases.

		Frequency (n)	Percent (%)
Age			
	< 12 months	44	72.1
	12-23 months	11	18
	24-35 months	3	4.9
	36-48 months	3	4.9
Gender			
	Male	28	45.9
	Female	33	54.1

Aetiologic agents identified in fatal Paediatric SARI cases.

The most common aetiologic causes of fatal paediatric SARI were viral 29 (47.5%) and bacterial 25 (41%) infections (Figure 2).

Figure 2: Aetiologic agents identified in fatal paediatric SARI cases.



There was no association between patient age and the aetiologic agent isolated in SARI (Table 2). The mean age of infection with bacterial and viral agents in fatal paediatric SARI was 11.7 ± 12.8 and 10.1 ± 6.4 months (p = 0.562), respectively. There were two fatal paediatric SARI cases directly attributable to protozoal infections and five (mean age 4.2 ± 3.3 months) to fungal infections.

Table 2: Mean age (SD) of pediatric patients according to identified aetiologic cause

	n	Mean	SD	p value
Bacterial	25	11.7	12.8	0.562*
Viral	29	10.1	6.4	
Fungal	5	4.2	3.3	
Protozoal	2	23.5	17.7	

based on a comparison of mean age for bacterial and viral infections.

Co morbidities identified in fatal Paediatric SARI.

Analysis of the final cause of death in the 61 cases showed that 56 (91.8%), had at least one co morbid condition contributing to death. Gastroenteritis with dehydration (21.4%) and meningitis (17.9%) were identified as the major co morbidities associated with fatal SARI. Table 3 presents the co morbid conditions identified during clinical course in these cases.

Table 3: Co morbidities identified in fatal paediatric SARI cases.

Co morbidity	Frequency (n)	Percent (%)
Gastroenteritis with dehydration	12	21.4
Meningitis	10	17.9
Severe malnutrition	7	12.5
HIV	6	10.7
Congenital malformation/disease	5	8.9
Sepsis	5	8.9
Prematurity	4	7.1
Rickets	2	3.6
Intestinal obstruction	1	1.8
Leishmaniasis	1	1.8
Sickle cell disease	1	1.8
Liver disease	1	1.8
Malaria	1	1.8
	56	**100**

Gross morphologic changes to the kidneys in fatal paediatric SARI

Twelve (19.7%) of fatal paediatric SARI cases had abnormal general appearance of the kidneys on autopsy (Table 4). The predominant morphological abnormality identified was a pale cortex in 5 (8.2%) of cases. Incidental renal cortical cysts were identified in one case (1.6%). Each of the remaining seven presentations of morphological abnormalities shown in table 4 (hilar haematoma, exudate within the medulla, dilated calyceal system, calyceal stones, underdeveloped kidneys, and a kidney within the pelvis) occurred in a single child. No abscesses were identified on gross examination of the kidneys.

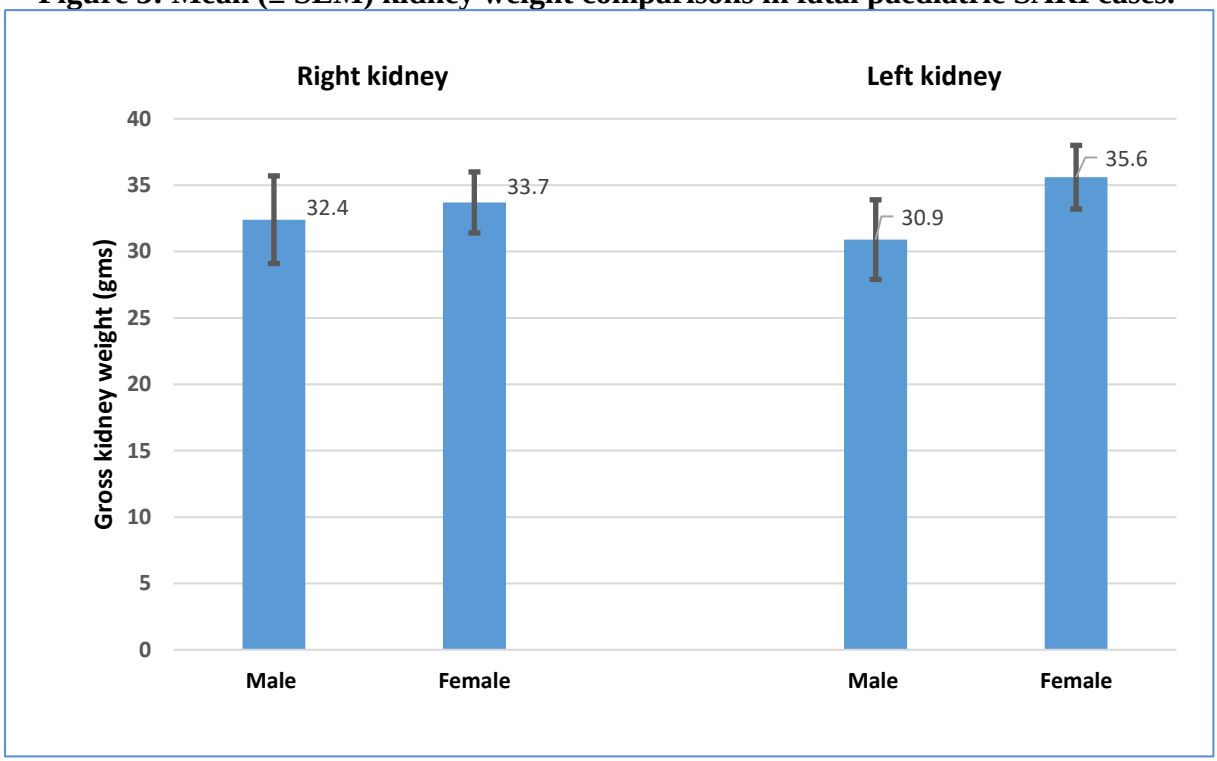
Table 4: Gross morphological changes to the kidneys identified at autopsy in fatal paediatric SARI cases.

	Frequency (n)	Percent (%)
General Appearance of the Kidneys		
Normal	49	80.3
Abnormal	12	19.7
Morphological Anomalies		
Pale cortex	5	8.2
Underdeveloped kidneys	1	1.6
Renal cortical cysts	1	1.6
Hilar haematoma	1	1.6
Exudate in the medulla	1	1.6
Dilated calyceal system	1	1.6
Kidney within pelvis	1	1.6
Renal calyceal stones	1	1.6
Abscesses	0	0

Mean kidney weight recorded at autopsy.

The mean kidney weight (± SEM) for all fatal paediatric SARI cases was 33.5 ± 1.9gms for the right kidney compared to 33.1 ± 1.9 for the left kidney (p = 0.626). Figure 2 compares mean unfixed kidney weights in males and females and shows no significant differences in mean kidney weights between males and females for either the right (32.4 versus 33.7gms, p = 0.291) or left (30.9 versus 35.6gms, p = 0.751) kidney.

Figure 3: Mean (± SEM) kidney weight comparisons in fatal paediatric SARI cases.



Comparison between mean unfixed kidney weights in males and females showing no significant differences in mean kidney weights between males and females for either the right (32.4 versus 33.7gms, p = 0.291) or left (30.9 versus 35.6gms, p = 0.751) kidney. There is also no significant difference in mean combined unfixed kidney weight between right (33.5 ± 1.9gms) and left (33.1 ± 1.9gms, p = 0.626) kidneys.

Glomerular histopathologic patterns identified in fatal paediatric SARI.

A Glomerular histopathologic abnormality was identified on light microscopy in 60 (98.4%) of fatal paediatric SARI cases. Table 5 shows the distribution of the glomerular histopathologic changes identified. Majority of cases exhibited a diffuse endocapillary proliferative glomerulonephritis (64.1%). Focal segmental glomerulonephritis was identified in 18%, membranoproliferative or membranous glomerulopathy in 13.1% and cellular crescents in 3.2% of the cases. There were no light microscopic changes (abnormality detected on light microscopy) in 1 (1.6%) case.

Table 5: Glomerular morphologic findings in fatal paediatric SARI cases.

Glomerular histopathologic pattern	Frequency (n)	Percent (%)
Diffuse Endocapillary Proliferative GN	39	64.1
Focal Segmental Glomerulosclerosis (FSGS)	11	18
Membranous/ Membranoproliferative GN	8	13.1
Crescents	2	3.2
Normal/ Minimal change disease	1	1.6
Total	**61**	**100**

There was no correlation between glomerular histopathologic patterns identified on light microscopy and SARI aetiologic agent, age or gender (p values of >0.05) in fatal paediatric SARI cases.

Table 6, 7 and 8 show correlations between histopathologic patterns identified in the glomerulus with SARI aetiologic agent, age and gender.

Table 6: Correlation between glomerular histopathologic changes identified and gender in fatal paediatric SARI cases.

Glomerular histopathologic pattern	Gender		
	Male (n)	Female (n)	p value
Diffuse Endocapillary proliferative GN	16	23	0.136
FSGS	6	5	0.740
Membranous/Membranoproliferative GN	3	5	0.647
No morphologic abnormality/ MCD	1	0	0.459

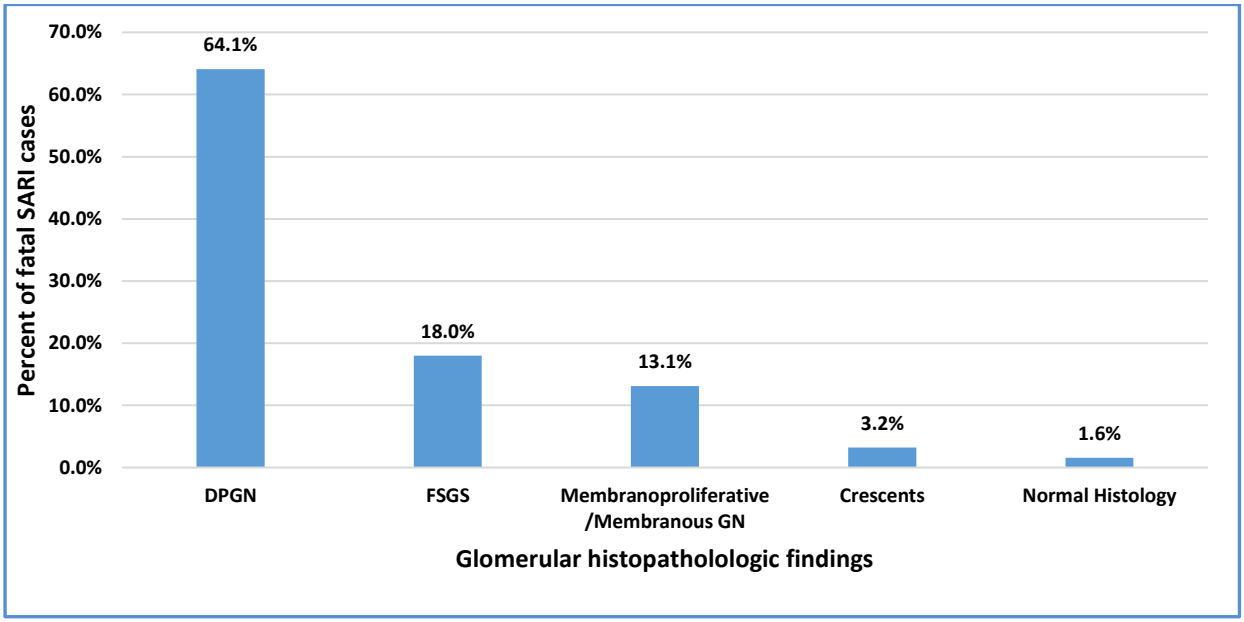
Table 7: Correlation between glomerular histopathologic changes identified and age in fatal paediatric SARI cases.

Glomerular histopathologic pattern	Age in months				
	< 12 months (n)	12-23 months (n)	24-35 months (n)	36-48 months (n)	p value
Diffuse Endocapillary proliferative GN	26	11	2	0	0.165
FSGS	8	2	0	1	0.907
Membranous/Membranoproliferative GN	5	2	1	0	0.649
No morphologic abnormality/ MCD	1	0	0	0	1

Table 8: Correlation between glomerular histopathologic changes identified and causative agent in fatal paediatric SARI cases.

Glomerular histopathologic pattern	SARI aetiologic agent				
	Bacterial (n)	Viral (n)	Fungal (n)	Protozoal (n)	p value
Diffuse Endocapillary proliferative GN	17	19	2	1	0.617
FSGS	2	5	3	1	0.027
Membranous/Membranoproliferative GN	3	5	0	0	0.529
No morphologic abnormality/ MCD	0	1	0	0	1

Figure 4: Distribution of glomerular histopathologic patterns identified in fatal Paediatric SARI cases.



Distribution of glomerular histopathologic changes in fatal Paediatric SARI. Diffuse Proliferative GN in 64.1% (n=39), FSGS in 18% (n=11), Membranoproliferative/Membranous in 13.1% (n=8), Crescents in 3.2% (n=2), Normal histology/MCD in 1.6% (n=1). There is no co relation between glomerular histopathologic patterns observed on light microscopy with SARI aetiologic agent, age and gender (p = >0.05 for bacterial, viral, fungal and protozoal infections)

Tubulointerstitial histopathologic changes in fatal paediatric SARI cases.

Tubulointerstitial pathology was identified in 37 (60.6%) of fatal paediatric SARI cases. Acute Tubular necrosis was the most commonly observed histopathologic change identified on conventional light microscopy in fatal SARI cases with 34.4% of all cases exhibiting this change. In 16 (26.2%) cases, focal interstitial nephritis was observed comprising of lymphocytic (21.3%), lymphoplasmacytic (3.3%) and eosinophilic type in 1.6% of cases (Table 9).

Table 9: Tubulointerstitial histopathologic changes in fatal paediatric SARI cases.

Tubulointerstitial histopathologic pattern	Frequency (n)	Percent (%)
Acute Tubular Necrosis	21	34.4
Focal Interstitial Nephritis		
Lymphocytic type	13	21.3
Lymphoplasmacytic type	2	3.3
Eosinophilic type	1	1.6
Normal appearing tubulointerstitium	24	39.3
Total	**61**	**100**

There was no correlation between Tubulointerstitial histopathologic patterns identified on light microscopy and SARI aetiologic agent, age or gender (p values of >0.05) in fatal paediatric SARI cases.

Table 10, 11 and 12 show correlations between histopathologic patterns identified in the tubulointerstitium with SARI aetiologic agent, age and gender.

Table 10: Correlation between tubulointerstitial histopathologic changes and age in fatal pediatric SARI cases.

Tubulointerstitial histopathologic finding.	Age in months				
	< 12 months (n)	12-23 months (n)	24-35 months (n)	36-48 months (n)	p value
Focal Interstitial Nephritis	11	4	1	0	0.259
Acute Tubular Necrosis	15	4	1	1	0.330

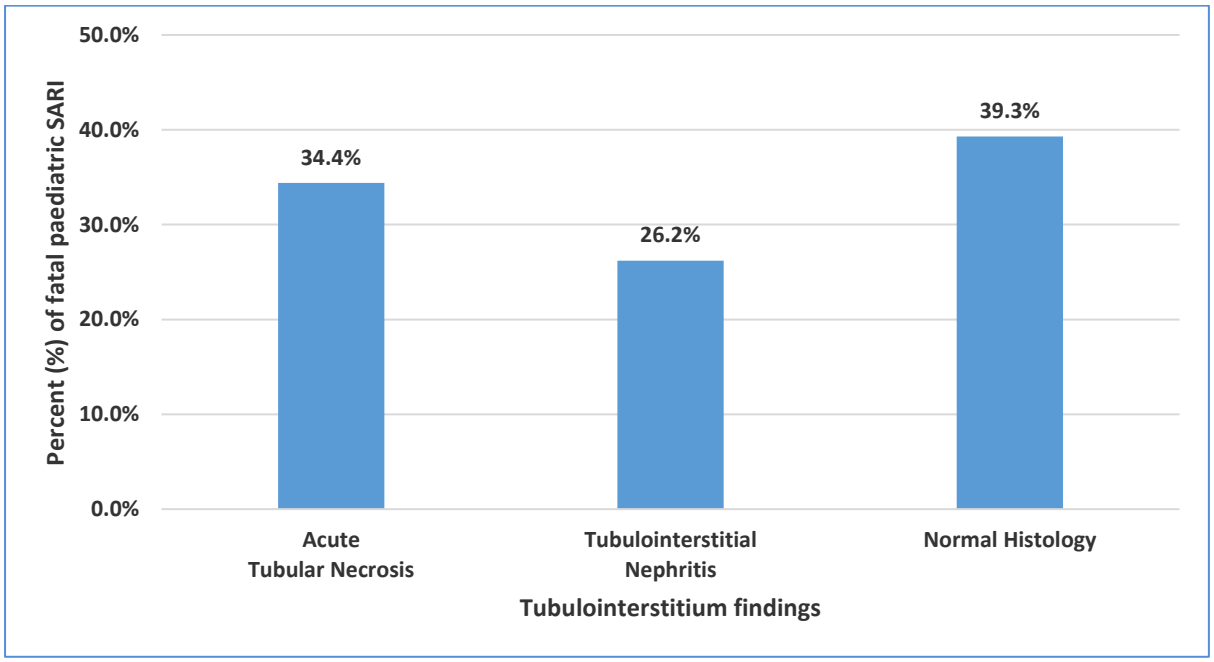
Table 11: Correlation between tubulointerstitial histopathologic changes and gender in fatal pediatric SARI cases.

Tubulointerstitial histopathologic finding	Gender		p value
	Male	Female	
Focal Interstitial Nephritis	7	9	0.716
Acute Tubular Necrosis	14	7	0.418

Table 12: Correlation between tubulointerstitial histopathologic changes and causative agent in fatal paediatric SARI cases.

Tubulointerstitial histopathologic finding	SARI etiologic agent				
	Bacterial (n)	Viral (n)	Fungal (n)	Protozoal (n)	p value
Focal Interstitial Nephritis	4	9	3	0	0.525
Acute Tubular Necrosis	10	9	2	0	0.209

Figure 5: Distribution of tubulointerstitial histopathologic patterns identified in fatal paediatric SARI cases.



Distribution of tubulointerstitial histopathologic changes in fatal paediatric SARI cases. Acute tubular necrosis in 34.4% (n=21), focal tubulointerstitial nephritis in 26.2% (n=16), Normal histology in 39.3% (n=24). There is no co relation between tubulointerstitial histopathologic patterns observed with SARI aetiologic agent, age and Gender (p = >0.05 for bacterial, viral, fungal and protozoal infections)

There were associated tubulointerstitial pathologic observations seen in 33 (89%) of cases in which either tubular necrosis or interstitial nephritis was observed. PAS positive distal hyaline casts characteristic of Tam-Horsfall protein were seen in 11 cases, intratubular mineral deposition in 9 cases, distal tubular RBC casts in 5 cases while interstitial haemorrhage was observed in 4 cases. A further 4 cases showed viral cytopathic effects or viral inclusion bodies within tubular epithelial cells. CMV (Cytomegalovirus) like viral cytopathic effects were identified morphologically in tubular epithelial cells in 2 cases while influenza and RSV (Respiratory Syncytial Virus) type effect on tubular epithelial cells were identified in one case each (Table 13).

Table 13: Associated tubulointerstitial histopathologic features in fatal paediatric SARI cases.

	Frequency (n)
Hyaline pas positive casts	11
Intratubular mineral deposition (calcium)	9
RBC tubular casts	5
Interstitial haemorrhage	4
Viral cytopathic effects on tubular epithelial cells	4
Total	**33**

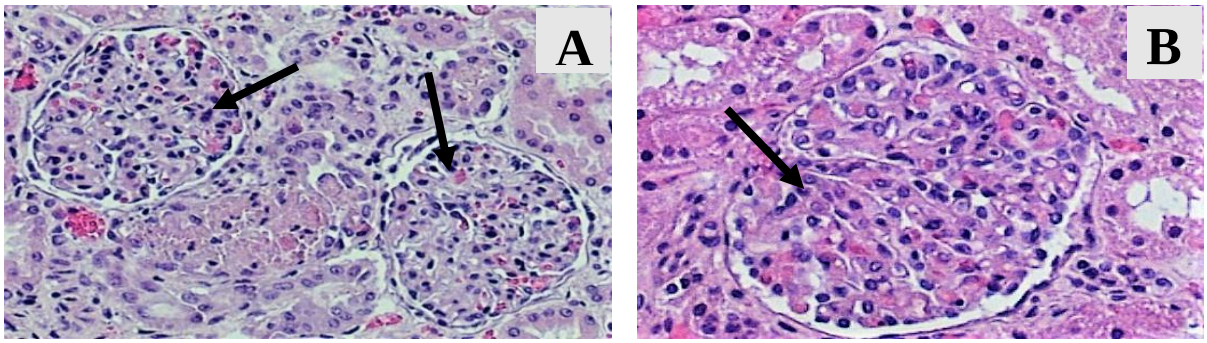
Renal vascular histopathologic changes in fatal paediatric SARI.

13 cases (21.3%) showed abnormal vasculature on light microscopic evaluation (Table 14). Majority (11.5%) showed arteriolar arteriolosclerosis within renal cortical blood vessel walls. Other findings included vascular congestion in 4 cases (6.3%) and thrombosis in 2 cases (3.3%).

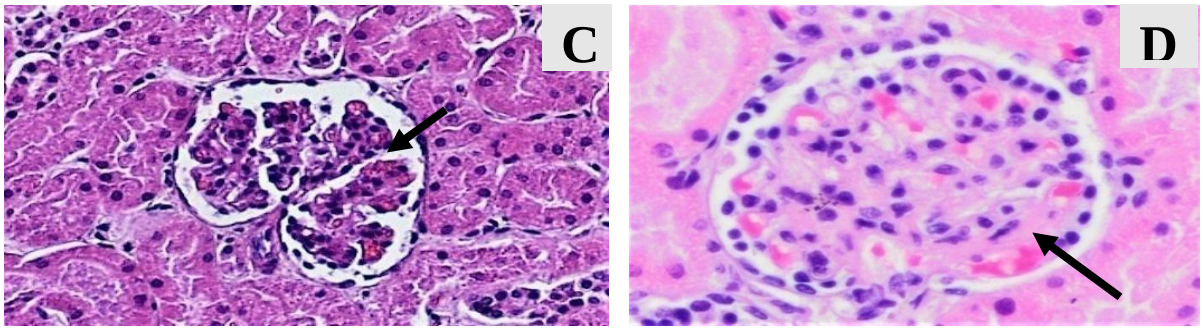
Table 14: Vascular histopathologic changes in fatal paediatric SARI cases.

Vascular histopathologic pattern	Frequency (n)	Percent (%)
Normal microvasculature	48	78.7
Arteriolar arteriolosclerosis	7	11.5
Vascular Congestion	4	6.3
Thrombosis	2	3.3
Total	**61**	**100**

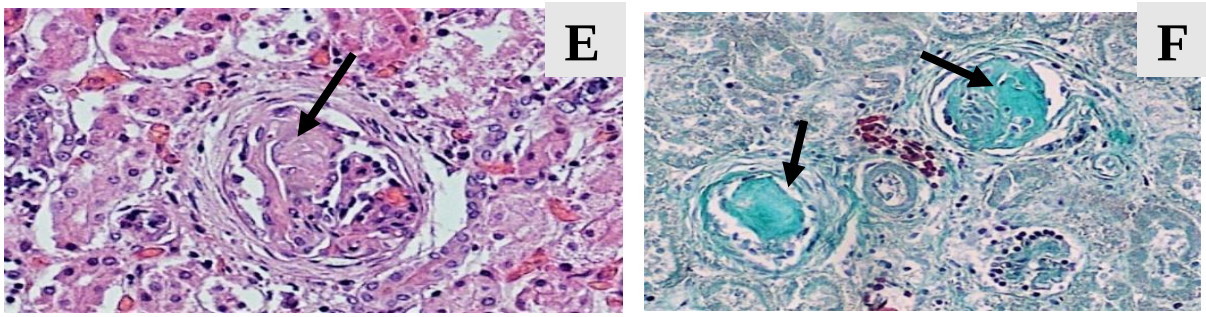
Figure 6. Glomerular histopathologic patterns on conventional light microscopy in fatal paediatric SARI cases.



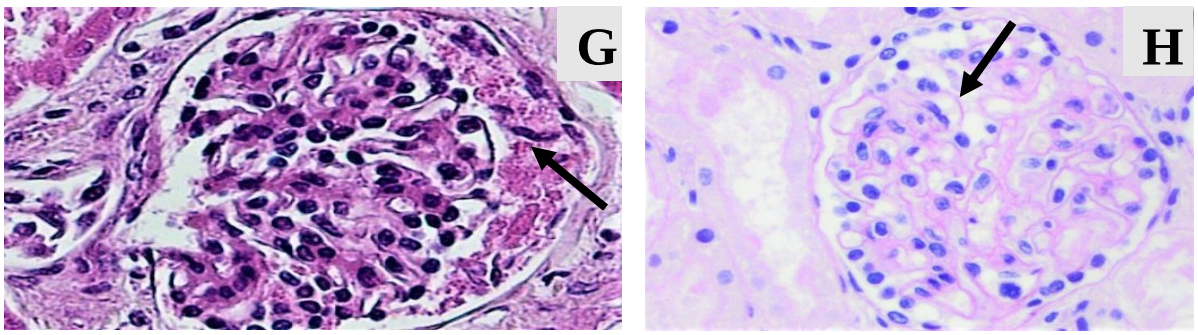
Panel A Shows two glomeruli (arrows) exhibiting lymphocytic and endocapillary proliferation in case 24 (H&E X200). **Panel B** shows a single glomerulus (arrow) exhibiting similar lymphocytic and endocapillary proliferation with marked hypercellularity in Case 21 H&E X400).



Panel C Shows consolidated expansion of mesangial segments with prominent lobulation as clearly demonstrable by urinary space demarcating the individual lobules (arrow) in case 52 (PAS X200). **Panel D** shows mesangial cell hypercellularity with increased mesangial matrix (arrow) incase 20 (H&E X400).

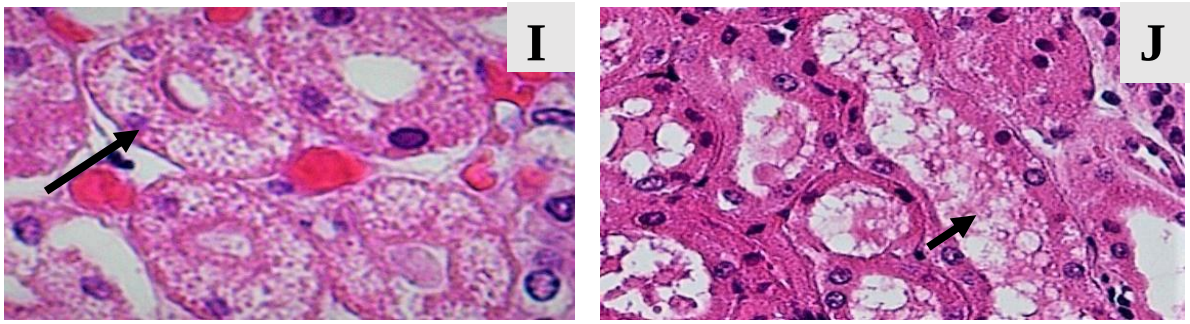


Panel E shows a single glomerulus exhibiting segmental glomerulosclerosis in case 61(H&E X200). **Panel F** shows two glomeruli (arrows) displaying similar segmental glomerulosclerosis (Masson's Trichrome X200).

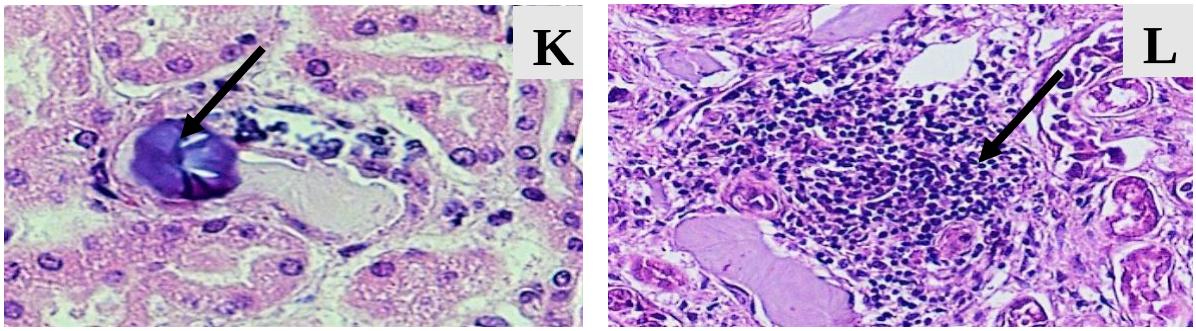


Panel G shows a single glomerulus exhibiting cellular epithelial crescent formation (arrow) (H&E X400) in case 24. **Panel H** shows a single glomerulus exhibiting prominent and thickened glomerular basement membrane (arrow) in case 21(PAS X400).

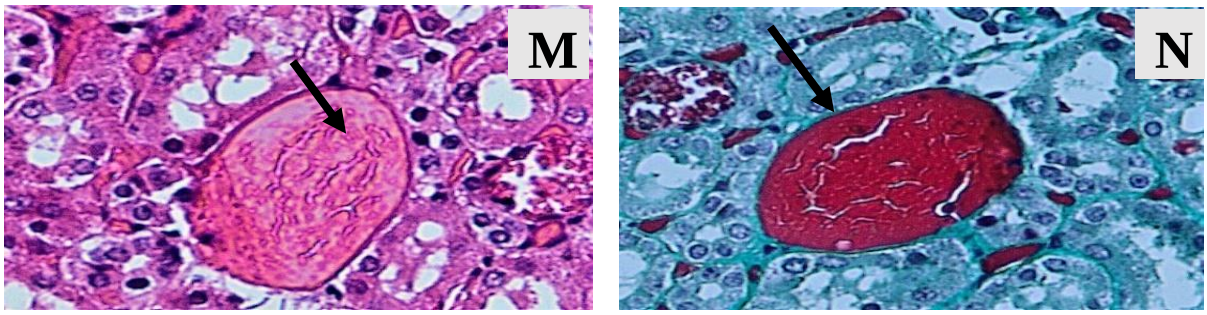
Figure 7: Tubulointerstitial histopathology on conventional light microscopy in fatal paediatric SARI cases.



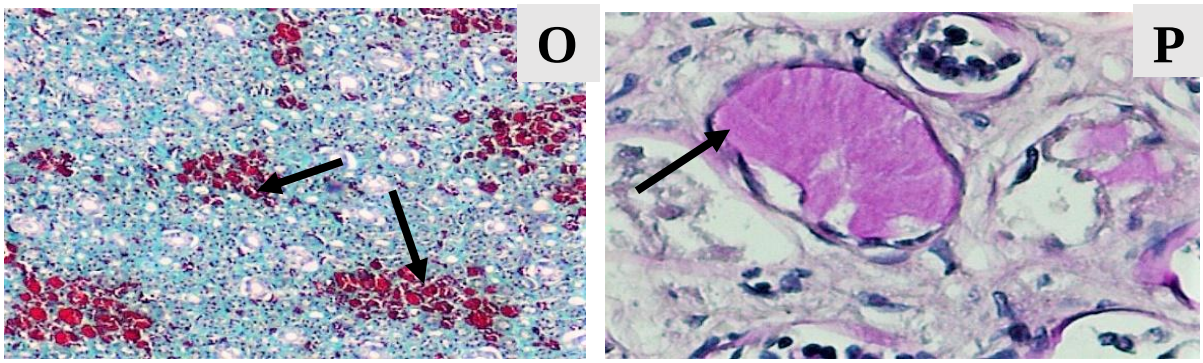
Panel I shows prominent vacuolation and hypopigmented nuclei in proximal tubular epithelial cells (arrow) signifying tubular cell injury in case 61 (H&E X400). **Panel J** shows dilated exudate filled proximal tubules (arrow) with resultant flattening of the lining epithelial cells in case 3 (H&E X200)



Panel K shows intratubular calcium deposition (arrow) appearing as an amorphous intratubular crystal in case 24 (H&E X200). **Panel I** shows a focus of interstitial inflammation (arrow) attended mainly by small mature lymphocytes in case 10 (H&E X100).

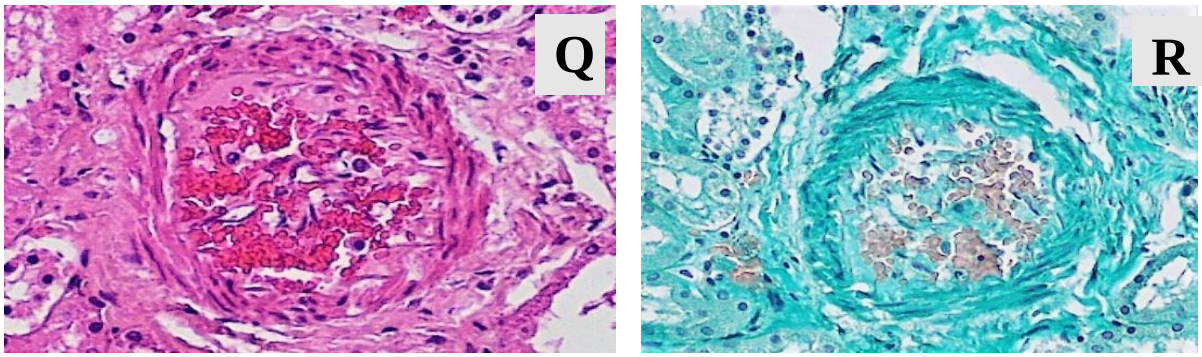


Panel M shows a dilated and thrombosed interstitial blood vessel Red blood cells are clearly visible within the lumen (arrow) (H&E X200). **Panel N** shows the same vessel at a deeper section where the vessel and its contents are elaborated in Masson's trichrome satin (X200).

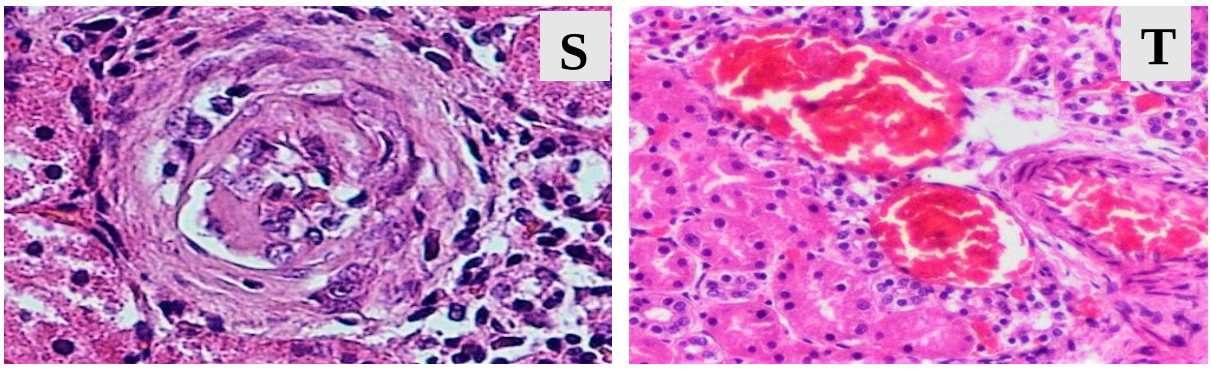


Panel O shows multiple foci of interstitial haemorrhage (arrows) in case 4 (Masson's Trichrome X40). **Panel P** shows a distal convoluted tubule dilated by PAS positive tubular material (arrow) consistent with Tam Horsfall protein in case 10 (PAS X200)

Figure 8: Arteriolar histopathology findings on conventional light microscopy in fatal paediatric SARI cases.

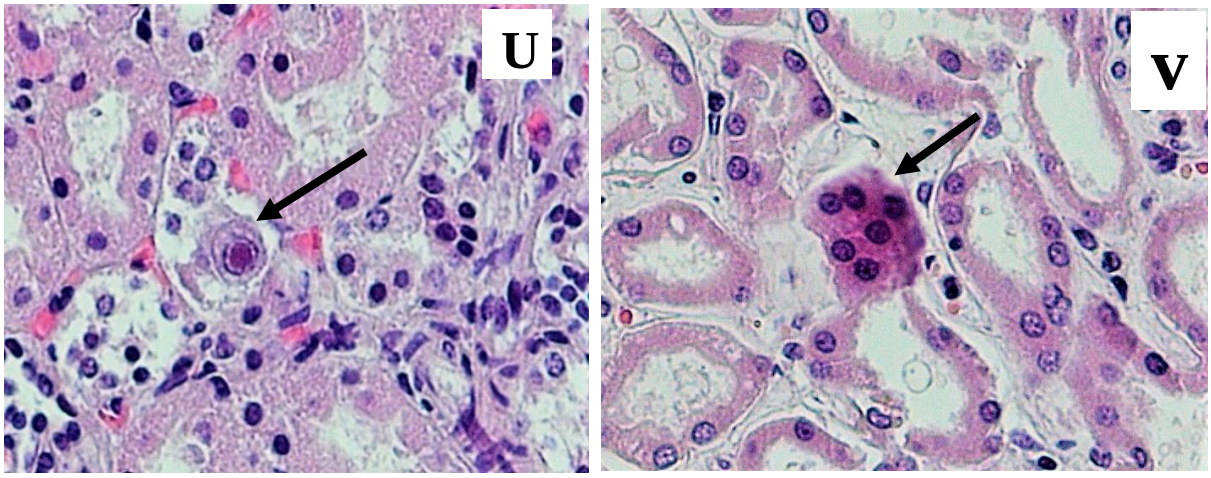


Panel Q shows a thrombosed interstitial arteriole within which there are numerous red blood cells within the arteriole in case 32 (H&E X100) **Panel R** shows the same vessel as demonstrated by Masson's Trichrome Stain



Panel S shows an arteriole demonstrating arteriolar arteriolosclerosis with a thickened vascular wall giving it an "onion skin" appearance in case 6 (H&E X200). **Panel T s**hows vascular congestion within venules in the interstitium in case 15 (H&E X100)

Figure 9: Viral inclusions in tubular epithelial cells in fatal paediatric SARI cases.



Panel U shows a viral inclusion body morphologically consistent with CMV within a tubular epithelial cell. The classic owl eye nuclear appearance is demonstrated by the arrow in case 17 (H&E X200). **Panel V** shows viral cytopathic effects morphologically consistent with RSV in case 24 (H&E X200). A characteristic multinucleated giant cell with round intracytoplasmic inclusions is demonstrated by the arrow.

10. DISCUSSION

Infections and sepsis are a leading cause of AKI in critically ill children. Literature on the morphologic and structural changes to the kidneys in children diagnosed with respiratory infections is extremely limited. Analysis of gross and histopathologic changes to the kidneys at autopsy presented a unique opportunity to elaborate these changes. This study evaluated the gross and histopathologic changes to the kidneys from 61 children who died due to Severe Acute Respiratory Illness in KNH between August 2014 and December 2015 from kidney tissue obtained at autopsy. The age of these children ranged from 1- 48 months with a mean age of 10.7 months (± 10).

Gross morphologic kidney changes in fatal paediatric SARI.

There was no significant variation in kidney weight distribution between the right and left kidneys in fatal paediatric SARI mortalities (Mean right kidney weight 33.5 ± 1.9gms and 33.1 ± 1.9gms for the left kidney ($p = 0.626$)). There was also no significant gender variation in kidney weights in these cases (Right kidney 32.4gms in males versus 33.7gms in females, $p = 0.291$ and left kidney 30.9gms in males versus 35.6gms in females, $p = 0.751$). The combined average kidney weights measured at autopsy were all within 95 percentiles for age in both boys and girls. This corresponds with normal reference ranges for autopsy kidney weights in infants at autopsy by Pryce (46). However, there is evidence demonstrating increased kidney weight at autopsy in critically ill children as described by Proulx (47). This study demonstrated no significant kidney weight changes. This may be explained by the acute nature and short duration between onset of a SARI and death and possible co morbidities in our study's population that may contribute to relative organ weight reduction where an increase was expected. Concurrent malnutrition seen in 12.7% of cases in this study has been associated with reduction in gross kidney weight in children as demonstrated by Ece (48). Moreover, prematurity which was a co morbidity in 7.1% of cases in this study, has been associated with reduction in gross kidney weights in children in a study by Shmidt, where premature children had smaller kidneys compared to mature at all ages, without any significant catch-up with progressing age (49).

Gross analysis of kidneys at autopsy also revealed at least one morphologic abnormality in 19.7% of cases. The most common grossly observable morphologic change of the kidneys in fatal paediatric SARI cases was bilateral pale cortices seen in 8.2% of the cases. A pale renal

cortex is indicative of renal cortical hypoperfusion as a result of congestion in cortical arterioles or by a drastic reduction in systemic blood pressure. Zhang has shown that 10% of all cases of renal cortical hypoperfusion and necrosis occur in infants and children (50). These cases are most commonly associated with infections and severe dehydration. This correlates well with this study where all cases were of mortality associated with complications arising from overwhelming severe acute respiratory illness. Moreover, in this study severe dehydration was observed as co morbidity in 24.1% of fatal SARI cases.

Spectrum of histopathologic changes to the kidneys in fatal paediatric SARI.

The analysis of data in this study revealed majority of paediatric SARI mortality cases had at least one histologic abnormality identified on conventional light microscopic examination. A Glomerular histopathologic pattern was identified in 98.4% of all cases, while tubulointerstitial histopathology was demonstrated in 60.6 % of cases. 21.1% of all cases showed abnormalities in renal vasculature on light microscopic examination. Autopsy studies detailing histomorphologic changes to the kidney in children are rare, however study on autopsy renal biopsy tissue by Rameshkumar established normal histology in 41.9%, tubular pathology in 30.6% and vascular abnormalities in 8% of critically ill children dying from severe infections (51). These findings differ significantly with results from this study suggesting SARI associated kidney disease is a unique entity with characteristic renal histopathology. Moreover, in this study 91.8% of fatal SARI cases were associated with co morbid conditions which independently shown to cause or contribute to renal disease.

<u>Glomerular histopathology in fatal paediatric SARI.</u>

The most common histopathologic pattern identified within in the glomerulus in fatal paediatric SARI cases was Diffuse Proliferative Glomerulonephritis (DPGN), seen in 64.1% of the cases. DPGN is well-established renal sequelae of systemic infections and is a true immune mediated glomerulonephritis. This type of injury results from the deposition of immune complexes within the mesangium and activation of complement system through the classical pathway and resultant recruitment of inflammatory cells i.e. lymphocytes and neutrophils with resultant proliferation of the mesangial and endothelial cells. The cellular immunologic attack of the glomerulus results in the glomerular basement membrane becoming permeable to proteins and red blood cells which is revealed as cellular casts in routine urinalysis with various degrees of proteinuria.

The classic clinical presentation is of acute glomerulonephritis. Balasubramanian described Group A gram positive β-hemolytic streptococcus bacteria as most common respiratory cause of acute glomerulonephritis in children but also showed viral infections and parasitic infections often cause acute glomerulonephritis attended by this classic endocapillary and mesangial hypercellularity (52). Lovett further demonstrated this specific mechanism in infections associated with gram negative bacteria mediated by antibodies produced against liposaccharides and protein 1 within gram negative bacterial cell walls (53). Influenza and Adenovirus infections have also been implicated in post infectious proliferative glomerulonephritis by Sotsiou et al where cell mediated mechanisms result in production of pro- inflammatory mediators including IL1 and TNF (54).

Acute post infectious glomerulonephritis with characteristic mesangial hypercellularity may further progress into a membranoproliferative type glomerulonephritis (MPGN) as the mesangium expands as immune deposits occur within it. Further transformation into membranous type glomerulonephritis (MGN) may also occur as subendothelial deposits become a prominent feature leading to a thickened glomerular basement membrane. This study found these characteristic histopathologic features in 13.1% of cases. In cases that are associated with very severe infections, further progression of this type of injury to the glomerular basement caused by immune deposition may result in epithelial crescent formation as seen in two cases in this study. In these particular cases, less than 50% of all glomeruli were involved, likely signaling a progression of DPGN rather than overt primary crescenteric glomerulonephritis.

The results in this study therefore indicate a clear progression from DPGN observed in majority of cases to rare crescent formation in extremely few cases indicating the nature of progressive glomerular injury in fatal paediatric SARI.

Focal Segmental glomerulosclerosis (FSGS) as identified in 18% of cases in this study is most common glomerular histologic pattern identified during investigation of idiopathic proteinuria in otherwise well children. FSGS has also been linked to HIV nephropathy (42). However, in this study the observed characteristic segmental solidifications of the glomerular tuft are also additionally likely to be secondary to persisting immune mediated inflammatory processes resulting from severe infection as studies linking Enterobacteriaceae infections, mainly *Klebsiella pneumoniae* with FSGS with have been described.

Data analysis in this study also showed no correlation between the glomerular histopathologic changes observed with SARI aetiologic agent, age or gender, (P values> 0.05) for bacterial, viral fungal and protozoal infections. This indicates the likelihood that the glomerular response is non-specific to causative organism and postulates that systemic inflammatory mediators to these microbes are responsible for pathologic changes within the glomerulus in fatal SARI cases.

Tubulointerstitial histopathologic features in paediatric SARI:

In this study histological evidence of Acute tubular necrosis (ATN) characterised by varying degrees of tubular cell structural alteration and damage was observed in 34% of fatal paediatric SARI cases. These changes were highlighted by characteristic patchy necrosis and loss of proximal tubular cell brush border, tubular dilation and evidence of attempts at regeneration. These histopathologic hallmarks of ATN commonly result from renal parenchymal injury through ischaemia, nephrotoxic agents and sepsis. In this study these features were notably associated with focal collections of various intra renal inflammatory cells which are characteristic of tubulointerstitial nephritis. In 26% of cases focal collections of lymphocytes, plasma cells and rarely eosinophils were present within the interstitium.

In overwhelming infections such as in cases of SARI, pro inflammatory cytokines produced by activated inflammatory cells have been demonstrated to be toxic to renal tubular cells. Baud showed the effect of TNF on tubular epithelial cells in animal-based study while Chwala demonstrated effects of IL6 (55, 56). Vasoactive compounds such as endothelins and reactive nitrous oxide which are also produced as a vascular response in overwhelming infections have also been shown to play a role in tubular cell injury by Heemskerk (57). The presence of inflammatory cell collections therefore suggests a source and modulator for these pro inflammatory mediators. Furthermore, local inflammation within the kidney has been recognised as a factor contributing to distant inflammatory injury in other organs as shown by Li (58), which may be a contributory factor to mortality in children with SARI.

To date comparative morphologic studies at autopsy in children have not been carried out to demonstrate these phenomena. However early autopsy studies in adults who died following overwhelming infections in intensive care units have demonstrated near normal tubulointerstitial kidney histologies. Hotchkiss and Karl showed normal kidney histology in 90% of cases while Langeenburg demonstrated only nonspecific histological changes in adults (59, 60). These findings contrast sharply with this study where significant renal tubular

injury and interstitial inflammation is demonstrated. This may be due to the contrasting paediatric population this study, and the specific host factors such as co morbidities, possible nephrotoxic therapeutics used during life and probable concurrent hypoxia in severe respiratory illness. Analysis of data in this study also showed no correlation between the histopathologic changes observed within the tubulointerstitium with SARI aetiologic agent, age or gender, (P values >0.05) for bacterial, viral fungal and protozoal infections. This further asserts the likelihood that inflammatory mediators and the host response to these organisms play the key role in tubulointerstitial disease in fatal SARI cases.

There were other concurrent histopathologic features observed within the tubulointerstitium in association with tubular necrosis and interstitial nephritis that demonstrated both intrinsic renal cellular injury and the response of the renal parenchyma to injury. PAS positive smooth textured distal intratubular casts characteristic renoprotective solidified Tam- Horsfall Protein secretion by the tubular epithelium were frequently identified. Intratubular mineral likely calcium was also observed in a significant number cases and likely indicates prior dehydration or use of nephrotoxic agents. Also identified clearly with the aid of trichrome staining were distal tubular RBC casts characteristic of severe tubular damage or leakage of red blood cells through a compromised glomerulus and patchy intraparenchymal haemorrhage which indicates increased vascular permeability and leakage of blood into the interstitium. Cellular casts indicative of proximal tubular cell fall off and collection further downstream at the distal tubules were also identified in fewer cases.

Further direct injury to the tubular epithelial cells was demonstrated by characteristic viral inclusion bodies within tubular epithelial cells in 4 cases. Viral Inclusion bodies have been described commonly in histologies of renal transplant patients and HIV in the background of immunosuppression. The immune status of the children in this study was however not fully documented from previous history and investigations therefore no correlation was made in regard to HIV infection. It is also noteworthy that co morbid conditions such as severe malnutrition, prematurity and rickets can also lead to immunosuppression.

Renal vascular changes in fatal Paediatric SARI**.**

This study demonstrated morphologic changes to the microvasculature in 21.3% of SARI associated paediatric mortalities. Majority (11.5%) of cases exhibiting vascular abnormalities showed arteriolar arteriolosclerosis. Thrombosis was observed in 6.3% while 6.3% demonstrated vascular congestion. Comparatively, Rameshkumar had demonstrated the main

vascular abnormalities in sepsis associated AKI as microthrombi in blood vessels and vascular congestion (51). Shear and Rosner have also postulated that renal congestion is a key mechanism in kidney injury in sepsis especially in capillary leakage (61). However, early onset of severe infections has been associated with increased intrarenal vascular resistance and may explain the predominance of arteriolar arteriolosclerosis as an adaptive feature of renal microvasculature in fatal paediatric SARI cases. Arteriolosclerosis is also seen in cases of severe tubulointerstitial damage and may indicate aggressive renal disease in cases where it is observed.

11. STUDY LIMITATIONS

1. Assessments of renal morphology in single time period may provide a limited perspective considering the adaptive and dynamic nature of kidney response injury in the background of clinically overwhelming infections. Furthermore, the interpretation of morphologic changes in histopathology may result from completely different molecular mechanisms as the kidney parenchyma responds in similar fashion to various injurious phenomena.

2. Conventional light microscopy even with additional special stains may not be adequate to extensively demonstrate kidney histopathology. Immunohistochemical and electron microscopy may demonstrate structural changes to the renal parenchyma with significantly better detail.

3. Gross morphologic features listed were obtained earlier and the principal investigator had no opportunity to examine the same.

12. CONCLUSIONS

1. There are significant gross and acute histopathologic changes in kidneys of children who succumbed to fatal paediatric SARI in KNH.

2. The most common glomerular histopathology in fatal Paediatric SARI is Diffuse Proliferative Glomerulonephritis (DPGN), while within the tubulointerstitium Acute tubular necrosis (ATN) and focal interstitial nephritis are a predominant finding. Comparatively, renal microvascular pathology is limited to much fewer cases.

3. There is no co relation between the histopathologic changes to the kidneys in fatal SARI cases with SARI aetiologic agent, age or gender. Together, the combined histopathologic changes observed in the kidneys in fatal paediatric SARI in each case are likely related to the overwhelming infection and the resulting systemic activation of broad spectrum pro- inflammatory pathways, cytokine release and immunologic dysregulation and dysfunction that is worsened by the myriad of co morbidities present in these children. This study demonstrates the kidney as a victim organ in a symphony of probable multiple end organ damage caused by aberrant systemic inflammation in fatal paediatric SARI.

13. RECOMMENDATIONS

1. There should be a high index of suspicion for acute kidney injury in children suffering from SARI regardless of clinical presentation. We therefore recommend that all children admitted for treatment of a SARI be routinely investigated through urinalysis and serum biochemical renal function tests as part of initial and periodic work up during admission.

2. There is extremely limited literature on SARI associated kidney pathology. Further clinicopathologic studies should be conducted to establish the prevalence of this entity and its contribution to morbidity and mortality in respiratory illness. The use of advanced immunohistochemical, biochemical and electron microscopy techniques should be included during these endeavors for demonstration of finer morphologic changes in acute kidney injury.

REFERENCES:

1. Hendrickse RG. Epidemiology and prevention of kidney disease in Africa. Trans R Soc Trop Med Hyg. 1980;74(1):8–16.
2. Ladapo TA, Esezobor CI, Lesi FE. Pediatric kidney diseases in an African country: prevalence, spectrum and outcome. Saudi J Kidney Dis Transplant. 2014;25(5):1110.
3. Cerdá J, Lameire N, Eggers P, Pannu N, Uchino S, Wang H, et al. Epidemiology of acute kidney injury. Clin J Am Soc Nephrol. 2008;3(3):881–6.
4. Mulholland K. Global burden of acute respiratory infections in children: implications for interventions. Pediatr Pulmonol. 2003;36(6):469–74.
5. Rudan I, Boschi-Pinto C, Biloglav Z, Mulholland K, Campbell H. Epidemiology and etiology of childhood pneumonia. Bull World Health Organ. 2008;86(5):408–416B.
6. Demographic K. Health Survey 2008–09 Calverton. Maryl KNBS ICF Macro. 2010;
7. Demographic K. Health Survey 2014: key indicators. Kenya Natl Bur Stat ICF Macro. 2014;
8. Ito M, Hirabayashi N, Uno Y, Nakayama A, Asai J. Necrotizing tubulointerstitial nephritis associated with adenovirus infection. Hum Pathol. 1991;22(12):1225–31.
9. da Silva Junior GB, Daher EDF. Tropical diseases-associated kidney injury. Rev Bras Clin Med São Paulo. 2013;11(2):155–64.
10. Montseny J-J, Meyrier A, Kleinknecht D, Callard P. The Current Spectrum of Infectious Glomerulonephritis: Experience with 76 Patients and Review of the Literature. Medicine (Baltimore). 1995;74(2).
11. Watanabe T, Yoshikawa H, Abe Y, Yamazaki S, Uehara Y, Abe T. Renal involvement in children with influenza A virus infection. Pediatr Nephrol. 2003;18(6):541–4.
12. Naicker S, Aboud O, Gharbi MB. Epidemiology of acute kidney injury in Africa. In: Seminars in nephrology. Elsevier; 2008. p. 348–53.
13. Esezobor CI, Ladapo TA, Osinaike B, Lesi FEA. Paediatric acute kidney injury in a tertiary hospital in Nigeria: prevalence, causes and mortality rate. PLoS One. 2012;7(12):e51229.
14. Andreoli SP. Acute kidney injury in children. Pediatr Nephrol. 2009;24(2):253–63.
15. Chintu C, Mudenda V, Lucas S, Nunn A, Lishimpi K, Maswahu D, et al. Lung diseases at necropsy in African children dying from respiratory illnesses: A descriptive necropsy study. Lancet. 2002;360(9338):985–90.
16. Abdelraheem MB, Ali E-TMA, Mohamed RM, Hassan EG, Abdalla OA, Mekki SO, et al. Pattern of glomerular diseases in Sudanese children: A clinico-pathological study. Saudi J Kidney Dis Transplant. 2010;21(4):778.
17. Buuren AJ Van, Bates WD, Muller N. Nephrotic Syndrome in. 1999;(10):4–7.
18. Michelow IC, Olsen K, Lozano J, Rollins NK, Duffy LB, Ziegler T, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics. 2004;113(4):701–7.
19. Jackson SJ, Steer AC, Campbell H. Systematic Review: Estimation of global burden of non-suppurative sequelae of upper respiratory tract infection: rheumatic fever and post-streptococcal glomerulonephritis. Trop Med Int Heal. 2011;16(1):2–11.
20. Martin J, Kaul A, Schacht R. Acute poststreptococcal glomerulonephritis: a manifestation of immune reconstitution inflammatory syndrome. Pediatrics. 2012;130(3):e710–3.
21. Phillips J, Palmer A, Baliga R. Glomerulonephritis associated with acute pneumococcal pneumonia: a case report. Pediatr Nephrol. 2005;20(10):1494–5.
22. Forrest JWJ, John F, Mills LR, Buxton TB, Moore WLJ, Hudson JB, et al. Immune complex glomerulonephritis associated with Klebsiella pneumoniae infection. Clin

Nephrol. 1977 Feb;7(2):76–80.
23. Saïd M-H, Layani M-P, Colon S, Faraj G, Glastre C, Cochat P. Mycoplasma pneumoniae-associated nephritis in children. Pediatr Nephrol. 1999;13(1):39–44.
24. Sánchez-Vargas FM, Gómez-Duarte OG. Mycoplasma pneumoniae—an emerging extra-pulmonary pathogen. Clin Microbiol Infect. 2008 Feb 1;14(2):105–15.
25. Waris ME, Toikka P, Saarinen T, Nikkari S, Meurman O, Vainionpää R, et al. Diagnosis of Mycoplasma pneumoniae Pneumonia in Children. J Clin Microbiol. 1998 Nov 5;36(11):3155–9.
26. del Carmen Laso M, Cadario ME, Haymes L, Grimoldi I, Balbarrey Z, Casanueva E. Mycoplasma pneumoniae detection with PCR in renal tissue of a patient with acute glomerulonephritis. Pediatr Nephrol. 2006;21(10):1483–6.
27. Narita M. Pathogenesis of extrapulmonary manifestations of Mycoplasma pneumoniae infection with special reference to pneumonia. J Infect Chemother. 2010;16(3):162–9.
28. Kaehny WD, Ozawa T, Schwarz MI, Stanford RE, Kohler PF, McIntosh RM. Acute nephritis and pulmonary alveolitis following pneumococcal pneumonia. Arch Intern Med. 1978;138(5):806–8.
29. Siomou E, Kollios KD, Papadimitriou P, Kostoula A, Papadopoulou ZL. Acute nephritis and respiratory tract infection caused by Mycoplasma pneumoniae: case report and review of the literature. Pediatr Infect Dis J. 2003;22(12):1103–6.
30. Cohen C, Walaza S, Moyes J, Groome M, Tempia S, Pretorius M, et al. Epidemiology of Severe Acute Respiratory Illness (SARI) among Adults and Children Aged ≥5 Years in a High HIV-Prevalence Setting, 2009–2012. Hill PC, editor. PLoS One. 2015 Feb 23;10(2):e0117716.
31. Gulati S, Kher V, Gulati K, Arora P, Gujral R. Tuberculosis in childhood nephrotic syndrome in India. Pediatr Nephrol. 1997;11(6):695–8.
32. Santra A, Mandi F, Bandyopadhyay A. Renal Tuberculosis Presenting as a Mass Lesion in a Two-year-old Girl: Report of a rare case. Sultan Qaboos Univ Med J. 2016 Feb 2;16(1):e105–8.
33. Wardle EN. Toll-like receptors and glomerulonephritis. Saudi J Kidney Dis Transplant. 2007;18(2):159.
34. Wenderfer SE. Viral-associated glomerulopathies in children. Pediatr Nephrol. 2015;30(11):1929–38.
35. Bigogo GM, Breiman RF, Feikin DR, Audi AO, Aura B, Cosmas L, et al. Epidemiology of respiratory syncytial virus infection in rural and urban Kenya. J Infect Dis. 2013;208(suppl 3):S207–16.
36. Breiman RF, Cosmas L, Njenga MK, Williamson J, Mott JA, Katz MA, et al. Severe acute respiratory infection in children in a densely populated urban slum in Kenya, 2007–2011. BMC Infect Dis. 2015;15(1):1.
37. de Suremain A, Somrani R, Bourdat-Michel G, Pinel N, Morel-Baccard C, Payen V. Néphrite tubulo-interstitielle aiguë au cours d'une virose respiratoire à adénovirus et virus respiratoire syncytial. Arch Pédiatrie. 2015;22(5):528–32.
38. Shenouda A, Hatch FE. Influenza A viral infection associated with acute renal failure. Am J Med. 1976;61(5):697–702.
39. Watanabe T. Renal complications of seasonal and pandemic influenza A virus infections. Eur J Pediatr. 2013;172(1):15–22.
40. Ardehali H, Volmar K, Roberts C, Forman M, Becker LC. Fatal disseminated adenoviral infection in a renal transplant patient. Transplantation. 2001;71(7):998–9.
41. Pretorius MA, Tempia S, Walaza S, Cohen AL, Moyes J, Variava E, et al. The role of influenza, RSV and other common respiratory viruses in severe acute respiratory infections and influenza-like illness in a population with a high HIV sero-prevalence,

South Africa 2012-2015. J Clin Virol. 2016 Feb 1;75:21–6.
42. Ramsuran D, Bhimma R, Ramdial PK, Naicker E, Adhikari M, Deonarain J, et al. The spectrum of HIV-related nephropathy in children. Pediatr Nephrol. 2012;27(5):821–7.
43. Strauss J, Abitbol C, Zilleruelo G, Scott G, Paredes A, Malaga S, et al. Renal disease in children with the acquired immunodeficiency syndrome. N Engl J Med. 1989;321(10):625–30.
44. Singh NP, Ganguli A, Prakash A. Drug-induced kidney diseases. J Assoc Physicians India. 2003;51(OCT):970–9.
45. World Health Organization. Global Epidemiological Surveillance Standards for Influenza. Igarss 2014. 2014;(1):1–5.
46. Pryce JW, Bamber AR, Ashworth MT, Kiho L, Malone M, Sebire NJ. Reference ranges for organ weights of infants at autopsy: results of> 1,000 consecutive cases from a single centre. BMC clinical pathology. 2014 Dec;14(1):18.
47. Proulx F, Guilemette J, Roumeliotis N, Emeriaud G. Organ weight measured at autopsy in critically ill children. Pediatric and Developmental Pathology. 2015 Sep;18(5):369-74.
48. Ece A, Gözü A, Bükte Y, Tutanç M, Kocamaz H. The effect of malnutrition on kidney size in children. Pediatric nephrology. 2007 Jun 1;22(6):857-63.
49. Schmidt ID, Chellakooty M, Boisen KA, Damgaard ID, Kai CM, Olgaard K, Main KM. Impaired kidney growth in low-birth-weight children: distinct effects of maturity and weight for gestational age. Kidney international. 2005 Aug 1;68(2):731-40.
50. Zhang, Z. (2018). Cortical Necrosis of the Kidneys - Kidney and Urinary Tract Disorders - MSD Manual Consumer Version. [online] MSD Manual Consumer Version. Available at: https://www.msdmanuals.com/home/kidney-and-urinary-tract-disorders/blood-vessel-disorders-of-the-kidneys/cortical-necrosis-of-the-kidneys [Accessed 4 Jun. 2018].
51. Rameshkumar R, Krishnamurthy S, Ganesh RN, Mahadevan S, Narayanan P, Satheesh P, Jain P. Histopathological changes in septic acute kidney injury in critically ill children: a cohort of post-mortem renal biopsies. Clinical and experimental nephrology. 2017 Dec 1;21(6):1075-82.
52. Balasubramanian R, Marks SD. Post-infectious glomerulonephritis. Paediatrics and international child health. 2017 Oct 2;37(4):240-7.
53. Lovett DH, Bursten SL, Gemsa D, Bessler W, Resch K, Ryan JL. Activation of glomerular mesangial cells by gram-negative bacterial cell wall components. The American journal of pathology. 1988 Dec;133(3):472.
54. Sotsiou F. Postinfectious glomerulonephritis. Nephrology Dialysis Transplantation. 2001 Sep 25;16(suppl_6):68-70.
55. Baud L, Oudinet JP, Bens M, Noe L, Peraldi MN, Rondeau E, Etienne J, Ardaillou R. Production of tumor necrosis factor by rat mesangial cells in response to bacterial lipopolysaccharide. Kidney international. 1989 May 1;35(5):1111-8.
56. Chawla LS, Seneff MG, Nelson DR, Williams M, Levy H, Kimmel PL, Macias WL. Elevated plasma concentrations of IL-6 and elevated APACHE II score predict acute kidney injury in patients with severe sepsis. Clinical Journal of the American Society of Nephrology. 2007 Jan 1;2(1):22-30.
57. Heemskerk S, Masereeuw R, Russel FG, Pickkers P. Selective iNOS inhibition for the treatment of sepsis-induced acute kidney injury. Nature Reviews Nephrology. 2009 Nov;5(11):629.
58. Li X, Hassoun HT, Santora R, Rabb H. Organ crosstalk: the role of the kidney. Current opinion in critical care. 2009 Dec 1;15(6):481-7.

59. Hotchkiss RS, Karl IE. The pathophysiology and treatment of sepsis. New England Journal of Medicine. 2003 Jan 9;348(2):138-50.
60. Langenberg C, Bagshaw SM, May CN, Bellomo R. The histopathology of septic acute kidney injury: a systematic review. Critical care. 2008 Apr;12(2): R38.
61. Shear W, Rosner MH. Acute kidney dysfunction secondary to the abdominal compartment syndrome. Journal of nephrology. 2006;19(5):556-65.
62. Sethi S, Haas M, Markowitz GS, D'Agati VD, Rennke HG, Jennette JC, Bajema IM, Alpers CE, Chang A, Cornell LD, Cosio FG. Mayo clinic/renal pathology society consensus report on pathologic classification, diagnosis, and reporting of GN. Journal of the American Society of Nephrology. 2015 Nov 13: ASN-2015060612.

APENDIX I: DATA COLLECTION PROFORMA.

KIDNEY MORPHOLOGY IN PEDIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT THE KENYATTA TEACHING AND REFERRAL HOSPITAL

1.0. Demographic information.

1.1. Study number _____
1.2. Corresponding PRESS Study Number _____
1.3. Sex: Male ☐ Female ☐
1.4. Age _____ months

2.0. Kidney gross morphology at autopsy.

3.0 Unfixed kidney weight:
3.0.1. RIGHT _____ grams
3.0.2. LEFT _____ grams

3.1 General Appearance of the Kidneys
Normal YES ☐ NO ☐

3.2 Morphological Anomalies
3.2.1Cysts YES ☐ NO ☐
3.2.2Abscesses YES ☐ NO ☐

3.3 Other abnormality _____

4.0. SARI aetiologic agent identified at Autopsy:

4.1. Bacterial ☐ 4.3. Fungal ☐
4.2. Viral ☐ 4.4. Protozoal ☐

APPENDIX II: KIDNEY HISTOPATHOLOGY REPORTING PROFORMA.

KIDNEY MORPHOLOGY IN PEDIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT THE KENYATTA TEACHING AND REFERRAL HOSPITAL

Study Number: _____

Clinical History/data:	
Brief Summary of clinical data	
Gross Description	
5.0 Light Microscopic Description.	
5.0.1 Histologic Stains performed	H/E ☐ PAS ☐ PASM ☐ MT ☐
5.0.2 Presence of Cortex/medulla capsule / calyceal mucosa (Sample adequacy)	Present ☐ Absent ☐
6.0 Glomeruli.	
6.0.1 Number of Glomeruli	
6.0.2 Number of (%) global Sclerosis (if present)	
6.0.3 Number of (%) segmental sclerosis (if present)	
6.0.4 Number of (%) crescents, cellular to fibrocellular (if present)	
6.0.5 Number of (%) fibrinoid necrosis (if present)	
6.0.6 Additional Abnormalities (e.g., hypercellularity, deposits, thrombosis, double contours, spikes)	Hypercellularity ☐ Deposits ☐ Thrombosis ☐ Double contours ☐ Spikes ☐ Other ☐
7.0 Tubulointerstitium.	
7.0.1 Extent of interstitial fibrosis/tubular atrophy, at least semiquantitative	
7.0.2 Interstitial inflammation, tubular injury, crystals	
8.0 Arteries/arterioles.	
8.0.1 Intimal fibrosis (absent/present/severity)	Absent ☐ Present ☐
8.0.2 Arteriolar hyalinosis (absent/present/severity)	Absent ☐ Present ☐
9.0 FINAL DIAGNOSTIC COMMENT	
SIGNED	Pathologists Student Date:

APPENDIX III: PROCEEDURE FOR SECTIONING OF PARRAFIN EMBEDDED TISSUE BLOCKS.

Method:

1. Release the brake and rotate the hand wheel until the handle is at 1 o'clock position and re-apply the brake.
2. Push the quick release lever of the cassette clamp backward, insert the cassette clamp backward, insert the cassette, release the lever and check that the cassette is firmly clamped.
3. Use the vertical and horizontal tilt controls to orientate the specimen correctly with the knife edge and lock the orientation head.
4. Release the brake and turn the coarse advance knob clockwise and anticlockwise to bring the tissue block closer or away from the cutting edge.
5. Trim the block using the coarse advance knob until the full face is attained.
6. Set the section thickness with thickness control knob.
7. Turn the hand wheel to cut the sections.
8. Pick the sections and float in warm water to remove the creases.
9. Fish the sections and mount on clean microscope slides. Label the slides with a lead pencil or diamond pencil.
10. Put the slides in a hot air oven at 56c for 1hour. Remove the slides and stain.

APPENDIX IV: HAEMATOXYLIN AND EOSIN STAINING PROCEDURE.

Method:

1. Take sections to water.
2. Place sections in haematoxylin for 5 minutes.
3. Wash in tap water.
4. 'Blue' sections in lithium carbonate or Scott's tap water.
5. Wash in tap water.
6. Place sections in 1% acid alcohol for a few seconds.
7. Wash in tap water.
8. Place sections in eosin for 5 minutes.
9. Wash in tap water.
10. Dehydrate, clear.
11. Mount With DPX.

Results:

- Nuclei - Blue-black
- Cytoplasm - Varying shades of pink

APPENDIX V: PERIODIC ACID SCHIFF (PAS) STAINING PROCEDURE.

Method.

1. Bring Section to water.
2. Oxidise for 10 minutes in 1% Periodic acid (aq)
3. Wash in running tap water for 5 minutes.
4. Rinse in distilled water.
5. Place in Schiff's reagent for 15 minutes or until section turns magenta colour.
6. Rinse in 3 changes of freshly prepared 0.5% sodium metabisulphite.
7. Wash in running tap water for 10 minutes.
8. Stain in alum haematoxylin for 6 - 7 minutes
9. Differentiate in 1% Acid alcohol - 3 dips
10. Blue in running tap water for 10 minutes or Scott's tap water for 1 minute.
11. Counterstain in 0.3% tartrazin in cellosolve for 3 minutes
12. dehydrate in absolute ethanol, clear in Xylene and mount in DPX

Results.

- Nuclei - blue
- Cytoplasm - Yellow
- Positive controls - Red or Magenta.

APPENDIX VI: PERIODIC ACID METHENAMINE SILVER (PASM) STAINING PROCEDURE.

FOR DEMONSTRATION OF GLOMERULAR BASEMENT MEMBRANE

Method:

1. Bring section to distilled water.
2. Rinse in 3 changes of distilled water.
3. Place in Periodic Acid for 10 minutes.
4. Place in Filtered Pre -warmed working silver nitrate solution at 60°C for 1 hour. the section should be yellowish brown.
5. Rinse in distilled water and check the impregnation, if unsatisfactory return to the silver nitrate.
6. Tone in gold chloride for 1 minute.
7. Fix in 5% sodium thiosulphate for 5 minutes (to remove undiluted silver).
8. Wash in water for 5 minutes.
9. Stain nuclei in Mayer's Haematoxylin for 2 minutes.
10. Differentiate in 1% acid alcohol.
11. Blue in running tap water for 10 minutes.
12. Dehydrate in 3 changes of ethanol.
13. Clear in xylene.
14. Mount in DPX.

Results:

- Basement Membrane - Black
- Background - Greyish
- Nuclei - Blue
- Positive control - Normal Kidney.

APPENDIX VII: MASSON'S TRICHROME STAINING PROCEDURE

Method.

1. Bring Section to water.
2. Stain nuclei with Celestin blue for 5 minutes, rinse in water then stain in alum haematoxylin for 5 minutes.
3. Differentiate in 1% acid alcohol until only the nuclei are stained.
4. Blue in running tap water for 10 minutes.
5. Stain in 1% ponceau2R for 5 minutes.
6. Rinse in distilled water.
7. Mordant in 1% phosphomolybdic acid for 5 minutes.
8. Rinse in distilled water.
9. Counter stain in 2% light green for 3 minutes.
10. Rinse off excess stain in distilled water.
11. Wash well in 1% acetic acid to remove excess light green from the cytoplasmic structures.
12. Dehydrate in 3 changes of absolute ethanol.
13. Clear in 3 changes of xylene.
14. Mount in DPX.

Results.

- Rbc - Red
- Nuclei – Blue/ Black
- Muscles - Red
- Cytoplasm – Red.
- Keratin – Red.
- Collagen fibers – Green.
- Mucin – Green.
- Positive controls – Stomach, Appendix or Liver tissue.

APPENDIX VIII: PRESS CONSENT FOR POST MORTEM STUDY.

Flesch-Kincaid readability level – 8.1

Consent form for postmortem specimen collection

Today's date	☐☐/☐☐/☐☐	Decedents unique identification number	☐☐☐/☐☐☐,☐
Name of decedent:			
Name next of kin:		**File num**:	

Receive our condolences on the death of (name). (Name) had an illness affecting his/her lungs which may have led to his/her death. Lung illnesses are one of the leading causes of sickness and death in our country. We would like to find out the cause of the lung illness in (name) that may have led to his/her death. This will enable us to know the leading germs that cause lung illnesses. This knowledge will then help us choose vaccines and other prevention and treatment options that might help us to avoid similar deaths in future.

What we will do

We will take some bone marrow trephine biopsy and bone marrow imprints. As is normally done in an autopsy we will open the chest and take samples of the lungs and other organs in the chest that may be damaged or have disease causing germs. These specimens will be sent to laboratories in KNH and KEMRI/CDC in Nairobi, and to CDC Laboratories in Atlanta, Georgia, USA for analysis so as to identify germs that may have led to your child's illness and death. For each specimen, we will place a number that uniquely identifies the samples. This number is similar to the one on questionnaires that collected clinical data when the child was in the ward. In addition, we will collect some blood and test it for HIV and influenza.

We will let you know the results of tests done once they are ready and the cause of death.

Benefit from being in this study:

The study will provide no direct benefit to you. In general, the study will help us to learn more about causes of lung illnesses and bone marrow illness that lead to death in our country so that we can provide better care to prevent similar deaths in the future. If you would like we will call you and tell you what we think was the cause of death for your child. It may take up to six months for us to determine the final cause of death.

Risks from being in this study:

The body may have cuts on the chest and abdomen which will be sewed together after the post mortem.

How will the deceased information be protected?

No names shall appear on samples collected. Instead, numbers will be used to identify the samples. Most of the samples collected will be tested here in the hospital and at KEMRI/CDC laboratory. However, for some tests that cannot be done here, part of the samples may be sent to special laboratories in CDC in the United States.

All the study records will be kept secretly and securely. There will be people involved in the study who will need to see the deceased's health records. These people may include members of the study team, the study monitors, and members of the ethics committees that oversee the study. In addition, the information collected about the deceased will be shared with our data team, who are located at KEMRI/CDC offices in Nairobi. The information shared with the data team will not contain names or any other personal identifying information. Reports and publications from this study will not contain decedent's name or any other personal identifying information.

What happens if I do not want a post mortem for the deceased?

You can choose not to have the deceased participate in this study. The body will still receive the usual care at the mortuary.

Will it cost anything?

It will not cost you anything to have the deceased participate in this study. We will meet the post-mortem costs, costs of any laboratory tests done and mortuary fees for a period of up to 5 days since death.

Who do I call if I have questions or problems?

- If you have questions or complaints about this study, you can call the principal investigator, Henry Njuguna, at phone#0724256803
- If you have questions about rights as a study participant, call the Ethical Review Committee for Research in Human Subjects**.** You should contact the ethics committee if you feel you have not been treated fairly or if you have other concerns. The ethics committee contact information is: 0722205981

What does your signature (or initials/mark) on this consent form mean?

Your signature (**or initials/mark)** on this form means:

- You have been informed about this study's purpose, procedures, possible benefits and risks.
- You have been given the chance to ask questions before you sign.
- You have voluntarily agreed to have the deceased body to be used in this study.

Please indicate Yes or No:

I agree to have the deceased body be used in this study

☐Yes ☐No

I agree to allow the deceased medical records to be reviewed by study staff, ethics committee members, and legal authorities:

☐Yes ☐No

I agree to allow specimens from the deceased to be stored and possibly used after this study is over to test for things related to respiratory disease:

☐Yes ☐No

I agree to specimens and data being sent outside the country for research:

☐Yes ☐No

Consent Agreement:

I have read or had the consent form read to me and had a chance to ask questions. I agree to have the deceased body to take part in the study. I understand that I am free to choose not to have his/her body take part in this study and that saying "NO" will have no effect on post-mortem care.

Next of kin	Name:	Signature:	Date☐☐/☐☐/☐☐
Witness*	Name:	Signature:	Date☐☐/☐☐/☐☐

*Next of kin can sign or make a mark and have his/her consent confirmed by the signature of a witness

APPENDIX IX: PRESS APPROVAL LETTER FROM KEMRI CDC ERC.

KENYA MEDICAL RESEARCH INSTITUTE

P.O. Box 54840-00200, NAIROBI, Kenya
Tel (254) (020) 2722541, 2713349, 0722-205901, 0733-400003; Fax: (254) (020) 2720030
E-mail: director@kemri.org info@kemri.org Website:www.kemri.org

31/1/2014

KEMRI/RES/7/3/1 **January 24, 2014**

TO: **DR. HENRY NJENGA NJUGUNA (PRINCIPAL INVESTIGATOR)**

THROUGH: **DR. STEPHEN MUNGA,**
ACTING DIRECTOR, CGHR,
KISUMU

Dear Sir,

RE: **SSC PROTOCOL NO. 2692 (*RESUBMISSION*): ETIOLOGY OF PEDIATRIC RESPIRATORY MORTALITY AT KENYATTA NATIONAL HOSPITAL, NAIROBI, KENYA**

Reference is made to your letter dated 16th January, 2014. The ERC Secretariat acknowledges receipt of the revised document on 22nd January, 2014.

This is to inform you that the Ethics Review Committee (ERC) reviewed the document submitted, and is satisfied that the issues raised at the 221st meeting of 26th November 2013, have been adequately addressed.

This study is granted approval implementation effective this **January 24, 2014.** Please note that authorization to conduct this study will automatically expire on **January 23, 2015**. If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the ERC secretariat by **December 12, 2014.**

You are required to submit any amendments to this protocol and other information pertinent to human participation in this study to the SSC and ERC for review prior to initiation.

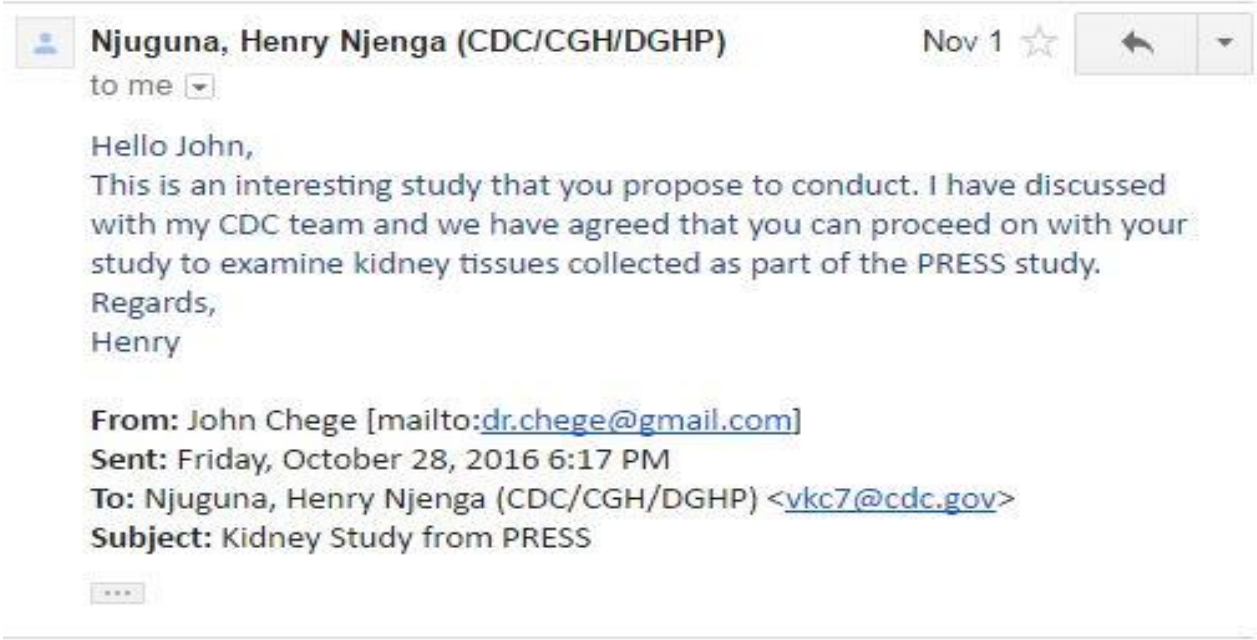
You may embark on the study.

Yours faithfully,

EAB

DR. ELIZABETH BUKUSI,
ACTING SECRETARY,
KEMRI/ETHICS REVIEW COMMITTEE

APPENDIX X: APPROVAL FOR USE OF MATERIALS BY PRESS PRINCIPLE INVESTIGATOR.



Njuguna, Henry Njenga (CDC/CGH/DGHP) Nov 1

to me

Hello John,
This is an interesting study that you propose to conduct. I have discussed with my CDC team and we have agreed that you can proceed on with your study to examine kidney tissues collected as part of the PRESS study.
Regards,
Henry

From: John Chege [mailto:dr.chege@gmail.com]
Sent: Friday, October 28, 2016 6:17 PM
To: Njuguna, Henry Njenga (CDC/CGH/DGHP) <vkc7@cdc.gov>
Subject: Kidney Study from PRESS

APPENDIX XI: KNH RESEARCH AND ETHICS COMMITTEE APPROVAL.

UNIVERSITY OF NAIROBI
COLLEGE OF HEALTH SCIENCES
P O BOX 19676 Code 00202
Telegrams: varsity
Tel:(254-020) 2726300 Ext 44355

KNH-UON ERC
Email: uonknh_erc@uonbi.ac.ke
Website: http://www.erc.uonbi.ac.ke
Facebook: https://www.facebook.com/uonknh.erc
Twitter: @UONKNH_ERC https://twitter.com/UONKNH_ERC

KENYATTA NATIONAL HOSPITAL
P O BOX 20723 Code 00202
Tel: 726300-9
Fax: 725272
Telegrams: MEDSUP, Nairobi

Ref: KNH-ERC/A/154

5th May 2017

Dr. John Chege Njuguna
Reg. No.H58/70149/2013
Dept.of Human Pathology
School of Medicine
College of Health Sciences
University of Nairobi

KENYATTA NATIONAL HOSPITAL APPROVED 05 MAY 2017 KNH/UoN-ERC P.O. Box 20723-00202 NRB.

Dear Dr. Njuguna

REVISED RESEARCH PROPOSAL – KIDNEY MORPHOLOGY IN PAEDIATIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT THE KENYATTA NATIONAL TEACHING AND REFERRAL HOSPITAL (P922/12/2016)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above revised proposal. The approval period is from 5th May 2017 – 4th May 2018.

This approval is subject to compliance with the following requirements:

a) Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b) All changes (amendments, deviations, violations etc) are submitted for review and approval by KNH-UoN ERC before implementation.
c) Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d) Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e) Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
f) Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.`
g) Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website **http://www.erc.uonbi.ac.ke**

Protect to discover

Yours sincerely,

PROF M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Director, CS, KNH
The Assistant Director, Health Information, KNH
The Chair, KNH-UoN ERC
The Dean,School of Medicine, UoN
The Chair, Dept. of Human Pathology, UoN
Supervisors: Dr. Joseph Ndungu, Dr. Edwin O.Walong, Dr.Deepa Patel

APPENDIX XII: PLAGIARISM DECLARATION

KIDNEY MORPHOLOGY IN PAEDIATRIC MORTALITIES ASSOCIATED WITH SEVERE ACUTE RESPIRATORY ILLNESS AT THE KENYATTA NATIONAL TEACHING AND REFERRAL HOSPITAL

Submission date: 12-Jul-2018 04:16AM (UTC-0400)
Submission ID: 982045517
File name: DISSERTATION.docx (3.25M)
Word count: 13446
Character count: 78673

ORIGINALITY REPORT

10%	7%	8%	2%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS