THE UNIVERSITY OF ZAMBIA

SCHOOL OF MEDICINE

The Histological Appearances of the Adult Kidney in HIV Infection at Autopsy at the University Teaching Hospital in Lusaka.

By

Mucheleng'anga Luchenga Adam

DISSERTATION SUBMITTED TO THE UNIVERSITY OF ZAMBIA IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF MEDICINE, PATHOLOGY.

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BY_____

DR. CLEMENCE MARIMO

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I HEREBY STATE THAT THIS DISSERTATION IS ENTIRELY THE RESULT OF MY OWN PERSONAL EFFORT. THE VARIOUS SOURCES TO WHICH I AM INDEBTED HAVE BEEN CLEARLY INDICATED IN THE BIBLIOGRAPHY AND ACKNOWLEDGMENT.

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ABSTRACT

Introduction: Kidney disease in acquired immunodeficiency syndrome is very common. The cause of the various histological appearances include HIV infection of the kidney, immunologic responses to the virus, opportunistic infections, neoplasms and consequences of anti-retroviral therapy that characterize the acquired immunodeficiency virus (AIDS). There are no autopsy studies conducted in HIV-infected cases in sub-Saharan Africa on the histological lesions of the kidney.

Methods: A descriptive study of 200 paraffin blocked kidney autopsy tissue samples collected between 2010 and 2012 in the Zambia Neuro-AIDS study (Subtype C Neuro-AIDS and pathogenesis in Zambia) was conducted at the University Teaching hospital in Lusaka, Zambia. The study aimed at describing the histological appearances of the adult kidney in Human Immunodeficiency Virus infection.

The study population consisted of all HIV infected cases above the age of 16years that had been on anti-retroviral therapy and those that were not commenced. The decedents were unselected for the presence of overt kidney disease. Paraffin blocked kidney tissue samples were processed according to standard histopathology laboratory protocols at the University Teaching Hospital and examined at light microscopic level.

Results: Two hundred (200) cases were examined of which 128 cases were male and 72 were female. One hundred and sixty nine cases (84.5%) revealed renal lesions. The renal histopathological lesions included; tubulointerstitial 65%, glomerular 59%, vascular 2%, and 19.5 % of cases revealed renal tuberculosis. Fungal, viral infections and malignancies were not identified.

Conclusion: The investigation has shown that renal lesions are highly prevalent (84.5%) among HIV infected cases at the University Teaching Hospital.

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ABBREVIATIONS

AIDS	Acquired immunodeficiency syndrome
ATN	Acute tubular necrosis
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CVA	Cerebral vascular accident
CD4+	Cluster differentiation
CMV	Cytomegalovirus
CR	Congo red
EVF	Efavirenz
FSGS	Focal segmental glomerulosclerosis
GMS	Gomori methenamine silver
ART	Antiretroviral therapy
H&E	Hematoxylin and eosin
HIV	Human immunodeficiency virus
HIVAN	Human immunodeficiency virus associated nephropathy
K.S	Kaposi sarcoma
IN -	Interstitial nephritis
LM	Light microscopy
MTS	Masson trichrome stain
NC	Nephrocalcinosis
NVP	Nevirapine
PAS	Periodic acid schiff
PTB	Pulmonary tuberculosis
Pre-ART	Patients who are antiretroviral therapy naive
SOP	Standard operating procedures
T.B	Tuberculosis

UTH	University Teaching Hospital
WHO	World Health Organisation
ZN	Ziehl Neelsen

DEDICATION

This dissertation is dedicated to my late father Greene Chitaku Mucheleng'anga for his interest in biology and my mother Jennipher N. Phiri for teaching me mathematics.

Chapter 1: Background and Introduction

Kidney disease in Acquired Immunodeficiency Syndrome (AIDS) patients was described before human immunodeficiency virus (HIV) was demonstrated to be the causative agent in 1984 and since then many studies have provided evidence that the kidneys harbor histologically diverse lesions in HIV-infected cases [1, 2, 3]. Antiretroviral therapy (ART) in the treatment of HIV infection has reduced the mortality and morbidity rates among the HIV population; however prolonged use of ART has brought about alterations in lipid metabolism[4, 5] leading to an increase in the prevalence of secondary diabetic and hypertensive kidney damage. Furthermore antiretroviral drugs such as indinavir and tenofovir have been associated with nephrotoxic effects [5].

The kidney is divided into four components (the glomeruli, tubules, interstitium and the blood vessels) for the purpose of histological description. HIV affects all the compartments and produces varied histological appearances[6, 7]. Although the most common histologic lesion seen among HIV-infected patients is HIV-associated nephropathy (HIVAN) a spectrum of other kidney histologic lesions in HIV disease have been described [2, 3, 4]. The causes of the various histological appearances include; HIV infection of the kidney, immunologic responses to the virus, opportunistic infections, neoplasms and consequences of various therapies or hemodynamic derangements that characterize AIDS [8]. Literature review shows that all renal pathologies are seen in HIV infection in Europe and America [9, 10] however, there is no literature on autopsy studies conducted in sub-Saharan Africa.

This descriptive study investigated renal lesions of the adult kidney in HIV infected patients at the University Teaching Hospital in the period 2010 to 2012 using blocked autopsy kidney samples were collected from the Zambia Neuro-AIDS study (subtype C neuro-AIDS and pathogenesis in Zambia) which was set up to investigate the effects of HIV on the brain at autopsy.

1.1 Statement of the problem.

The histological appearances of the kidney in HIV-infected adults have not been described in Sub-Saharan Africa where the disease burden is HIV/AIDS is high.

1.2 Justification and significance of the study.

The histological appearances of the kidney in HIV infection have not been described in the Zambian population despite Banda *et al*, 2010 observing a renal dysfunction prevalence rate of 42% among hospitalized HIV-infected patients at the UTH[11].

This autopsy study provided a unique opportunity to study kidney histological appearances in HIV-infected patients in Zambia.

Morbidity rates in HIV-infected patients are affected by the early recognition of renal disease[12] hence, this study identified kidney lesions that did not present with indications for kidney biopsy when the descendant was alive for example renal granulomas.

Chapter 2: Review of literature.

The initial description of AIDS brought to the fold a discovery that the kidneys were affected by HIV [12, 13]. Rao *et al*, 1984 described an entity which is now known as HIVAN. HIVAN is characterised histologically by focal and segmental glomerulosclerosis. These cases presented with nephrotic syndrome and died six months after the initial diagnosis [14, 15]. Approximately 60% of cases with AIDS manifest with kidney dysfunction including; acid-base, electrolyte disturbances, and parenchymal damage. The nature of the lesions varies considerably based on racial and geographic considerations [16, 17].

The number of cases living with HIV-infection has increased due to ART and so has the burden of renal disease among HIV-infected cases [17]. Numerous studies previously documented collapsing focal segmental glomerulosclerosis (FSGS) as very prevalent in HIV cases, but now a whole range of kidney diseases is observed in HIV cases [15]. Banda *et al*, 2010 showed a kidney dysfunction prevalence rate of 42% among hospitalized cases infected with HIV at the University Teaching Hospital, Lusaka in a cross-sectional study. For the purpose of their study renal dysfunction was defined as the rise of serum creatinine 1.5 times the upper limit of normal, i.e. ≥ 180 micromoles per liter, with or without the decrement in urine output [11].WHO stage III-HIV disease and vomiting were associated with renal dysfunction among HIV infected patients. Tenofovir was not associated with kidney disease[11].

As concerns autopsy studies, Hailemariam *et al*, 2001, van der Reijden *et al*, 1989 and Niedt *et al*, 1985 demonstrated direct renal infection in up to 20% of cases with AIDS[19,20,21]. The kidney is a site of isolated infection and at times part of a systemic process. Fungal organisms (candidiasis and cryptococcosis) infect the kidney more often than viral organisms [19, 20]. van der Reijden *et al*, 1989, D'Agati *et al*, 1989 and Nochy *et al*, 1993 revealed that the kidneys are affected by malignant lesions in 4% to 8% of cases with AIDS [20, 21, 22]. Seney *et al*, 1990 and Pollok *et al*, 1995 described neoplastic processes such as lymphomas and Kaposi's sarcoma at autopsy [23, 24].

Wyatt *et al*, 2009 conducted a prospective study of antiretroviral treatment-experienced cases with advanced HIV/AIDS in New York, revealed a high prevalence of both chronic kidney disease and subclinical renal pathology. This was a cohort with advanced HIV/AIDS. The inclusion criteria included HIV-positive cases with advanced HIV disease (a pre-specified AIDS-defining condition or a CD4 cell count persistently ≤50 cells per mm^3). There were eighty-nine (89) kidney tissue donors examined at autopsy. The histologic evaluation was conducted using stains; Haematoxylin and Eosin (H& E) and Periodic Acid Schiff (PAS) -stained slides. These were prepared from an area consisting of the cortex and medulla that had been fixed in formalin at the time of autopsy. Histopathologic examination identified 46 (51.7%) cases of subclinical kidney pathology. There were 75 cases that revealed renal lesions. Twenty-two (22) cases showed glomerular lesions, 77 cases-tubulointerstitial, 15 cases-vascular, and no cases revealed neoplastic lesions. Factors such as HIV viral load, CD4+ cell count, and Hepatitis C coinfection were not found to be associated with the presence of renal pathology. HIVAN and glomerulonephritis were two of the most frequent diagnoses [25].

Seife *et al*, 2001 conducted a national Swiss cooperative study on a Caucasian population. Two hundred and thirty-nine (239) autopsies were conducted prospectively of cases that had died of AIDS between April 1981 and August 1989 at various hospitals in Switzerland. The histological evaluation was done by examining slides stained using H& E, PAS, Mucicarmine and Congo red (CR). There were one hundred and two (102) cases that revealed renal lesions. Eighty-nine cases showed glomerular lesions, 3 cases-tubulointerstitial, 7 cases-vascular and 3 cases revealed neoplastic lesions. Ischemic nephropathy showed the highest prevalence. There was sclerosis of the glomeruli in 7% of cases. Focal segmental glomerulosclerosis was identified in 4 cases. Only one case showed features of HIVAN. Nonspecific interstitial changes and pyelonephritides were also identified. The prevalence of nephrocalcinosis was similar to the prevalence of the pyelonephritides. Lymphoma was found in 3% of the kidneys. Tubulo-interstitial involvement due to infectious agents was noted in 3% of cases showing that the kidneys do have varied lesions in AIDS[26].

Sarino-Rosas *et al*, 1998 in Mexico examined 138 cases retrospectively of HIV cases at the Mexico City General Hospital the period 1986 to 1991 at autopsy in a Hispanic population. The histologic evaluation was conducted using stains; Haematoxylin and Eosin (H& E) and Periodic Acid Schiff (PAS) -stained slides. Eighty-seven (87) cases showed renal lesions. Sixty-two (62) cases revealed glomerular lesions, 80 cases tubulointerstitial, 2 cases neoplasia and no vascular lesions were identified. Glomerular tuft collapse was identified in 30 cases (48%), mesangial expansion in 22 cases (35%), focal and segmental glomerulosclerosis in 20 cases (32%) and glomerular proliferation in eight cases (11 %) respectively. Nephrocalcinosis was noted in 25 cases (31 %). Thirty-six cases (45%) presented tubulointerstitial infections; mycobacterium tuberculosis in 19 cases (23%), cryptococcus in 10 cases (12.5%), cytomegalovirus (CMV) in eight cases (10%), Gram-negative bacteria in 3 cases (3.7%), and one case had histoplasmosis. There were six cases that revealed multiple pathologies. Two cases revealed infiltration by large-cell non-Hodgkin lymphoma [27].

Monga *et al*, 1997 examined a Caucasian population in Italy at autopsy. One hundred and twenty (120) AIDS cases were consecutively autopsied and the kidneys were evaluated by light microscopy using stains; H& E and PAS stained slides. There were 82 cases that revealed renal lesions. Twenty-five (25) cases showed glomerular lesions, 30 cases-tubulointerstitial, 27 cases-vascular, and no cases revealed neoplastic lesions. There were 16 mesangial glomerulonephritis, 4 membranous glomerulonephritis, 2 cirrhotic glomerulosclerosis; and 3 lupus-like glomerulonephritis. Glomerular diseases were significantly associated with chronic hepatitis and liver cirrhosis. Interstitial inflammation was present in 19 cases; 2 chronic pyelonephritis, 5 focal nephritis, 7 multiple cortical abscesses, and 5 granulomatous nephritis. Cryptococci were found in one case and undetermined microorganisms in two cases of multiple cortical abscesses. Atypical mycobacteria were revealed in two (2) cases of granulomatous nephritis. Mycotic infections were identified in 6 cases, where an inflammatory response was not elicited. Focal tubular necrosis was noted in 15 cases. Benign nephrosclerosis was the most common vascular change (27 cases) [28].

In Madrid, Spain, Martinez *et al*, 1996 conducted a total of 85 autopsies on HIV-positive cases from 1985 to 1993. The histologic evaluation was conducted using

stains; H& E and PAS stained slides. There were 50 cases that revealed renal lesions. 30 cases showed tubulointerstitial lesions, 4 cases-neoplastic lesions and no cases for glomerular and vascular lesions. Light microscopy showed renal changes; 15.2% acute tubular necrosis (ATN), 7% tumours, 55.8% interstitial nephritis, 5.8% nephrocalcinosis and 11.7% other conditions. The presence of disseminated mycobacteriosis at autopsy was significantly higher in the group with nephropathy than in the group without nephropathy (11 vs. 1, $p < 0.05$). The renal lesions were influenced by the use of nephrotoxic drugs, the presence of HBsAG, and opportunist infections among these cases [29].

van der Reijden *et al*, 1989 in Amsterdam performed 47 autopsies of AIDS cases during the period 1982 to 1987. None of the cases presented with kidney disease clinically. The histologic evaluation was conducted using stains; H& E and PAS stained slides. On microscopic examination of the kidney tissue obtained at autopsy, no abnormalities were seen in 12 cases. There were 35 cases that revealed renal lesions. Twenty-four (24) cases showed glomerular lesions, 29 cases-tubulointerstitial, 5 cases-vascular and 2 cases revealed neoplastic lesions. Glomerular changes included; 22 cases-fibrous caps in Bowman's space, 5 cases-mesangial and 5 cases intracapillary lesions. Tubular atrophy was found in 14 cases and sparse interstitial inflammation in 15 cases. Opportunist infections were found in 11 cases; comprising 4 CMV, 2 tuberculosis, 1 mycobacterium avium intracellulare and 4 Cryptococcal infections. One case revealed renal Kaposi sarcoma and another renal non-Hodgkin lymphoma [19].

Autopsy examinations of unselected cases with HIV infection have indicated that many of the lesions encountered in renal biopsy series are rarely observed in autopsies [24].

Below is a table which highlights major autopsy study outcomes conducted in the developed world discussed in the literature.

Table showing autopsy studies of HIV infected patients in Europe and America.

Author	Race	Country, City and number of autopsies done	Type of Study	Inclusion criteria	Methods	Renal lesions [n, (%)]	Glomerular lesions [n, (%)]	Tubulo-interstial lesions [n, (%)]	Vascular lesions [n,(%)]	Neoplasms [n, (%)]
Wyatt *et al*, 2009.	Blacks and a few Hispanics	USA, New York. 89	Prospective	Advanced HIV patients	LM: H&E and PAS-stained slides	75 (84)	22 (29)	77 (97)	15 (20)	0 (0)
Seife *et al*, 2001.	Caucasian	Switzerland, countrywide. 239	Prospective	Advanced HIV patients	LM: H&E,PAS,Mucicarmine and CR	102 (43)	89 (87)	3 (3)	7 (7)	3 (3)
Sarino-Rosas *et al* 1998.	Hispanics	Mexico, Mexico city. 138	Retrospective	Advanced HIV patients	LM:H&E, PAS, Jones Methenamine Silver.	87 (63)	62 (71)	80 (92)	0 (0)	2 (2.3)
Monga *et al*, 1997.	Caucasian	Italy, Milan. 120	Prospective	Patients with HIV	LM:H&E,PAS, CR	82(68.3)	25 (30)	30 (37)	27 (33)	0 (0)
Martinez *et al*, 1996.	Caucasian and Negros	Spain, Madrid 85	Prospective	Patients with HIV	LM: H&E,PAS,CR	50 (58.8)	0 (0)	50 (100)	0 (0)	4 (7)
van der Reijden *et al*, 1989.	Caucasian and Negros	Holland, Amsterdam 47	Prospective	Patients with HIV. No renal disease premortem	LM. H&E,PAS, CR	35 (75)	24 (68)	29 (82)	5 (14)	2 (6)

The table shows major autopsy studies in Europe and America and how their findings compare.

2.2. Research Question.

What are the histological appearances of the adult kidney in HIV infection at autopsy at the UTH Lusaka, Zambia?

2.3. Aim.

To describe kidney histological appearances in HIV infection using autopsy material at the UTH, Lusaka.

2.4 Specific objectives.

2.4.1 To determine glomerular, tubular, interstitial and vascular histological appearances.

2.4.2 To determine kidney histological appearances consistent infections in HIV cases.

2.4.2 To determine kidney neoplasms in HIV cases.

2.5. Ethical issues.

The waiver was granted by ERES Converge IRB for this nested study, see appendix 13. The study used blocked autopsy material (kidneys) from the Zambia Neuro-AIDS (subtype C neuro-AIDS and pathogenesis in Zambia) study. Permission to perform the study was granted by the office of the Senior Medical Superintendent of the University Teaching Hospital, refer to appendix 12.

2.5.1. Consent.

The Zambia Neuro-AIDS (subtype C neuro-AIDS and pathogenesis in Zambia) study obtained consent from the next of kin, see to appendix 2.

Chapter three: Methodology.

3.1. Research design and setting.

This was descriptive study that investigated blocked autopsy adult kidney samples of HIV infected patients at the University Teaching Hospital in the period 2010 to 2012.It was part of Zambia Neuro-AIDS study (subtype C neuro-AIDS and pathogenesis in Zambia) that was to investigating effects of HIV on the brain at autopsy. The principal investigator of this study performed and assisted in 40 autopsies out of the 466 total autopsies between 2011 and 2016. The study was conducted in the Department of Pathology and Microbiology at The UTH, Lusaka, Zambia.

3.2. Sample size, sampling frame and sample selection.

Two hundred (200) pairs of paraffin blocks (400 paraffin blocks) collected in the period 2010 to 2012.

These were samples from autopsies conducted in the period 2010-2012 collected by convenient sampling from participants in the Zambia Neuro-AIDS study (subtype C neuro-AIDS and pathogenesis in Zambia).

3.3. Inclusion Criteria.

Paraffin blocked kidney samples from the decedents who were HIV infected and aged above 16 years in the Zambia Neuro-AIDS study (subtype C neuro-AIDS and pathogenesis in Zambia).The age 16 years is used because of the criteria of admission at the UTH where all patients above 16 years are admitted to the adult wards and not the paediatric ward.

3.5. Exclusion criteria.

Specimens from cases below 16 years and any paraffin blocked kidney samples that were not part of the Zambia Neuro-AIDS study (subtype C neuro-AIDS and pathogenesis in Zambia).

3.6. Review of available clinical data.

A review of available clinical data which included age, sex, past medical history, drug history, CD4+ count results, urea, creatinine, proteinuria and clinical diagnosis was conducted. Refer to appendix 14.

3.7. Tissue processing and microscopic examination.

Paraffin blocks were processed according to the standard operating procedures (SOP) at the University Teaching Hospital. This was done by a qualified histopathology laboratory technician.

3.7.1. Specimen collection and specimen labelling.

Paired formalin-fixed paraffin embedded kidney specimens were collected from the tissue bank for the Zambia Neuro-AIDS study (Subtype C Neuro-AIDS and pathogenesis in Zambia).

Each formalin-fixed paraffin embedded kidney specimen block was given a new identification number (i.e. 000) in this study.

3.7.3. Microtomy procedure.

Refer to appendix 4.

3.7.2. Staining.

For staining procedures (H& E, PAS, CR, MTS, ZN and GMS). Refer to appendix 5, 6,7,8,9 and 10.

3.7.3. Slide mounting.

Refer to appendix 11.

3.7.4. Microscopic examination and clinical case notes review.

Microscopic examination to evaluate; glomerular, interstitial, tubular and vascular appearances was conducted using an Olympus CX 31 binocular biological microscope at the following power of magnifications: x 20, x 40, x 100, x 200 and x400.

The tissue was evaluated to identify renal lesion and if any was identified, independent of compartment, it was enumerated in the data collection sheet (refer to appendix 15, 16, 17, 18, 19, and 20). The case was counted once even if there were multiple lesions identified in one compartment or different compartments to come up with the total number of renal lesions. The different tissue compartments were evaluated systematically, i.e. glomeruli, tubules, interstitium, and vasculature guided by the definitions, refer to appendix 21, 22, and 23. This meant therefore that the renal lesions and percentages of in various compartments lesions did not add up to the total number of cases with renal lesions.

Infections and neoplasms as suggested by the histological appearances were listed. See appendix 14, 15, 16, 17, 18, 19 and 20.

3.8. Statistical analysis.

Data was entered into Epi infor 7 and analyzed using the statistical software package SPSS version 21. Descriptive analytical statistics were used to provide simple summaries about kidney samples and descriptions thereof. Continuous variables were expressed as percentages and as actual numbers. Categorical variables were expressed as percentages. Descriptive statistics were shown by graphical representation. All statistical tests were at

5% significance level. The Pearson's chi-squared test was used for comparison of proportions between groups. The Fisher's exact test was used when one or more of the cells had an expected frequency of five or less. The Independent Samples T-test and Mann-Whitney U Test were used to compare means and medians, respectively. Selection for entry into logistic regression model was based on $P < 0.20$ or clinical significance.

Chapter 4: Results.

4.1 Clinical, demographic characteristics and renal lesions.

There were 200 decedents, 128 (64%) male and 72 (36%) female. The mean age was 35.6 ± 8.70 years. Categorically, there were 60 (30%) cases between the age 16 – 30 years, 120 (60%) between 31 – 45 years, 18 (9%) between 46 – 60 years, and 2 (1%) between 61 – 75 years. Refer to figure 1.

One hundred and three cases (51.5%) had a diagnosis of TB medical, 8 (4%) had a clinical diagnosis of renal disease, 80 (40%) had other medical history, and 14 (7%) had no medical history.

There were 65 (32.5%) cases on ART and 135 (67.5%) cases not on ART. Out of the 65 cases on ART 63 cases had information of drug combinations. Thirty seven cases (58.7%) were on Truvada/EFV and 18 (28.6%) were on Atripla. Refer to table 4.

Twenty nine (29) cases had CD4 count results; twenty three (23) cases had urea results, with median urea value of 5.1 Thirty nine (39) cases had creatinine results with median creatinine value of 90.1. Refer to Table 1.

At 5% significance level, none of the study variables were significantly associated with the presence of renal lesions. Refer to Table 2.

Logistic regression analysis done for presence of renal lesions showed that cases on ART had on average 86% reduced odds for presence of renal lesions compared to cases not on ART [Odds Ratio (OR) = 0.14, 95% Confidence Interval (CI) = 0.06 – 0.34, P <0.001). Cases with no TB infection had on average 69% reduced odds for presence of renal lesions (OR = 0.31, CI = 0.18 – 0.53, P <0.001). Refer to table 3.

4.2 Renal lesion in the various compartments.

Two hundred (200) cases were examined and 169 (84.5%) cases revealed renal lesions, and this proportional difference was significant (P<0.001). Tubulointerstitial lesions were most prevalent, 130 (65%) cases, glomerular lesions 118 (59%) cases, and 4 (2%) had

vascular lesions. There was no neoplasm identified in the study population. There were 39 (19.5%) cases renal tuberculosis. Refer to table 1 and figure 1 and 2.

Table 1. Below summarizes the characteristics of the study cases.

Variable	**Frequency (n = 200)**	
	n	**%**
Sex		
Female	72	36
Male	128	64
Age category		
16 - 30 years	60	30
31 - 45 years	120	60
46 - 60 years	18	9
61 - 75 years	2	1
Medical history		
TB	103	51.5
Renal Lesions	8	4
Other	80	40
None	14	7
ART History		
Pre-ART	135	67.5
ART	65	32.5
Renal Lesions		
Present	169	84.5
Absent	31	15.5
Glomerular		
No	118	59
Yes	82	41
Tubules		
No	70	35
Yes	130	65
Blood vessels		
No	196	98
Yes	4	2
Interstitium		
No	85	42.5
Yes	115	57.5
TB infection		
Yes	39	19.5
No	161	80.5
Age (n, mean, SD)	200, 35.6, 8.70	
Urea (n, median, IQR)	23, 5.1, 40.1	
Creatinine (n, median, IQR)	39, 90.1, 81	

Bivariate analysis

At 5% significance level, none of the study variables were significantly associated with presence of renal lesions (Refer to Table 2.)

Table 2. Bivariate analysis for association with renal lesions

Variable	Renal Lesions Present		Renal Lesions Absent		P-value
	n	%	n	%	
Sex					
Female	62	36.7%	10	32.3%	0.64[c]
Male	107	63.3%	21	67.7%	
Age category					
16 - 30 years	51	30.2%	9	29.0%	0.99[f]
31 - 45 years	101	59.8%	19	61.3%	
46 - 75 years	17	10.1%	3	9.7%	
TB medical history					
No	79	46.7%	18	58.1%	0.25[c]
Yes	90	53.3%	13	41.9%	
Renal Lesions medical history					
No	162	95.9%	30	96.8%	0.99[f]
Yes	7	4.1%	1	3.2%	
Other medical history					
No	103	60.9%	17	54.8%	0.52[c]
Yes	66	39.1%	14	45.2%	
ART History					
Pre-ART	110	65.1%	25	80.6%	0.09[c]
ART	59	34.9%	6	19.4%	
CD4 count					
0 - 100	11	47.8%	4	66.7%	0.65[f]
Above 100	12	52.2%	2	33.3%	
HAART Regimen					
Atripla	15	26.3%	3	50.0%	0.34[f]
Truvada	42	73.7%	3	50.0%	
TB infection					
Yes	36	21.3%	3	9.7%	0.13[c]
No	133	78.7%	28	90.3%	
Age (n, mean, SD)	169, 35.5, 8.89		31, 36.2, 7.65		0.68[t]
Urea (n, mean rank)	16, 13.2		7, 9.2		0.20[m]
Creatinine (n, mean rank)	32, 21.28		7, 14.14		0.14[m]

[c]=Chi Square Test, [t]=Independent samples T-test, [f]=Fisher's exact test, [m]=Mann-Whitney U test

Table 3. Logistic regression analysis predicting renal lesions

Variable	Crude Odds Ratio (95% CI)	Adjusted Odds Ratio (95% CI)	P-value
Drugs History			
Pre-HAART	1	1	
HAART	0.10 (0.04 - 0.24)	0.14 (0.06 - 0.34)	<0.001
TB Infection			
Yes	1	1	
No	0.23 (0.14 - 0.38)	0.31 (0.18 - 0.53)	<0.001

Table 3 below shows logistic regression analysis predicting presence of renal lesions. Cases on ART had on average 86% reduced odds for presence of renal lesions compared to cases on Pre-ART [Odds Ratio (OR) = 0.14, 95% Confidence Interval (CI) = 0.06 – 0.34, P < 0.001). Cases with no TB infection had on average 69% reduced odds for presence of renal lesions (OR = 0.31, CI = 0.18 – 0.53, P <0.001).

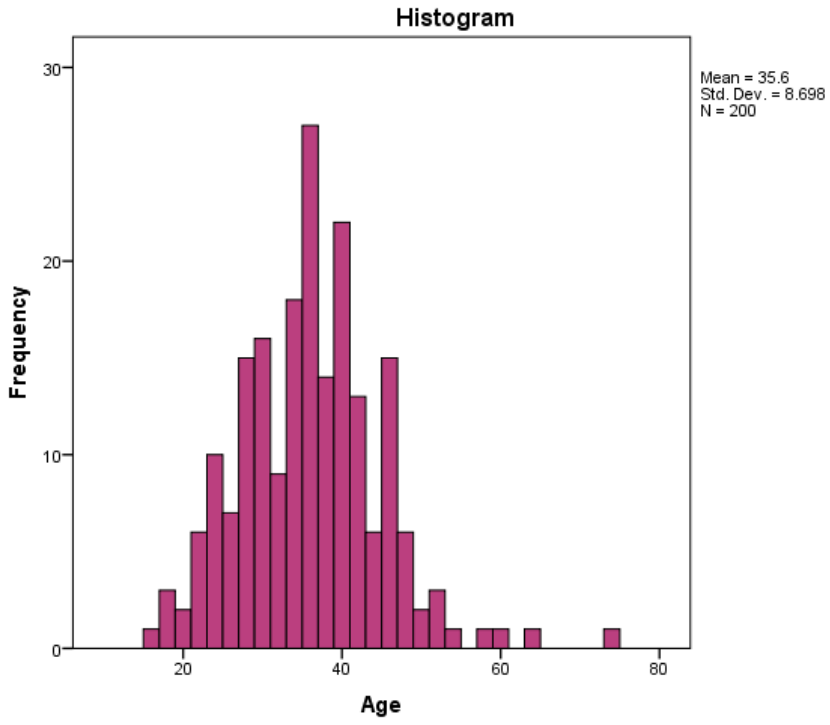


Figure 1. Case age distribution

Table 4. ART combination frequency distribution

ART Combination	Frequency	Percentage (%)
ATRIPLA	18	28.6
TRUVADA/EFV	37	58.7
TRUVADA/NVP	7	11.1
TRUVAVDA/EFV	1	1.6
Total	63	100

The most used combination was TRUVADA/EFV with 37 cases and the least used combination was TRUVADA/EFV with one case.

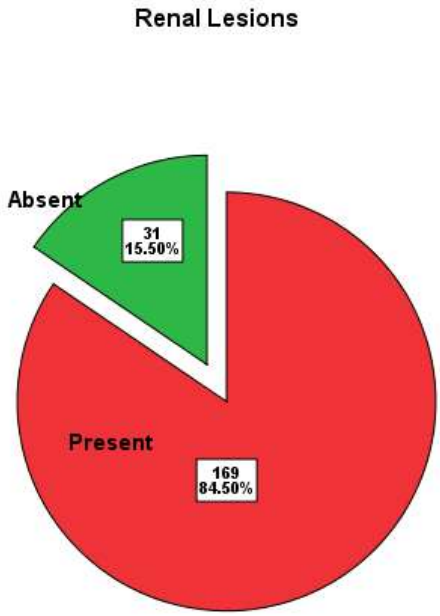


Figure 2. Renal lesions frequency pie chart

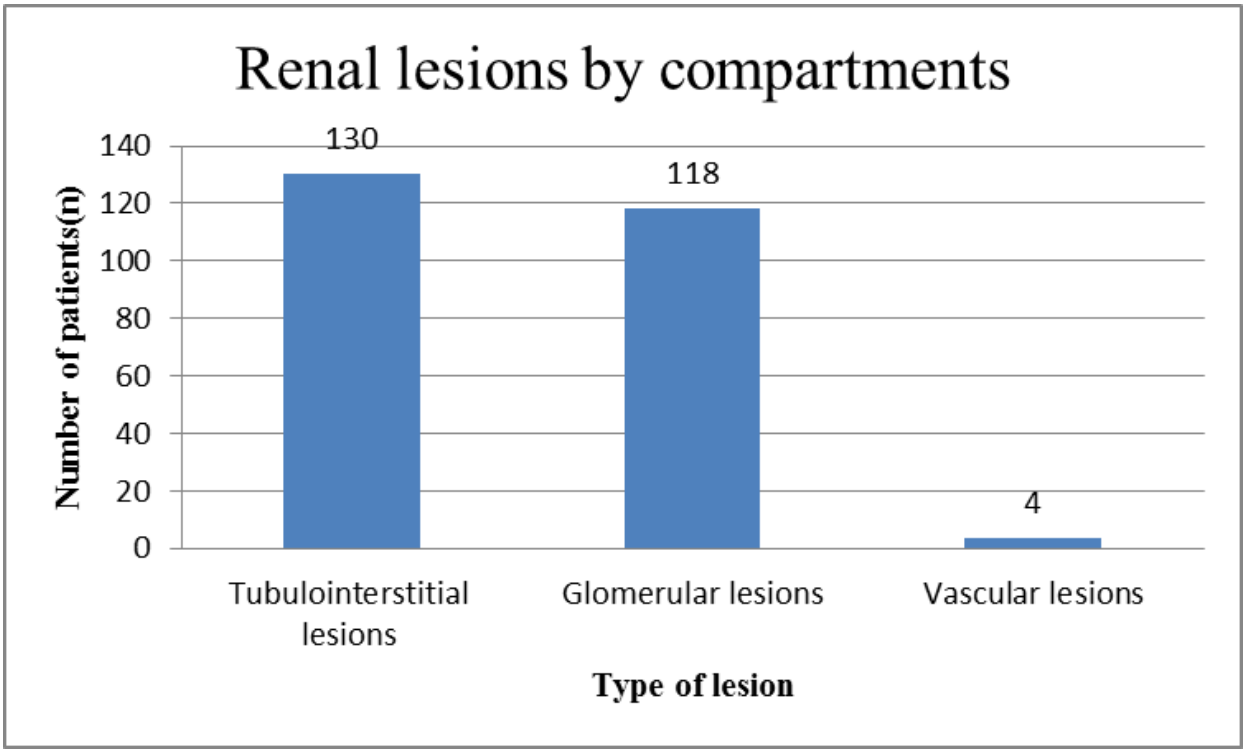


Figure 3. Distribution of renal lesions in the study population.

Tubulo-interstitial lesions were most prevalent 130 cases, glomerular lesions with 118 cases and least were the vascular lesions.

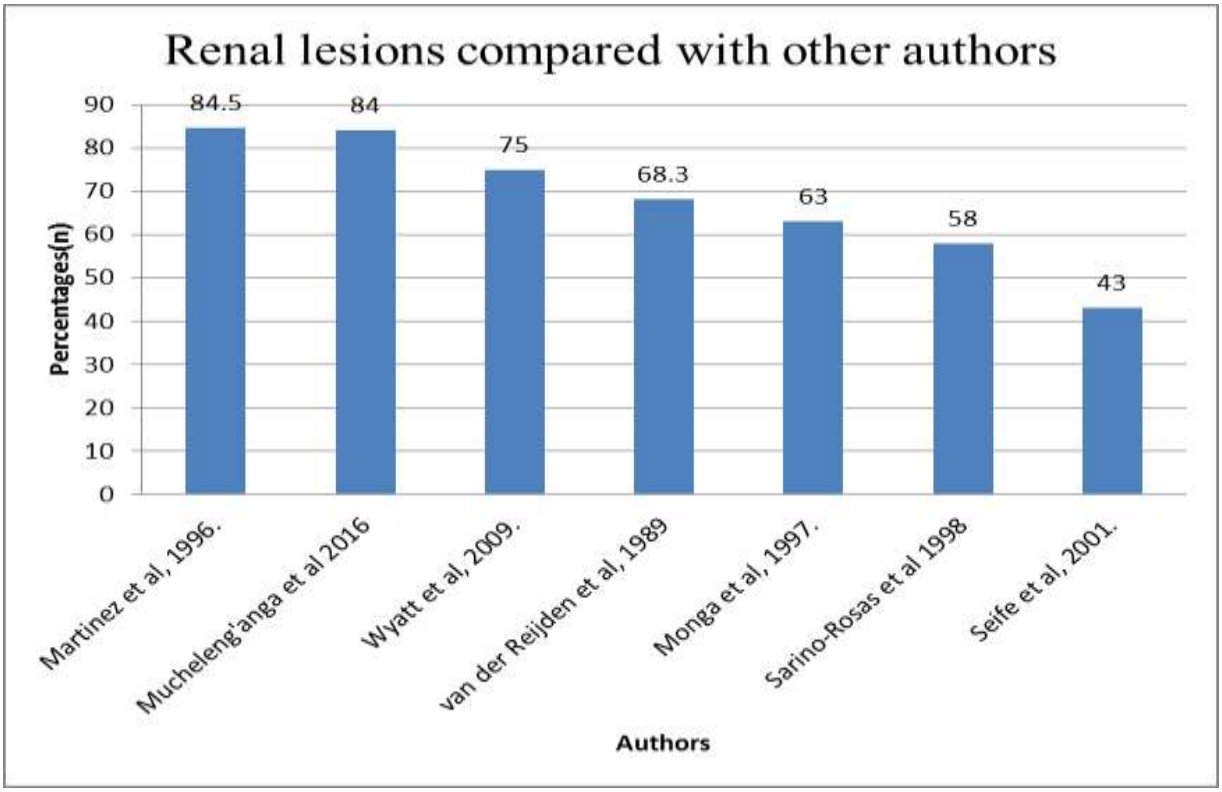


Figure 4. Renal lesions compared to studies conducted in Europe and America.

This study revealed a high prevalence of renal lesions compared to the quoted autopsy studies in literature. Martinez *et al*, 1996; Wyatt *et al*, 2009; Sarino-Ross *et al*, 1998; and van der Reijden *et al*, 1989; all showed high prevalence of renal similar to this study. Seife *et al*, 2001 had a lower prevalence.

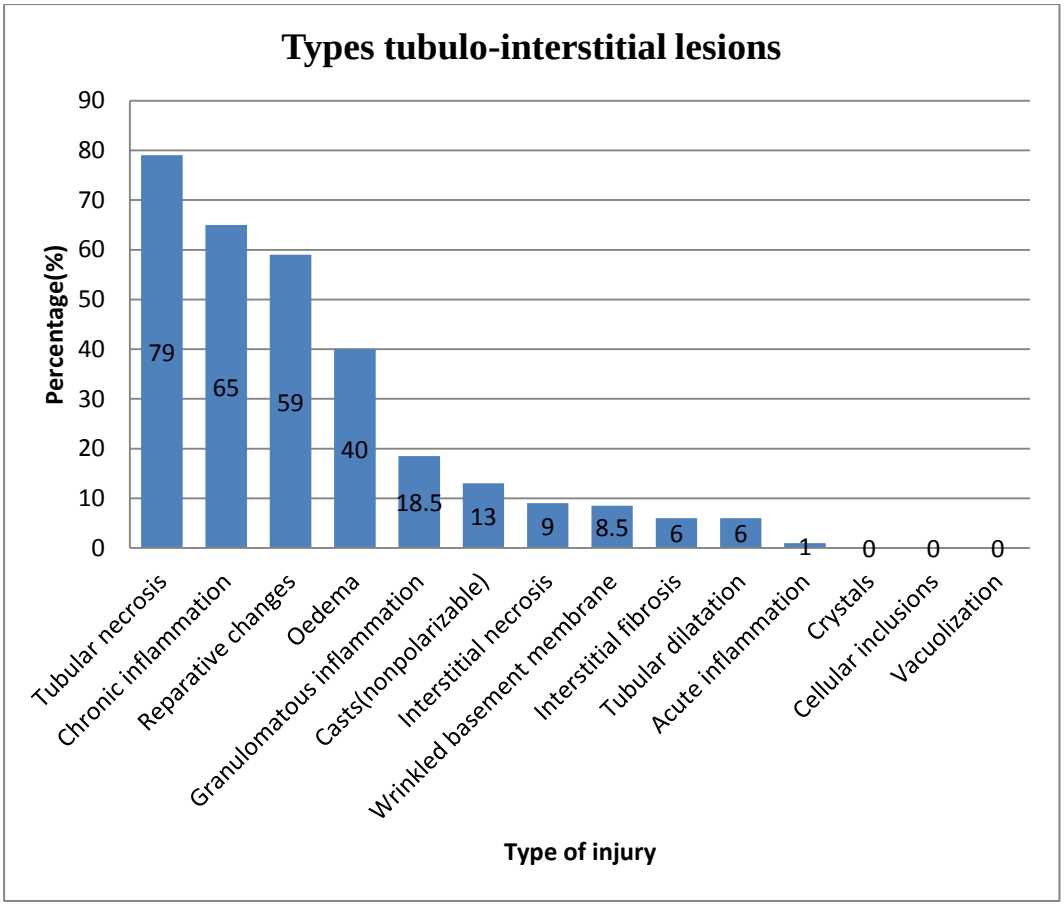
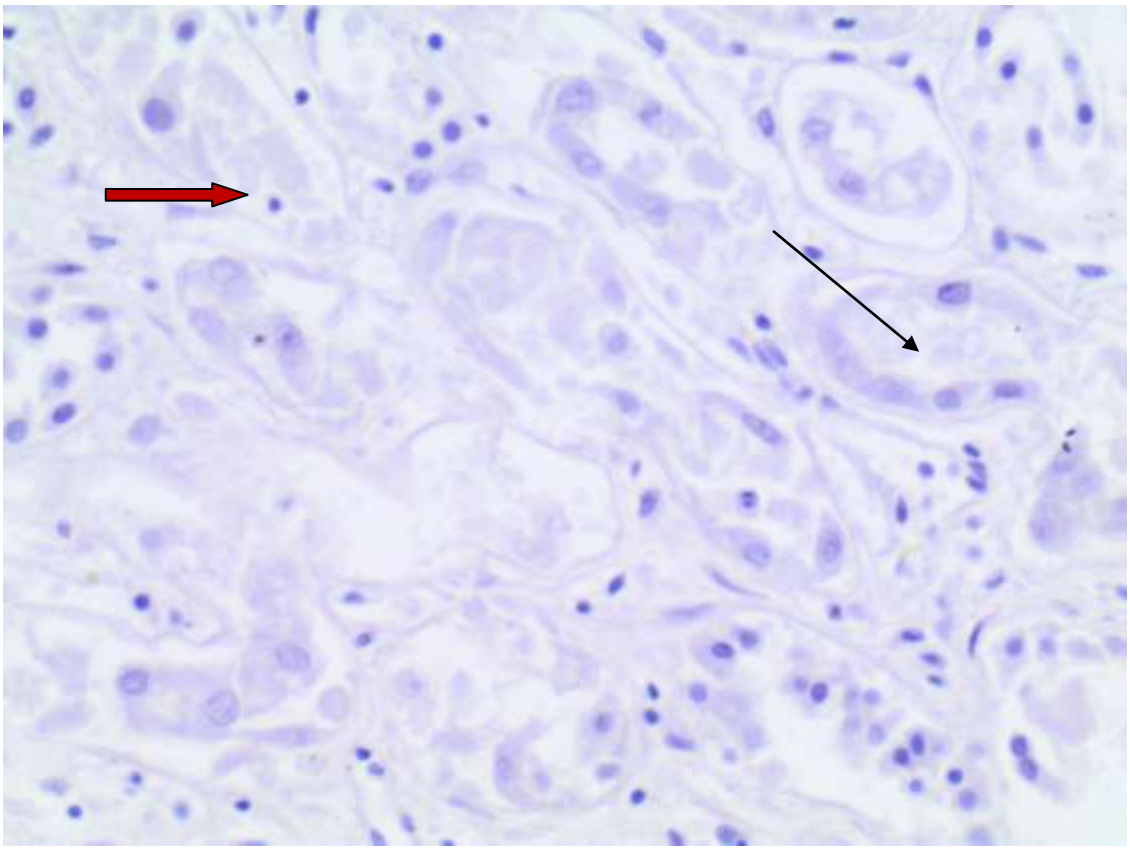


Figure 5. Types of tubulo-interstitial lesions in the study population.

Tubular necrosis (79%), reparative changes (59%).Refer to figure 5. Sixty five percent (65%) Chronic inflammation was among the predominant interstitial responses to injury. Refer to figure 6. Granulomatous inflammation and non-polarizable casts were 18.5% and 13% respectively. Refer figures 8 and 9. Acute inflammation, interstitial fibrosis, edema, interstitial necrosis, wrinkled basement membranes and tubular dilation were below 10%. Cellular inclusions and vacuolization were not observed.

Figure 6: Micrograph showing tubular necrosis and reparative changes.



This micrograph of part of kidney shows sloughing of epithelial cells in tubular epithelium with maintenance of the basement membrane.

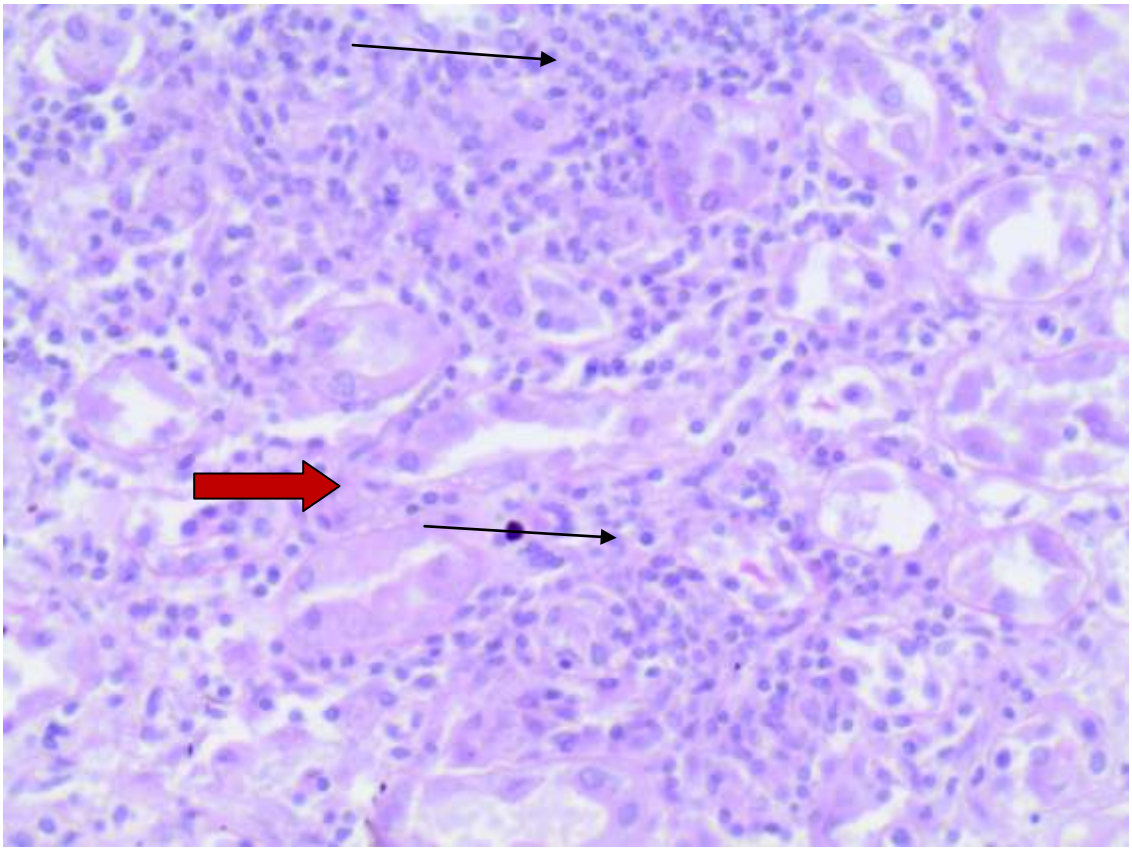
PAS x 100.

Key:

Red arrow- intact membrane.

Thin black arrow-necrosis

Figure 7: Micrograph showing interstitial nephritis with reparative changes.



This micrograph of part of kidney shows infiltration of the interstitium by mononuclear cells.

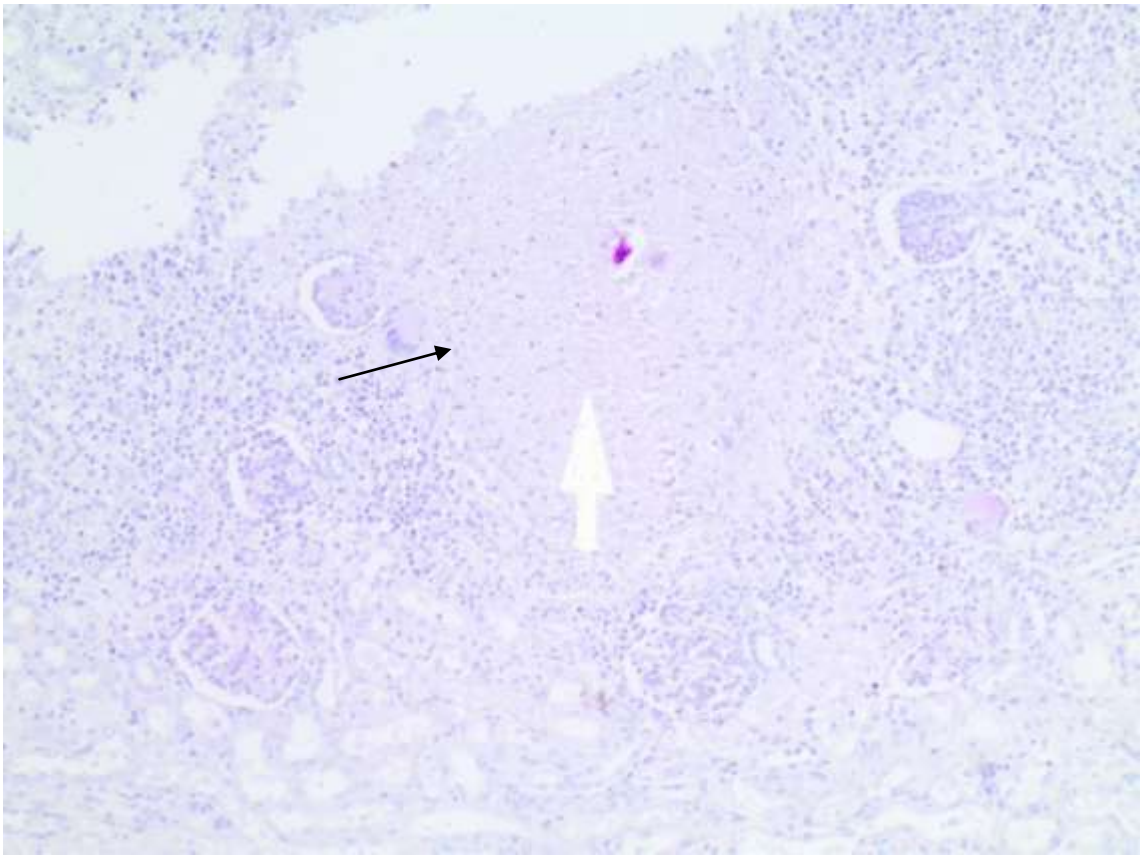
PASstain x 100.

Key:

Thin black arrows- mononuclear cells.

Big red arrow- necrosis.

Figure 8. Micrograph showing granulomatous inflammation in a tuberculosis case.



This micrograph of part of kidney shows chronic granulomatous inflammation composed of macrophages, lymphocytes and giant cells surrounding an area of necrosis.

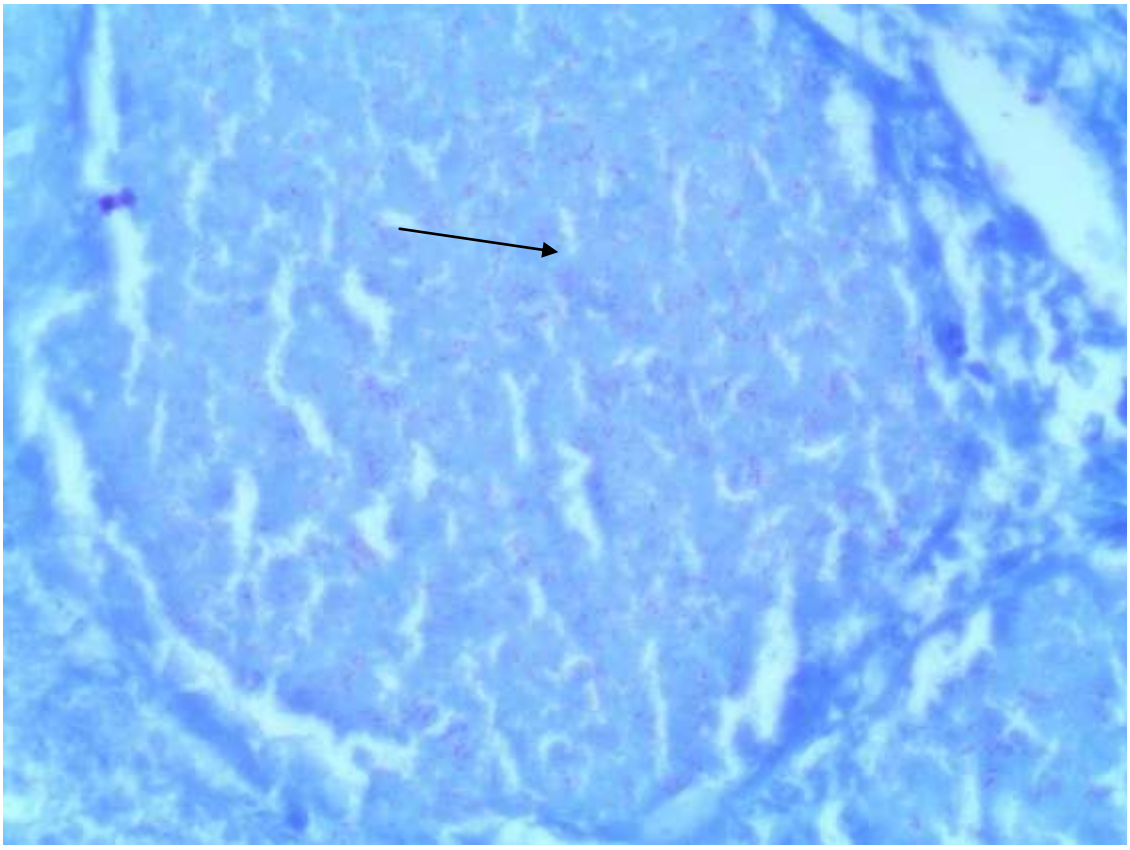
PAS stain x 100

Key:

Yellow arrow-necrosis.

Thin black arrow-Langhans giant cell.

Figure 9. Micrograph showing tubercle bacilli in a tubule in a kidney in a tuberculosis case.



This micrograph of part of kidney shows tubercle bacilli in a tubule with granulomatous inflammation.

ZN Stain x 400

Key:

Black arrow-tubercle bacilli

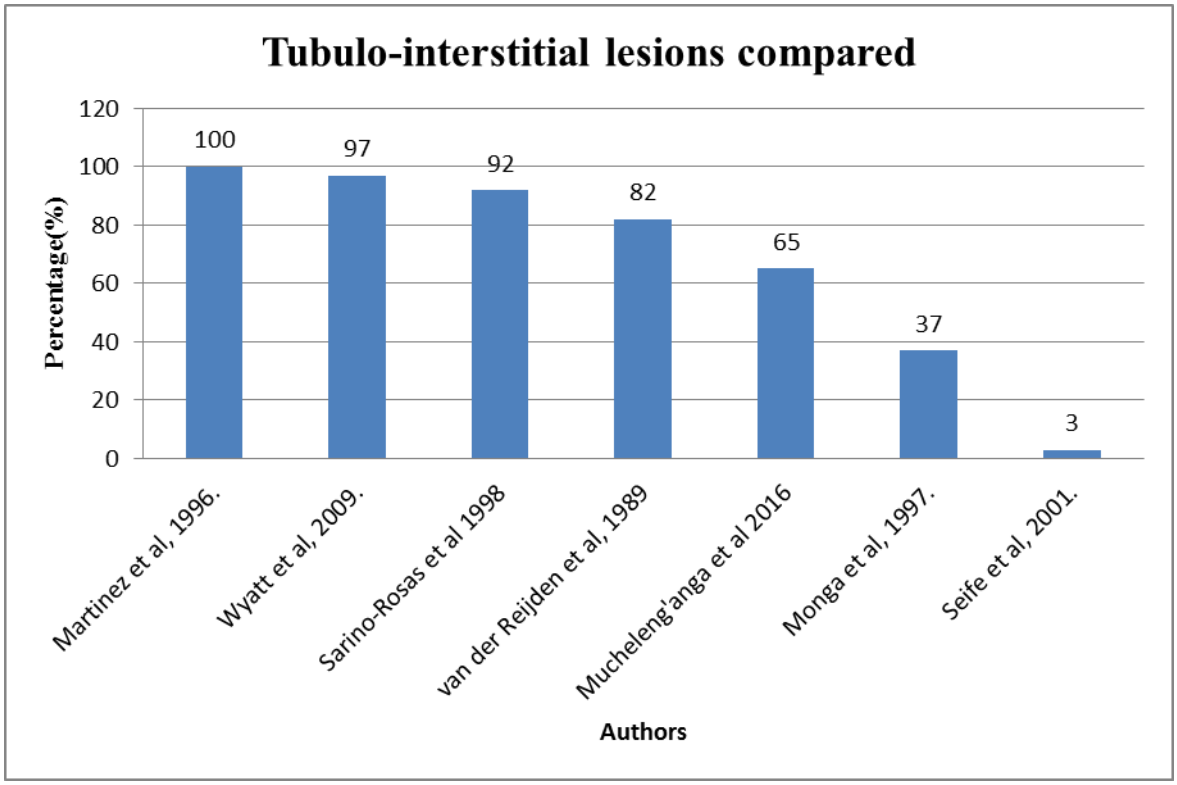


Figure 10. Tubulo-interstitial compared to studies conducted in Europe and America.

Martinez *et al*, 1996; Wyatt *et al*, 2009; Sarino-Ross *et al*, 1998 and van der Reijden *et al*, 1989 all showed a higher prevalence of tubulo-interstitial lesions than this study. Seife *et al*, 2001 had a lower prevalence.

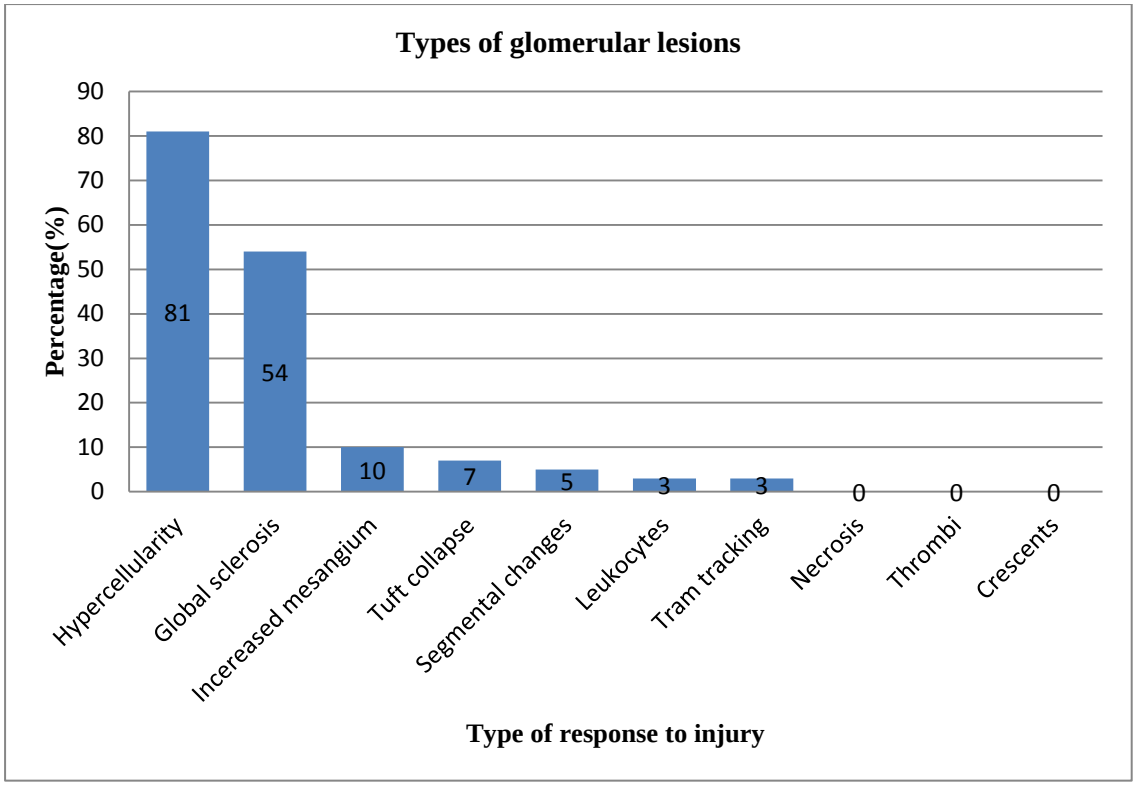
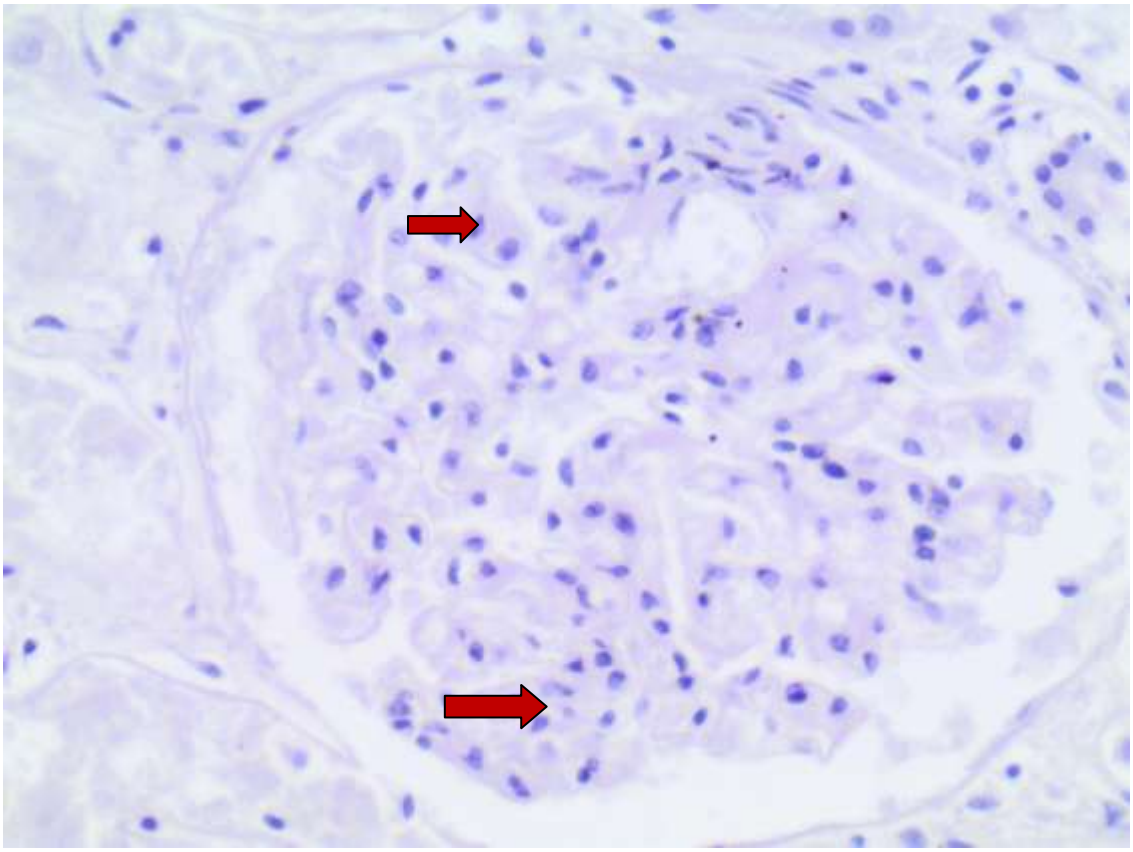


Figure 11: Types of glomerular lesions in the study.

Hypercellularity of mesangium was the most prevalent pattern 81% while global sclerosis was 54%. Refer to figure 12 and 13). The rest of the responses to injury were below10%. There was no necrosis, thrombi and crescents.

Figure 12: Micrograph showing hypercellularity.



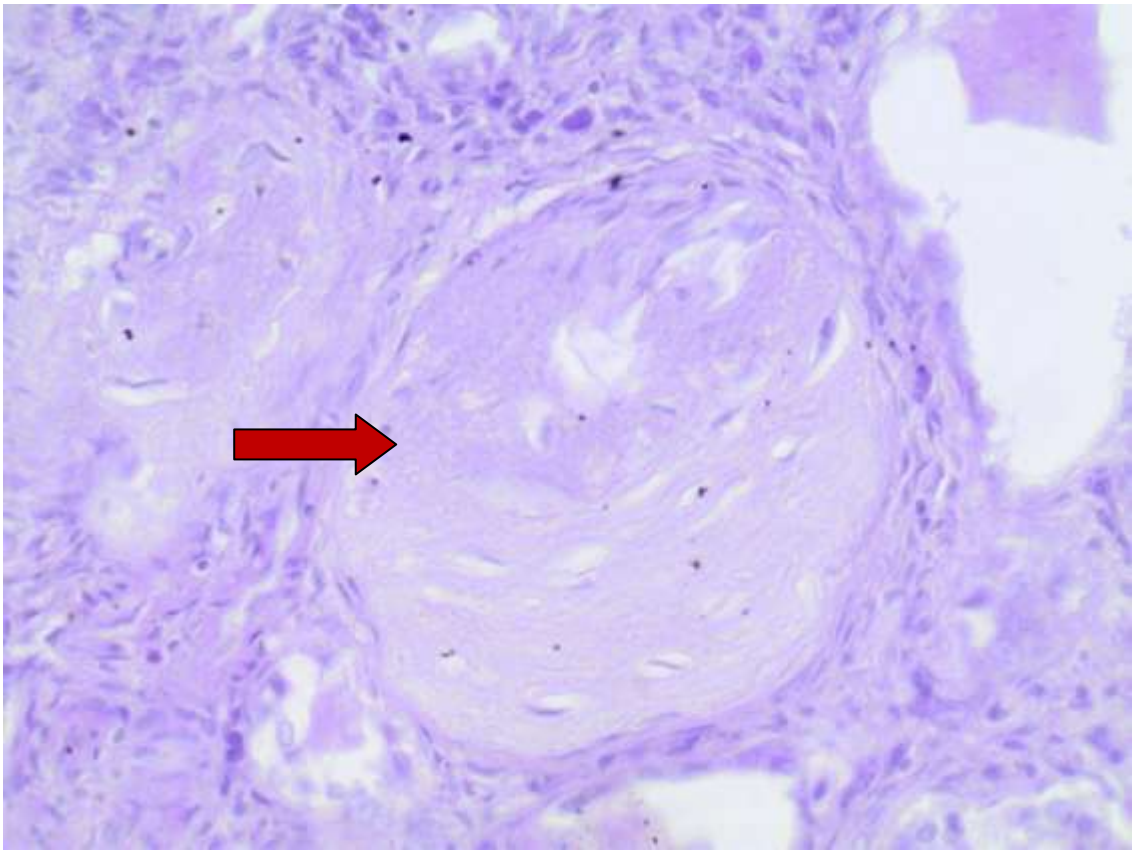
This micrograph of part of kidney shows a glomeruli with 4 or more nuclei in a peripheral mesangial segment or glomerular tuft.

PAS Stain x 400.

Key:

Red arrow-areas of hypercellularity.

Figure 13: Micrograph showing global sclerosis.



This micrograph of part of kidney shows increased collagenous extracellular matrix that has expanded the mesangium, obliterating capillary lumens.

PAS Stain x 400.

Key:

Red arrow -collagenous extracellular matrix

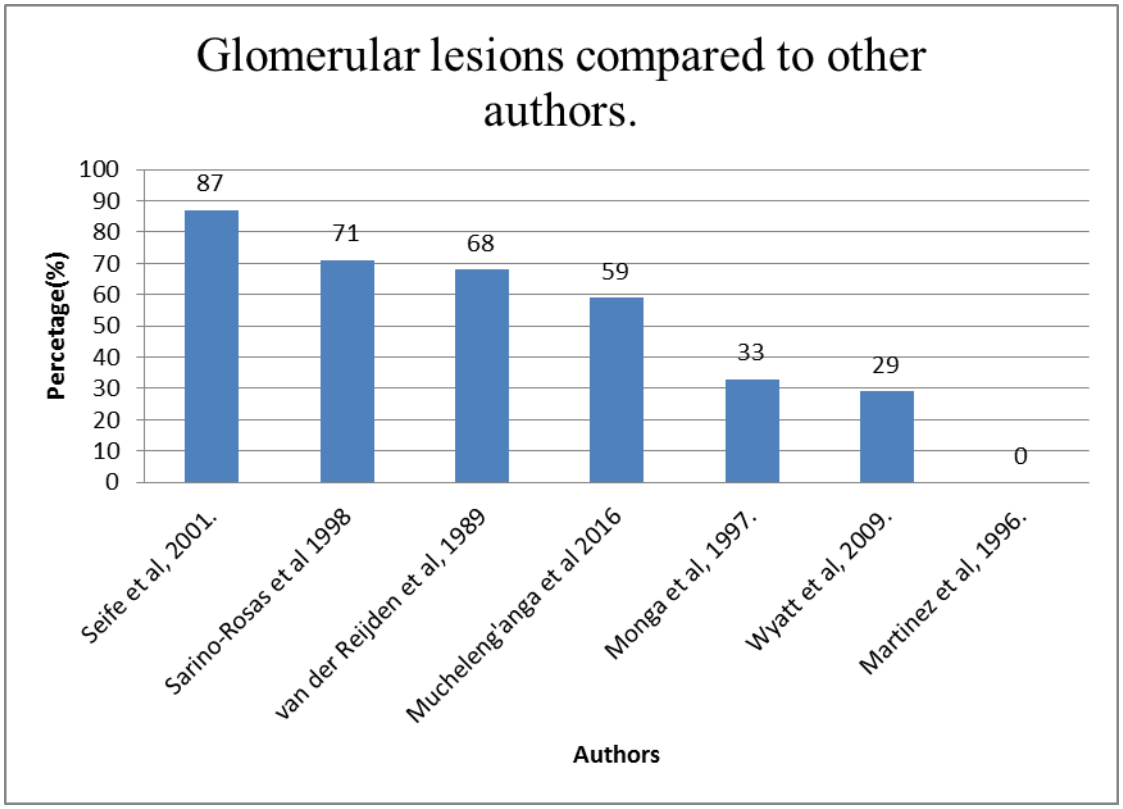


Figure 14: Glomerular lesions compared to studies conducted in Europe and America.

Seife *et al*, 2001; Sarino-Ross *et al*, 1998; van der Reijden *et al*, 1989; showed a higher prevalence of glomerular lesions than this study. Wyatt *et al*, 2009 and Monga *et al*, 1997 revealed a lower prevalence of glomerular lesions. Martinez *et al*, 1996 showed no glomerular lesions.

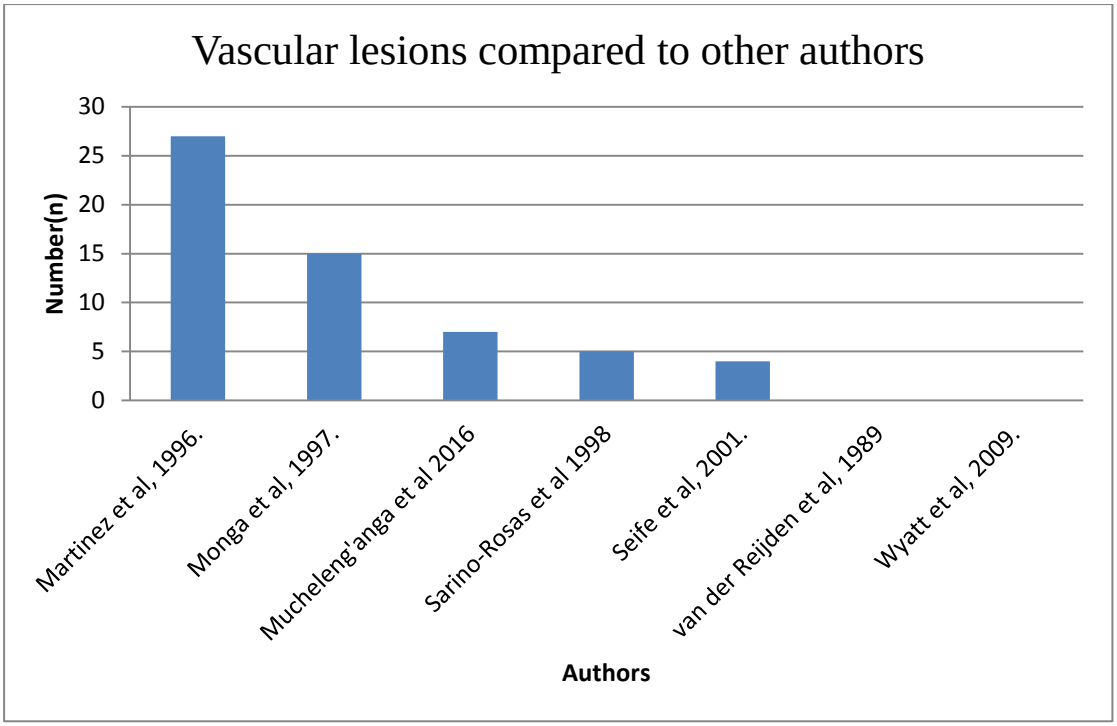


Figure 15: Vascular lesions compared to studies conducted in Europe and America.

Martinez *et al*, 1996; Monga *et al*, 1997; showed a higher prevalence of vascular lesions than this study. Seife *et al*, 2001; Sarino-Ross *et al*, 1998 showed a lower prevalence than this study. Wyatt *et al*, 2009 and van der Reijden *et al*, 1989 revealed a no vascular lesions.

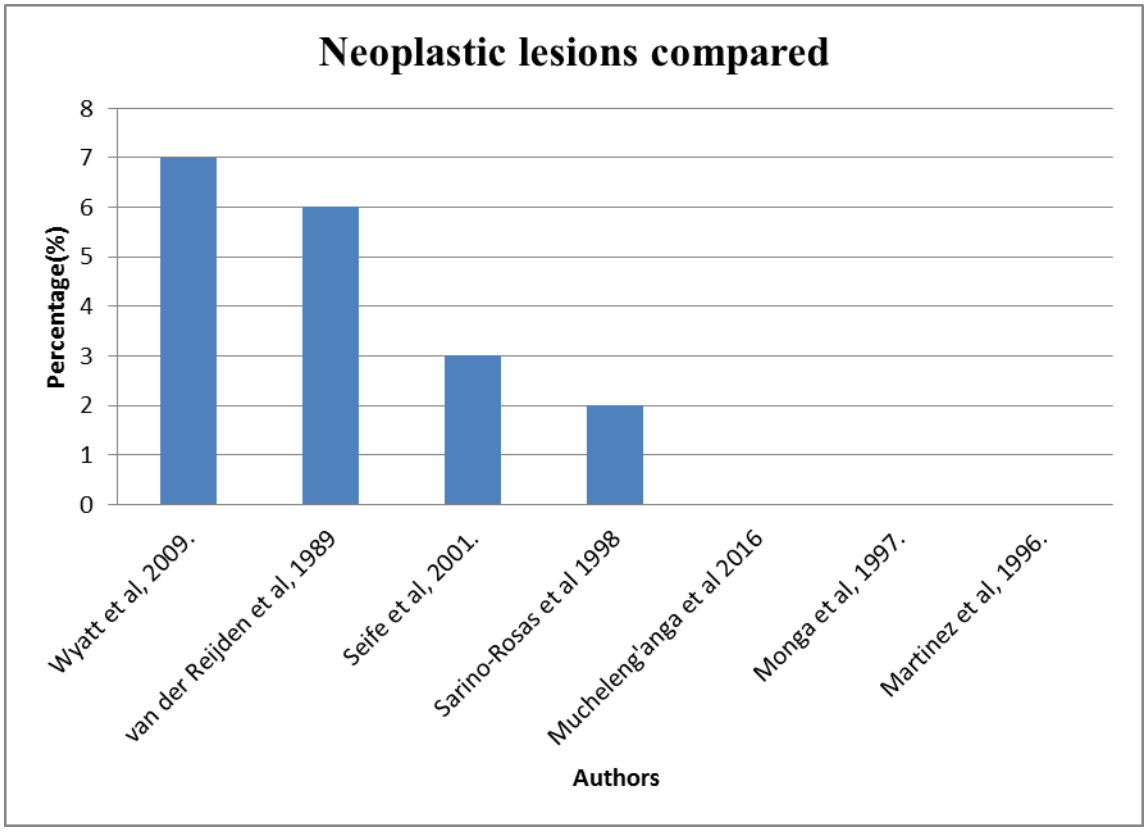


Figure 16. Neoplastic lesions compared to studies conducted in Europe and America.

Wyatt *et al*, 2009; van der Reijden *et al*, 1989; Seife *et al*, 2001; Sarino-Ross *et al*, 1998showed a higher prevalence of neoplastic lesions than this study. Martinez *et al*, 1996, Monga *et al*, 1997, showed a zero prevalence of neoplastic lesions.

Chapter 5: Discussion.

This study provided a window into the range of renal histological lesions seen in HIV-infected cases that were unselected for the presence of kidney disease at UTH in Lusaka. The study population consisted of two hundred (200) cases and 165 (84.5%) of cases revealed renal lesions, the cases being black Africans only.

Clinical and demographic characteristics

In this study sex, age, history of kidney diseases, level of CD4 count, level of urea and creatinine were not significantly associated with the presence of renal lesions. The findings are at variance with recent data from biopsies that show an association between age and renal lesions [28, 30], however this difference is supported by the idea that clinical and autopsy kidney studies show different statistics for the same lesions [24].

Kenneth *et al*, 2001 in this population-based study stated that some of the predictors of renal disease among women with proteinuria included an absolute CD4 cell count of ⩽200 cells/μL14. This investigation shows a different result due to the population differences such as having both males and females and the fact that in our study the patients were unselected for kidney disease and their study was limited by its inability to define histologic lesions and may have included women with HIV-related renal diseases other than HIVAN[31].

Logistic regression done the absence of Tb clinically and HIV infected cases on ART in this study showed an association, and this is in line with studies conducted by (Rule *et al*, 2010 and Tan et al, 2010). The findings are also supported by Quesada *et al*, (2015) who showed that several types of ART are associated with kidney dysfunction [32, 33].

ART contributes to renal dysfunction directly by inducing acute tubular necrosis, acute interstitial nephritis, crystal nephropathy, and renal tubular disorders [36]. This explains the renal lesions observed in cases on ART.

ART renal toxicity occurs in HIV cases with pre-existing kidney disease, poorly controlled HIV disease, long use of HAART duration, elevated baseline creatinine concentration, female gender, African ethnicity, CD4+ cell count less than 200 cells/mm3, and where other nephrotoxic drugs are administered [38,39]. This then entails that clinicians treating HIV cases must be on the lookout for renal pathology.

Light microscopy

This study provided a detailed histological description of the various compartments of the kidney in adults infected with HIV/AIDS at autopsy. One hundred and sixty-five cases showed renal lesions. This study compares very well with studies in literature [19, 25, 29]. Wyatt *et al*, (2009), van der Reijden *et al*, (1989) and Martinez *et al*, (1996) had a mixed population with blacks in their study hence the similarity in prevalence of renal lesion.

One hundred and thirty (130) cases showed the tubulointerstitial group of lesions. The tubulointerstitial group was composed of the following lesions in order of frequency of lesions; tubular necrosis (79%), chronic inflammation (65%) and reparative changes were among the predominant interstitial responses to injury. Acute inflammation, interstitial fibrosis, edema, interstitial necrosis, wrinkled basement membranes and tubular dilation were below 10%. Tubular necrosis in this study was caused by HIV infection of the epithelial cells in tubules [34, 35].

Quoted autopsy studies in literature [19, 25, 27, 29,] showed a higher prevalence of tubulo-interstitial lesions than this study. This study recorded a lower prevalence of tubulointerstitial lesions (65%). This is due to the differences in sample sizes; this study had a high sample size. Most of the tubulointerstitial lesions found in this study represented renal disease that was not diagnosed clinically.

Seife *et al*, (2001) had a lower prevalence.

One hundred and eighteen (118) - 59% cases revealed glomerular lesions. The most prevalent lesion was extracapillary hypercellularity (81%) followed by global sclerosis at 54%. The causes of hypercellularity in the study were immune-mediated

which were not acutely severe enough to cause crescent formation. Global sclerosis indicates and end-stage glomerulus[35].

Autopsy studies [19, 26, 27] showed a higher prevalence of glomerular lesions than this study. Wyatt *et al*, 2009 and Monga *et al*, 1997 revealed a lower prevalence of glomerular lesions. Martinez *et al*, 1996 showed no glomerular lesions. This study compares relatively well to reviewed autopsy studies [19, 27] due the composition of the study populations i.e. mixed race population including blacks and Hispanics. Literature shows that glomerular lesions are less likely to occur in a black race population compared to the white population [26]. This investigation revealed that glomerular lesions are as prevalent in the black population.

In this study four cases (4) showed a vascular reaction to injury. Two (2) cases showed hyperplastic sclerosis and two showed intimal thickening. The hyperplastic sclerosis was caused by undiagnosed hypertension. Martinez *et al*, 1996, Monga *et al*, 1997, showed a higher prevalence of vascular lesions than this study. Seife *et al*, 2001, Sarino-Ross *et al*, 1998 showed a lower prevalence than this study. Wyatt *et al*, 2009 and van der Reijden *et al*, 1989 revealed a no vascular lesions.

Studies show black ethnicity is a strong and independent risk factor for peripheral arterial disease [33]. This is in contrast to the findings of this study and autopsy studies [19, 25, 26,27,28,28] again underscoring the idea that clinical and autopsy studies in kidney pathology in HIV cases yields different results [24].

This study revealed a 19.5% prevalence of renal TB infection. This is because of the high number of decedents diagnosed with tuberculosis at the time of admission. No fungal organisms or changes consistent with viral infections were identified in this study. Literature states that the kidney is a site of isolated infection or part of a systemic process [20]. No fungal organisms or changes consistent with viral infections were identified in this study. The reasons for this finding are obscure.

HIV-associated nephropathy was not identified in this study. This is in contrast to the view that HIVAN is a common lesion in HIV cases [36]. The reason for the low

prevalence of HIV-associated nephropathy in this study is that the cohort was unselected for the presence of kidney disease.

There were no neoplasms identified in this study showing that they are uncommon in the kidneys of HIV cases. This is in keeping with the low prevalence of neoplasms in literature [19, 25, 26,27,28,28] all below 7%. Autopsy studies reviewed in literature showed neoplasms such as Kaposi's sarcoma and lymphoma [21, 22, 23] which were not identified in this study.

5.3. Limitations.

A number of important limitations must be noted that; autopsy studies are limited by the potential for postmortem tissue autolysis and that the design of this study was limited by the lack of immunofluorescence and electron microscopic analysis.

This study focused on glomerular, interstitial, and vascular pathology, which are less sensitive to postmortem alterations. In addition, the design of the parent study facilitated rapid postmortem examination and harvesting of tissue, thereby minimizing autolytic changes. The evaluation of over 350 glomeruli per case had the advantage of providing a broad sampling of both cortex and medulla. This was particularly helpful to describe relatively focal tubulointerstitial processes.

The results of this study do contribute a useful resource to HIV and renal pathology.

Chapter 6: Conclusions and Recommendations.

The investigation has shown that renal lesions (tubulointerstitial 65%, glomerular 59%, vascular 2% and renal infections-TB-19.5%) are highly prevalent (84.5%) among cases at the UTH infected with HIV.

6.1 Recommendations and Future Research.

There are lesions like basement membrane thickening, podocyte foot effacement, light chain deposition disease, immune complex deposition etc. that are not apparent on light microscopy, so future studies with the use of immunofluorescence and electron microscopy are required to note and refine the presence of those lesions.

References.

1. Bourgoignie J, Meneses R, Ortiz C, Jaffe D, Pardo V. The clinical spectrum of renal disease associated with human immunodeficiency virus. Am J Kidney Dis. 1988 Aug; 12(2):131-7.
2. Cohen S, Chawla L, Kimmel P. Acute kidney injury in patients with human immunodeficiency virus infection. Curr Opin Crit Care. 2008 Dec; 14(6):647-53.s
3. Varma P, Prasher P, Deshpande G. Spectrum of renal lesions in HIV patients. J Assoc Physicians India 2000; 48:1151.
4. Herman E, Klotman P (2003). HIV-associated nephropathy: epidemiology, pathogenesis, and treatment. Semin Nephrol 2003; 23(2): 200–208.
5. Adedeji T, Adedeji N, Adebisi S, Idowu A, Fawale M, Jimoh K. Prevalence and Pattern of Chronic Kidney Disease in Antiretroviral-Naive Patients with HIV/AIDS. J Int Assoc Provid AIDS Care 2015, 26:434-40.
6. Jennette J, Olson J, Schwartz M, Silva F. Primer on the Pathologic Diagnosis of Renal Disease in Heptinstall's Pathology of the Kidney, 6th Edition, 2007, Chapter 3.
7. Agaba E, Agaba P, Sirisena N. Renal disease in the acquired immunodeficiency syndrome in north central Nigeria. Niger J Med 2003; 12:120.
8. D'Agati V, Suh J, Carbone L. The pathology of HIV nephropathy: A detailed morphologic and comparative study. Kidney Int 1989; 35:1358.
9. Winston J, Klotman M, Klotman P. (1995). HIV-associated nephropathy is a late, not early, manifestation of HIV-1 infection. Kidney Int; 55:1036-40.
10. D'Agati V, Appel G. HIV infection and the kidney. J Am Soc Nephrol 1997; 8(1): 138–152.

11. Banda J, Mweemba A, Siziya S, Mweene M, Andrews B, and Lakhi S. Prevalence and Factors Associated with Renal Dysfunction in HIV Positive and Negative Adults at the University Teaching Hospital, in Lusaka. Med J Zambia. 2010 July-September; 37(3): 136–142.

12. Gardenswartz M, Lerner C, Seligson G, Zabetakis P, Rotterdam H et al. Renal disease in patients with AIDS: a clinicopathologic study. Clin Nephrol. 1984 Apr; 21(4):197-204.

13. Pardo V, Aldana M, Colton R. Glomerular lesions in the acquired immunodeficiency syndrome. Ann Intern Med 1984; 101:429–434

14. Rao T, Filippone E, Nicastri A .Associated focal and segmental glomerulosclerosis in the acquired immunodeficiency syndrome. N Engl J Med 1984; 310: 669–67

15. Chloe S, Richard S, Anju S, Robert D, John M, Anmol M. Imaging and Histopathologic Features of HIV-related Renal disease. Radiographics Volume 28 Number 5, education exhibit.

16. Cho M, Kopp J. HIV and the kidney: a status report after 20 years. Curr HIV/AIDS Rep2004; 1(3):109–115.

17. Lu T, Ross M. HIV-associated nephropathy: a brief review. Mt Sinai J Med 2005; 72(3): 193–199.

18. Hailemariam S, Walder M, Burger HR. Renal pathology and premortem clinical presentation of Caucasian patients with AIDS: An autopsy study from the era prior to antiretroviral therapy. Swiss Med Wkly 2001; 131:412.

19. van der Reijden H, Schipper M, Danner S. Glomerular lesions and opportunistic infections of the kidney in AIDS: An autopsy study of 47 cases. AdvExp Med Biol 1989; 252:181.

20. Niedt G, Schinella R. Acquired immunodeficiency syndrome: Clinicopathologic study of 56 autopsies. Arch Pathol Lab Med 1985; 109:727.

21. Nochy D, Glotz D, Dosquet P. Renal lesions associated with human immunodeficiency virus infection: North American vs European experience. Adv Nephrol Necker Hosp 1993; 22:269.

22. D'Agati V, Appel G . Renal pathology of human immunodeficiency virus infection. Semin Nephrol. 1998 Jul; 18(4):406-21.

23. Seney F, Burns D, Silva F. Acquired immunodeficiency syndrome and the kidney. Am J Kidney Dis 1990; 16:1.

24. Pollok R, Francis N, Cliff S. Kaposi's sarcoma in the kidney. Int J STD AIDS 1995; 6:289.

25. Wyatt M, Susan M, Rebecca K, Mary E, Paul E. The spectrum of kidney disease in patients with AIDS in the era of antiretroviral therapy. Kidney Int. 2009 February; 75(4): 428–434.

26. Seife H, Marcel W, Hans-Rudolf B, Gieri C, Michael M. Renal pathology and premortem clinical presentation of Caucasian patients with AIDS: An autopsy study from the era prior to antiretroviral therapy. Swiss med wkly 2001; 131:412–41

27. Soriano-Rosas J, Avilla Carsado M , Carrera-Gonzalez E, Chavez-Mercado L, Cruz-Ortiz H . AIDS-associated nephropathy: 5-year retrospective morphologic analysis of 87 cases. Pathology research and practice 1998; Volume 194, Issue 8, 1998, Pages 567–570.

28. Monga G, Mazzucco G, Boldorini R, Cristina S. Renal changes in patients with acquired immunodeficiency syndrome: a post-mortem study on an unselected population in north western Italy.Mod Pathol. 1997 Mar; 10(3):159-67.

29. Martinez P, Pena J, Arnalich F, Garcia M, Adridano O, Gonzalez J, et al. Spectrum of the renal pathology in HIV infection: description of 85 autopsies and clinico-pathologic correlation. Rev Clin Esp 1996; 196(9):577–83.

30. Tan J, Busque S, Workeneh B, Ho B, Derby G, Blouch KL, et al. Effects of aging on glomerular function and number in living kidney donors. Kidney Int. 2010, 78:686-692.24.

31. Kenneth H. Mayer, Section Editor and Lynda Anne Szczech. Renal Diseases Associated with Human Immunodeficiency Virus Infection: Epidemiology, Clinical Course, and Management. Clin Infect Dis. (2001) 33 (1): 115-119. doi: 10.1086

32. Rule A, Amer H, Cornell L, Taler S, Cosio F, Kremers WK et al., The association between age and nephrosclerosis on renal biopsy among healthy adults. Ann Intern Med. 2010, 152:561-567.

33. Michael H, Veronica V, Julie O, Elena H, Matthew, Robert D, et al 2005 Ethnicity and Peripheral Arterial Disease, The San Diego Population Study. Circulation. 2005; 112:2703-2707

34. Madeddu G, Quirino T, Carradori S, Ricci E, Grosso C. Renal toxicity in HIV-infected patients receiving HAART including tenofovir.Infez Med. 2006 Sep; 14(3):125-34.

35. Vinay K, Abul K. Abbas J, Aster C. Robbins and Cotran, Pathologic basis of disease, ninth edition. Pages 903-910. ISBN 978-1-4557-2613-4. Elsevier Saunders, 1600 John F.Kennedy Blvd. Ste 1800 Philadelphia.

36. Han T, Naicker S, Ramdial P, Assounga A. A cross-sectional study of HIV-seropositive patients with varying degrees of proteinuria in South Africa. Kidney Int. 2006 Jun; 69(12):2243-50 PubMed ID: 16672914.

37. Izzedine H, Harris M, Perazella MA. The nephrotoxic effects of HAART. Nature Reviews Nephrology. 2009 Oct; 5(10):563-73. doi: 10.1038/nrneph.2009.142.

38. Jao J, Wyatt C. Antiretroviral medications: adverse effects on the kidney. Adv Chronic Kidney Dis. 2010 Jan; 17(1):72-82. doi: 10.1053/j.ackd.2009.07.009.

39. Röling J, Schmid H, Fischereder M, Draenert R., and Goebel F. HIV-Associated Renal Diseases and Highly Active Antiretroviral Therapy—Induced Nephropathy Clin Infect Dis. (2006) 42 (10):1488-1495.doi: 10.1086/503566)

Appendixes.

Appendix 1: Zambia HIV Neuro-AIDS study (Sub-type c Neuro-aids and pathogenesis in Zambia project.)

The Zambia HIV Neuro-AIDS study (Sub type C Neuro-AIDS and pathogenesis in Zambia) was set up to investigate the effects of HIV on the brain at autopsy. The aims of the study were to

(i) Develop tools needed to evaluate the extent of HIV associated neurological disease in Zambia

(ii) Determine the prevalence and underlying pathology of HIV associated dementia in subtype C infected individuals in Zambia

(iii) Provide short term training for Zambian neurologist, pathologists, and neurovirologists both in Zambia and the United States and to develop the tools and technology needed to address the first two proposed aims and to develop the Neuro-AIDS research infrastructure.

There were 480 autopsies performed over a period of six years (2009-2016). Full autopsies were done. Organs including thyroid, heart, lungs, liver, kidneys, adrenals and colon were routinely sampled at autopsy. The inclusion criterion was an adult HIV infected patient admitted to the University teaching hospital aged between 16 to 45 years. The study was approved by The University of Zambia Biomedical Research Ethics Committee. Consent was given for a full autopsy to sample organs for further study and investigation to allow for a full comprehensive autopsy report. See consent form in appendix.

The Zambia Neuro-AIDS (Sub type C Neuro-AIDS and pathogenesis in Zambia) study was approved by the Biomedical Research Ethics Committee. The funding agency to the parent study considered it as a low risk based on non-human participants in accordance to the department of health and human services46CFR46.

The Zambia Neuro-AIDS (Sub type C Neuro-AIDS and pathogenesis in Zambia) study obtained consent from the next of kin. Participation in this study was strictly voluntary. The next of kin was not remunerated, and did not suffer any consequences if they decided

not to participate in the study and he/she was allowed to withdraw from the study at any time for any reason without consequences.

In the case that the next of kin had any questions or clarifications, they were advised to contact Dr Victor Mudenda, at The UTH, Department of Pathology and Microbiology.

Consent was obtained from the next of kin after a thorough explanation of what the Zambia Neuro-AIDS study was about. The next of kin was informed that doctors would choose not to proceed to collect specimens or use such specimens for such study without the next of kin‘s consent in the consent form for the next of kin.

Appendix 2: Autopsy consent for the next of kin in sub-type c Neuro-AIDS and pathogenesis in Zambia project.

This information is being provided to regarding the Zambia Neuro-AIDS study to enable to give informed and voluntary consent to participate in this study.

Kindly read it carefully or let someone read it to you before you sign the consent form.

Introduction

People infected with HIV, the virus that causes AIDS, with time may lose their memory and sometimes become confused or demented. Although sub African has highest level of HIV infection in the world, there is very little known of how HIV affects a person's brain function in this region. This research seeks to understand how HIV infests the brain, and how it affects it. In order to do this, we will test samples from people who have already died of various causes and ran tests on their blood samples to see if they are HIV positive. If they are, we will use the samples collected normally at autopsy to see the effects, if any that HIV has had on the brains. These samples collected will include will include brain samples, cerebrospinal fluid, the fluid that washes over the brain and blood samples. On these samples, we will do tests to verify if the virus (HIV) is present or not, and at what stage of infection the person was at by determining their CD4 counts (a type of white blood cell) and viral loads(amount of virus in these samples.)

Benefits and risks

There is no risk to the family or the family or to the deceased as no more will be done than the normal procedure for autopsy. The immediate benefit to the family is that the autopsy will be expedited and should the family need transport to move the body from the point of death to the hospital, the project will provide this at no cost to the family. There is, however, benefit to the community as doctors get to better understand how HIV affects the brain and so can treat those with HIV to better prevent the deleterious effects of HIV on the brain and mental function

Should you have any questions, please contact Dr Constantine Malama on 097-9-070477 and /or the research ethics committee (REC).

Confidentiality

All the information gathered in this study will be used in privacy and known only to members of the research team. The identity of the persons will not be disclosed to anyone outside the research team.

Declaration.

I give consent to have the deceased have tissues and blood samples be taken at the time of autopsy for the purposes of taking part in this Zambia HIV neuro AIDS study. I give my consent indicating voluntary and informed consent as next of kin to the deceased. I may withdraw my consent at anytime without penalty or loss of benefits or treatment to which i am entitled. Doctors may choose not proceed to collect specimens or use such specimens for such study without my consent**.** Additionally, my withdrawal will not in any way affect how doctors treat me in regard to the deceased / next of kin

Declaration:

I understand what the study is all about and what is expected of if I participate in this study.

Interviewees name.....

Signature / Thumb print.....

Date.....

Witness' name.....

Signature.....

Date.....

Should you have any questions, you can contact Dr Constantine Malama, Kalingalinga Health Centre or Dr Victor Mudenda, at the UTH, Department of Pathology. You can also contact the Research and Ethics committee (REC).

Dr Constantine Malama

Project study medical doctor and principal investigator.

Mobile 097-9-070477

Dr Victor Mudenda

University teaching hospital

Department of Pathology and Microbiology

0966-750646

Dr AdelinaHolgun

Research co-investigator

Mobile 0977-931184

Office260-252661

Dr KOR Chiyenu

Project medical doctor

Department of medicine

Mobile: 0955-999357

The Chairperson

University of Zambia Biomedical Research Ethics Committee

PO Box 50110,

Ridgeway campus,

Lusaka,

Zambia.

Appendix 3: Data collection sheet for sub-type c Neuro-AIDS and pathogenesis in Zambia project.

ID ___ ___ ___ -___ ___ ___ **Postmortem Information**

Zambia HIV Neuropathogenesis

PATHOLOGY

Ward: ___ ___ ___ ___

AGE: ___ ___ SEX: O Male O Female

Clinical Diagnosis:_____

DOD: __ __/__ __/__ __ __ __ Time of D: ___ ___:___ ___ hrs

Date of AUTOPSY: __ __/__ __/__ __ __ __

Specimen collected: Date: ___ ___ /___ ___/___ ___ ___ ___

O Blood Time: _____

Purple top: _____ ml, # of vials: ____

Red top: _____ ml, # of vials: ____

Analysis: O HIV

Results: HIV: O Positive O Negative

O Plasma stored in -80 degree freezer

O CSF Time: _____

_____ ml, # of vials: _____

Analysis: O HIV

Results: HIV: O Positive O Negative

O CSF stored in -80 degree freezer

TISSUES COLLECTED FOR MOLECULAR ANALYSIS

BRAIN REGION:	Hemisphere
□ Frontal Lobe	□ Right □ Left
□ Parietal Lobe	□ Right □ Left
□ Temporal Lobe	□ Right □ Left
□ Occipital	□ Right □ Left
□ Hippocampus	□ Right □ Left
□ Cerebellum	□ Right □ Left
□ Basal Ganglia (Caudate/Putamen)	□ Right □ Left

□ Lymph node □ Choroid Plexus □ Gut tissue

□Stored in cryovials, -80 degree freezer

Tissues collected for HISTOLOGICAL ANALYSIS

BRAIN REGION

□ Frontal Lobe	□ Right □ Left
□ Hippocampus	□ Right □ Left
□ Parietal Lobe	□ Right □ Left
□ Temporal Lobe	□ Right □ Left
□ Occipital	□ Right □ Left
□ Basal Ganglia	□ Right □ Left

□ Spleen □ Choroid Plexus □ Gut tissue □ Liver □ Lungs

□ Lymph node □ Kidneys/adrenals □ Pancreas □ Thyroid

□Stored in normal buffered formalin

Pathologist (Print)

Technician

_____

Date and Time picked up

ID ___ ___ ___ -___ ___ ___ Postmortem Information

Ward: ___ ___ ___ ___

AGE: ___ ___ SEX: O Male O Female

Clinical Diagnosis:_____

DOD: __ __/__ __/__ __ __ __ Time of D: ___ ___:___ ___ hrs

Date of AUTOPSY: __ __/__ __/__ __ __ __

Specimen collected: Date: ___ ___ /___ ___/___ ___ ___ ___

Tissues collected for histological analysis

Brain

Lymph node

Kidneys

Adrenals

Pancreas

Thyroid

Liver

□Stored in normal buffered formalin

Pathologist

Technician

Date and Time picked up

Appendix 4: Microtomy procedure.

1. Purpose

To manually create sections of specimens of varying thickness for use in routine histopathology procedures

2. Principle

Microtomy is dependent on the rotating of the microtome thereby cutting the specimens blocks into sections. The desired thickness of the sections is achieved by manipulating the clearance angle, the adjusting knob and the scale.

3. Equipment, Reagents, Supplies, Personal Protective Equipment (PPE)

Equipment	Reagents	Supplies	PPE
Microtome Water bath	NA	Microtome blades Tissue forceps Frosted slides Cleaning brush Sharps box	Lab Coat Safety Goggles (where necessary)

4. Specimen

Formalin fixed, paraffin embedded blocks.

5. Safety precautions

Refer to the UTH Laboratory Safety Manual (QMS-SFT-v1) and the Histopathology Safety handbook (HIS-SFT-v1) for details.

Only experienced personnel should use the microtome
a) Always observe the correct resting angle of the instrument to the table to avoid

	pinching your fingers.
b)	Make sure that all the necessary parts and tools are assembled before attempting to use the instrument.
c)	Always clamp the specimen block before clamping the knife or changing the specimen block and during all work breaks. The hand wheel must be locked prior to any manipulation of the knife.
d)	Be very careful when handling microtome blades. The cutting edge is extremely sharp and can cause severe injury.
e)	Always turn the hand wheel evenly in clockwise direction; otherwise the brake will not work properly. The rotation speed of the hand wheel must be adapted to sit the hardness of the specimen.
f)	Take care not to block the coarse driving wheel when turning the hand wheel otherwise there will be no feedback motion of the section thickness and thus no sectioning will occur.

6. Calibration procedures

Always ensure that the Microtome stage is well aligned and the desired thickness to be section is selected before use.

7. Procedure Step-by step

Step	TRIMMING
1.	Set the blocks on the cold plate. (Only large tissue can be trimmed first before sectioning)
2.	Insert a specimen block into the specimen clamp/holder. (Clean the Microtome prior to this as stipulated in the HIS-TECH-037v1)
3.	Carefully insert disposable blade into the knife holder and clamp making sure that the knife is clamped parallel to the upper edge of the pressure plate.
4.	Advance the block until it touches the knife block.
5.	Orient the position of the specimen surface (only in the case of specimen holders that can be oriented).
6.	Release the hand wheel lock or brake respectively

7.	Using the trimming lever selects the required trimming stage. Trimming is done at 10 -15 microns
8.	Begin the cutting process by turning the hand wheel.
9.	Trim the specimen block until the required specimen level has been reached.
10.	Let go of the trimming lever, clump the hand wheel lock and remove the specimen block from the specimen clamp and put it back on ice cold surface for further cooling. Tissue blocks are cooled for 1-3 minutes before they can be sectioned.
SECTIONING.	
11.	Mount a well trimmed and cooled specimen block into the specimen holder.
12.	Install a new blade or proceed with an unused area of the blade for sectioning.
13.	Set the required section thickness or check the value setting on the display respectively. Section cutting is done at 3-5 microns
14.	For sectioning, turn the hand wheel evenly in a clock wise direction ensuring that evenly sized section are produced. Ideally successive sections will stick edge to edge due to local pressure with each stroke forming a ribbon.
15.	Lock the hand wheel, pick up the sections using a forcep and place the sections in the water bath ensuring that the shiny surface faces down.
16.	Float the sections in the water bath.

8. Quality Control

a) Standardize all the steps involvede.g the temperature of the block when it is cut and the mode of cooling should be standardized to give a similar consistency
b) Never use a blunt bade for sectioning as this may injure the tissue.
c) Always refer to the document EXT-DOC-002v1 for troubleshooting on Microtomy for paraffin sectioning to solve problems encountered during Microtomy.
d) Tissue specimen is examined after staining for the quality of section and thickness.

9. Calculation of results

NA.

10. Reference range/Test Interpretation

NA.

11. Alert/critical values, where appropriate

NA

12. Notes, Limitations and Anything Else

a) Practical experience necessary to manipulate the microtome is gained under the guidance of the skilled tutor
b) It is possible to damage the tissue by gouging or scoring when trimming the block.
c) Blocks should be arranged in a numerical order for smart work.
d) Over cooling of the block may cause tissue distortion and expansion.
e) Always cut the sections at the required thickness.
f) Cutting speed and length of ribbon must also be standardized.

13. Reference

McGill (2005), Blood Laboratory, McGill University, Montreal, Canada.

Ministry of Health Standard operating Procedure for level lll Hospitals (2008 Revision), Lusaka, Zambia.

Monica Cheesbrough (2000), District laboratory Practice in Tropical Countries, Volume II Cambridge University Press, UK, Page 341.

Appendix 5: Hematoxylin and Eosin (H&E) Staining Protocol.

Principle.

The oxidation product of haematoxylin is haematin, and is the active ingredient in the staining solution. Haematoxylin is not classified as a dye since the molecule possesses no chromophore. The *in situ* oxidation of haematoxylin is effected by the addition of a strong oxidant to the stain, in this case sodium iodate.

Haematin exhibits indicator-like properties, being blue and less soluble in aqueous alkaline conditions and red and more soluble in alcoholic acidic conditions. In acidic conditions, haematin binds to lysine residues of nuclear histones by linkage via a metallic ion mordant, in this case aluminium. To ensure saturation of chemical binding sites, the stain is applied longer than necessary, resulting in the over staining of the tissues with much non-specific background colouration. This undesirable colouration is selectively removed by controlled leaching in an alcoholic acidic solution, (acid alcohol), the process being termed "differentiation". Differentiation is arrested by returning to an alkaline environment, whereupon the haematin takes on a blue hue, the process of "blueing-up". The haematin demonstrates cell nuclei.

Full cellular detail is obtained by counterstaining with the eosin mixture. Colour enhancement is achieved by fortifying the stain with phloxine, a chemical member of the same family as eosin (halogenatedfluorosceins). The mechanism of their staining is not fully understood, but is believed to be of an electrostatic nature. Visualisations most acceptable to the histologist are obtained by applying the dyes in acidic conditions, whereby more intense specific colourations are obtained, the more acidic tissue components taking up the dye to a greater intensity, hence the addition of acetic acid.

Technical Points.

1. (step 2) - The length of time necessary to over-stain the tissues will depend upon fixation and the type of alum haematoxylin employed. Lillie Mayer's alum haematoxylin-formalin fixed tissues should take 5 minutes.

Tissue Type	Haematoxylin	Acid alcohol 0.3%	Eosin	Comment
Routine tissues	4 minutes	See technical point 2	2 minutes	
Renal biopsies	10 minutes	1-2 seconds	2-4 minutes	Check staining
Decals	10 minutes	1-2 seconds	30 seconds	Check staining after blueing. Hx step may need to be repeated if prolonged decal.

2.(Step 4) - Differentiation with acid alcohol requires some practical experience to ascertain the correct end-point, since the acid solution alters the colour of the tissue to red. The correct end-point is when, after blueing up, the background is almost colourless. For renal biopsy sections, two quick dips in 0.3% acid alcohol are all that is required

3. (Step 6) - If Scott's tap water substitute is employed; blueing up is achieved in a much shorter time.

4. (Step 8) - Eosin is highly soluble in water. Over-staining is removed by washing in running water.

5. Fixation - Not critical. Acidic fixatives will give a more eosinophilic result. Picric acid containing fixatives give an overall enhanced result. Acidic decalcifying fluids give poor nuclear staining.

6. Renal biopsies - 10% buffered formalin. Sections cut at 2micrometers

Method.

1. Bring sections to distilled water
2. Stain nuclei with the alum haematoxylin (see note)
3. Rinse in running tap water

4. Differentiate with 0.3% acid alcohol (see note)
5. Rinse in running tap water
6. Rinse in Scott's tap water substitute (see note)
7. Rinse in tap water
8. Stain with eosin 2 mins
9. Dehydrate, clear and mount.

Results

Collagen.....pale pink
Muscle.....deep pink
Acidophilic cytoplasm.....red
Basophilic cytoplasm.....purple
Nuclei.....blue
Erythrocytes.....cherry red

Reagent Formulae

LillieMayer alum haematoxylin

aluminumammonium sulphate ----- 200 g
hematoxylin (CI 75290) ---------- 20 g
ethanol ---------- 40 ml
sodium iodate ---------- 4 g
acetic acid ---------- 80 ml
glycerol ---------- 1200 ml
distilled water ---------- 2800 ml

In a 4L Ehrlenmeyer flask, to 1000 mls of the distilled water add the aluminium ammonium sulphate. Place the flask on a heater/stirrer, turn on the heater and allow mixing until the alum dissolves - this takes about 15 minutes. Remove the flask from the heater/mixer, allow cooling, and then adding the remaining 1800 mls distilled water - this

will further cool the solution. Add the haematoxylin powder to the alcohol and dissolve as much of the powder as possible by shaking for a few minutes. Pour the strong alcoholic solution of haematoxylin into the cooled alum solution and stir to ensure all the powder is dissolved, preferably overnight. Add the sodium iodate, acetic acid, and finally the glycerol. Mix well, plug loosely and store.
It is appropriate to make up a batch of the required amount, dependent upon the usage rate.

2. Acid alcohol 0.3% Acid Alcohol

    commercial grade ethanol ----- 2800 ml
    distilled water ---------- 1200 ml
    conc. hydrochloric acid --------- 12 ml

In a sufficiently large container, add the acid to the water, then add the alcohol and mix thoroughly. The generation of fine bubbles is an indication that mixing is thorough.

3. Scott's tap water substitute

    sodium hydrogen carbonate --- 10 gm
    magnesium sulphate ---------- 100 gm
    distilled water ---------- 5 L

Dissolve the salts in the water.

Store stock solutions at room temperature.

4. alc. acetified eosin/phloxine TQEH

    1% eosin Y (CI 45380) ---------- 400 ml
    1% aqphloxine (CI 45405) ----- 40 ml
    95% alcohol ---------- 3100 ml
    gl acetic acid ---------- 16 ml

Mix the above reagents together, and stir well. The solution keeps well.

References

Mayer P, (1896), Mitt. zool. Stn. Neapel. 12,303

Lillie RD, (1965), Histopathologic Technique and Practical Histochemistry, 3rd edition, McGraw-Hill Book Co. New York

Lynch MJ, Raphael SS, Mellor LD, Spare PD and Inwood MJ, (1969), Medical Laboratory Technology and Clinical Pathology, 2nd edition, WB Saunders Co., Philadelphia London Toronto

LG Luna, Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology, third edition, McGraw Hill.

Appendix 6: Masson Trichrome Staining Protocol.

Principle.

A differential visualisation of tissue elements is achieved with two similar acid dyes. A dye with a small molecule size (Biebrich scarlet/acid fuchsin) is used first which stains all tissue elements in the section. This red stain is selectively removed from unwanted areas by differentiating with phosphomolybdic acid, which also acts as a mordant for the next step of the procedure. A dye with a large molecule (light green) is then progressively applied to the section. A Celestin blue/haematoxylin is used to stain the nuclei as it is more resistant than alum haematoxylins to removal by the acid solutions used in the method. This technique differentiates oedema from fibrosis and visualises immunological deposits. Post mordanting in bouins before staining with this method, gives enhanced acidophilic staining.

Technical Points

1. (Step 8) - The mordanting by dodecamolybdophosphoric acid is easily removed by washing.

Method

1. Sections to distilled water
2. Stain in Celestin blue 5 mins
3. Rinse in distilled water
4. Stain nuclei with alum haematoxylin 5 mins
5. Wash in running tap water 5 mins
6. Rinse in distilled water
7. Stain with Biebrich scarlet-acid fuchsin stain 10 mins
8. Rinse in distilled water
9. Treat with freshly prepared dodecamolybdophosphoric acid 10 mins

10. Drain, do not rinse
11. Stain with light green 10 mins
12. Rinse excess stain from slide with alcohol
13. Dehydrate, clear and mount.

Results.

Fibrinoid.....red

Immune deposits.....red

Basement membrane.....green

Collagen.....green

Nuclei.....blue

Reagent Formulae

1. Celestin blue

2. Biebrich scarlet

Biebrich scarlet (C.I. 26905)	0.9 g
Acid fuchsin (C.I. 42685)	0.1 g
Glacial acetic acid (Analar)	1.0 ml
Distilled water	100 ml

Mix the powders together then dissolve in distilled water. Add acetic acid.

2. 5% aqdodecamolybdophosphoric acid (Phosphomolybdic acid)

Prepare weekly

Phosphomolybdic acid	5 g
Distilled water	100 ml

4. 1% Light Green in 4% Acetic Acid stain

light green SF (CI 42095)	1.0 g
distilled water	96.0 m

Glacial Acetic Acid 4.0 ml

Dissolve the dye in the water, add Acetic Acid. Mix well. The stain keeps well.

References

1. Masson PJ, (1929), J.Technol.Methods

Appendix 7: Periodic Acid Schiff Protocol.

Principle.

The dialdehydes formed by treatment with periodic acid from certain carbohydrates, will selectively reduce an alkaline hexamine-silver salt mixture. This method is especially suitable for renal glomerular basement membranes. This method helps determine the thickness of glomerular basement membranes and mesangial matrix.

Method

1. Deparaffinise sections with xylene then take through alcohols to distilled water.
2. Rinse well in distilled water.
3. Treat with 0.5 % periodic acid 5 mins
4. Rinse well in distilled water.
5. Place in freshly filtered silver methenamine solution at 58 - 60 degrees Celsius. Check the slide microscopically after 20 minutes then every 5 minutes until the basement membrane is well demonstrated.
6. Rinse well in distilled water.
7. Tone in 0.2% gold chloride 2 mins
8. Rinse in distilled water.
9. Treat with 2% sodium thiosulphate (hypo) 2 mins
10. Wash in running tap water.

(NOTE: At this point sections can be differentiated using 0.5% sulphuric acid in 0.2% ferric chloride if over stained.)

Stain sections with either H&E orchromotrope if requested.

Hematoxylin and Eosin Counterstain.

1. Stain in Lillie Mayers Hematoxylin solution 5 mins
2. Wash in running tap water 1 min
3. Differentiate in acid alcohol (0.6%) 1-2 sec
4. Rinse in running tap water.
5. Blue in Scotts tap water
6. Rinse well in running tap water
7. Stain in Eosin / Phloxine solution 2 mins
8. Dehydrate, clear and mount in DPX.

Chromotrope Counterstain

1. Mordant in bouins fluid at 58 - 60 deg 30 mins
2. Wash well in water until sections lose their yellow colour
3. Treat with 1% phosphotungstic acid (make fresh weekly) 1 min
4. Wash well in water.
5. Stain with Chromotrope 2R 15 mins
6. Rinse quickly in distilled water.
7. Dehydrate in alcohols, clear in xylene and mount in DPX

Results.

Basement membranes .....Black

Background .....Red

Reagent Formulae

1. Gomori's silver methenamine solution

3% hexamine (methenamine)….....46 ml

5% silver nitrate ….....2.5 ml

5% borax ….....6 ml

Ensure that all glassware is thoroughly cleaned with glass cleaning solution and distilled water before measuring any solutions. Filter silver methenamine solution before use.

2. Chromotrope solution

Chromotrope 2R (C.I. 16570) .....2 g

1/10N hydrochloric acid .....100 ml

3. 1% phosphotungstic acid

Phosphotungstic acid .....1 g

Distilled water .....100 ml

References

Gomori, G. 1946, A new histochemical test for glycogen and mucin.American Journal of Clinical Pathology, V16, p177.

Jones, D.B. 1957, Nephrotic Glomerulonephritis.American Journal of Pathology, V33, p313.

Lillie, R.D. 1977, H.J. Conn's Biological Stains, 9th edition. Williams and Wilkins

Culling C.F.A. Handbook of Hitopathological and Histochemical techniques, Third edition, Butterworths

Appendix 8: Ziehl Neelsen Staining Protocol.

Principle

Mycobacterial cell walls contain a waxy substance composed of mycolic acids. These are ß-hydroxy carboxylic acids with chain lengths of up to 90 carbon atoms. The property of acid fastness is related to the carbon chain length of the mycolic acid found in any particular species (Lyon H 1991).

Basic fuchsin binds to negatively charged groups in bacteria. The mycolicacid (and other cell wall lipids) present a barrier to dye entry as well as elusion (washing out with solvent) and this is partly overcome by adding a lipophilic agent to a concentrated aqueous solution of basic fuchsin and partly by heating.

Technical Points.

1. Include a control

Method

1. Place the working solution in a coplin jar and pre-heat in 58 -60oC water bath 10 mins
2. Deparaffinise sections, bring to water.
3. Stain in the pre-heated working solution in the water bath 15 mins
4. Place the coplin jar containing the slides into running cold tap water 2 mins
5. Remove the slides from the coplin jar and wash in running water 1 min
6. Differentiate in 3% hydrochloric acid in 95% ethyl alcohol until no more colour runsfrom the slide.
7. Wash briefly in water to remove the acid alcohol.
8. Counterstain with 0.25% methylene blue in 1% acetic acid 15 to 30 secs
9. Wash in water, dehydrate, clear and mount in DPX.

Results

Acid fast bacilli..... Red

Nuclei..... Blue

Other tissue constituents..... Blue

Reagent Formulae

1. Staining solution

Stock Solution A (stable for 6 months)

L.O.C. High Suds (Amway) .....0.6 ml

Distilled water .....100 ml

Stock Solution B

Basic fuchsin .....1 g

Absolute ethyl alcohol .....10 ml

The two solutions can be kept as stock solution and mixed before use

2. Working Solution (stable for 1 month)

Mix 50ml of A with 5 ml of B.

3. 3% hydrochloric acid in 95% ethyl alcohol

Absolute ethyl alcohol..... 95ml

Distilled water..... 2 ml

Concentrated hydrochloric acid ..... 3 ml

Make up the alcohol solution then add the concentrated acid. Use extreme care when handling concentrated acid.

4. 0.25% methylene blue in 1% acetic acid

Methylene blue ..... 0.25 g

Distilled water ..... 99 ml

Acetic acid..... 1 ml

References.

Lillie, R.D. (1977). H.J. Conn's biological stains, 9th edition. William's and Wilkins.

Lyon, H (1991), theory and strategy in histochemistry. Springer - Verlag.

Neelsen, P. (1883). Zentralblatt fur de MedizinischenWissenschafen, V21, pg497.

Ziehl F. (1882) ZurFarbung des Tuberkelbacillum. Deutsche MedizinischeWochenschrift, V8, pg 451.

Ellis, R.C.Zabrowarny L.A. (1993) J. Clinical Pathology. V46, pg 559-560

Appendix 9: Bennhold's Congo red Staining Protocol for Amyloid.

Description:

This Bennhold's Congo red stain is used for the detection of Amyloid on formalin-fixed, paraffin-embedded tissue sections with amyloidosis, and may be used for frozen sections as well. The amyloid deposits will be stained red and the nuclei will be stained blue. The thickness of sections is usually 5 um. But in case of inadequate amyloid deposits, 10um thick sections will be more satisfactory. Congo red stains amyloid in tissue sections.

Fixation: 10% formalin.

Section: paraffin sections at 5 um (in case inadequate amyloid deposits, use 10 um thick sections).

Solutions and Reagents:

1% Congo red Solution:

Congo red (Sigma, Cat# C-6277) ------ 1 g
Distilled water ---------- 100 ml

1% Sodium Hydroxide:

Sodium hydroxide ---------- 1 g
Distilled water ---------- 100 ml

Alkaline Alcohol Solution:

1% Sodium hydroxide ---------- 1 ml
50% alcohol ---------- 100 ml

Procedure:

1. Deparaffinize and hydrate sections to distilled water.
2. Stain in Congo red solution for 30-60 minutes.
3. Rinse in distilled water.

4. Differentiate rapidly (5-10 dips) in alkaline alcohol solution.
5. Rinse in running tap water for 5 minutes.
6. Counterstain in Gill's hematoxylin for 30 seconds.
7. Rinse in tap water for 1 minute.
8. Dip in ammonia water (add a few drops of ammonium hydroxide to tap water and mix well) for 30 seconds or until sections turn blue.
9. Rinse in tap water for 5 minutes.
10. Dehydrate through 95% alcohol, 100% alcohol
11. Clear in xylene and mount with resinous mounting medium.

Results:

Amyloid, elastic fibers, eosinophil granules ---------- red
Nuclei ---------- blue

Positive Controls:

Know amyloidosis containing tissue (Tissue from a multiple myeloma sample).

Appendix 10: Grocott's Methenamine Silver Staining Protocol.

Principle.

Chromic acid oxidation forms aldehydes from fungal cell wall polysaccharide components, which are subsequently demonstrated by reduction of an alkaline hexamine-silver complex. The reaction may be compared to that of the periodic acid Schiff reaction, (see PAS).

Grocott's alkaline hexamine-silver solution represents a vehicle which, upon reduction, precipitates nascent silver ions, thus blackening the site. This is known as an "argentaffin reaction".

Argentaffin reaction - the ability of a silver complex solution to blacken a tissue element without the need of a reducing bath. The term is adjectival and is applied to many methods, (eg von Kossa). The term "argentaffin reaction" should therefore not be used as a proper name.

Technical Points

1. A known positive control section must be used to ensure correct differentiation has beenachieved.
2. Reagents should be prepared in a fume hood.

Method

1. Bring sections to distilled water.
2. Oxidise with 4% aq chromic acid at room temperature 1 hr
3. Wash in water for a few seconds.
4. Treat sections with 1% sodium metabisulphitee 1 min
5. Wash in running tap water3 mins
6. Rinse thoroughly in distilled water.

7. Place in pre-heated working silver solution in a water bath at 60°C for 15 to 20 mins until section turns yellowish-brown (Check microscopically after washing in distilled water – fungi should be dark brown).

8. Rinse well in distilled water

9. Tone sections with 0.2% gold chloride 2 mins

10. Rinse in distilled water

11. Treat sections with 2% sodium thiosulphate 2 mins

12. Wash with running tap water 5 mins

13. Counterstain in working light green 15 sec

14. Rinse excess light green off slide with alcohol

15. Dehydrate, clear and mount.

Results

Fungi, Pneumocystis carnii, histoplasmaspp ----------black

Inner parts of mycelia and hyphae ----------old rose

Leishmaniaspp, toxoplasma spp ----------negative

Mucin----------dark grey

Background ----------pale green

Reagent Formulae

Wear protective clothing, gloves and safety glasses when preparing reagents.

1. 4% aq Chromic Acid

   Chromium trioxide (analytical) ---- 4 g

   Distilled water ---------- 100 ml

2. Silver solution

3% methenamine (= hexamine) ---- 23 ml

5% silver nitrate ---------- 1.25 ml

5% borax (sodium tetraborate) ---- 3 ml

Distilled water ---------- 25 ml

3. 0.2% aq Sodium chloroaurate (yellow gold chloride)

   Gold Chloride (analytical) ---------- 1.0 g

   Distilled water ---------- 500 ml

4. 2% aq Sodium thiosulphate (hypo)

   Sodium thiosulphate ---------- 2.0 g

   Distilled water ---------- 100 ml

5. Working light green

   1% light green (CI 42095) in 1% acetic acid --- 10 ml

   Distilledwater ---------- 40 ml

References.

Grocott, R.G. 1955, A stain for fungi in tissue sections and smears.American Journal of Clinical Pathology, V25, p975

Luna L.G. Histopathological Methods and colour atlas of special stains and tissue artefacts, American Histo Labs Inc, Publications Division 1992.

Appendix 11: Slide Mounting Protocol

Purpose

To provide the maximum degree of transparency to stained tissue sections.

Principle

Mounting is achieved by using a medium that has a refractive index which is approximate to that of dried protein i.e. between 1.53 and 1.54. The refractive index of a mounting medium may change on drying due to evaporation of solvents.
Air bubbles should not be permitted to remain under cover-slips since these air bubbles tend to expand.

Equipment, Reagents, Supplies, Personal Protective Equipment (PPE)

Equipment	Reagents	Supplies	PPE
NA	DPX Mountant	Cover-slips (preferably 24 x 40mm or larger) Slides of interest Filter paper/ Kim wipes Orange sticks or Mountant dropper	Lab Coat Nitrile Gloves

Specimen

Stained slides.

Safety precautions

General safety precautions as described in the UTH Safety Manual including Universal Precautions must be adhered to.

Engage the use of proper body mechanics to prevent work related musculoskeletal related disorders. REFER TO THE HISTOPATHOLOGY SAFETY HANDBOOK (HIS-SFT-v1) FOR DETAILS.

Calibration procedures

NA

Procedure Step-by step

Step #	Action
17.	Remove slide from xylene and wipe off excess xylene and let it lie on a flat surface (Do not allow tissue on the slide to dry).
18.	Place 3-4 drops of mounting medium on the glass slide (with stained tissue) and lower the cover slip onto the slide by turning it right side up until it comes into contact with the edge of the slide then continue lowering it down until it completely covers the stained section on the slide.
19.	Gently tease bubbles from the coverslip with an applicator stick.
20.	Allow the slide to dry on a flat surface
OR	
21.	Place 3-4 drops of mounting medium on the coverslip and spread over the surface using a dropper or stick
22.	Remove slide from xylene and drain off excess xylene but do not allow tissues on the slide to dry
23.	Lower slide onto the coverslip by turning it right side up until it comes into contact with the edge of the coverslip then continue lowering it down until it completely covers the coverslip.
24.	Allow to dry on a flat, clean surface (Preferable an absorbable paper like a filter paper)

Quality Control

Always check for the presence of air bubbles macroscopically and microscopically and remount before taking the slides to the pathologist. QUALITY CONTROL checks must be documented in the SLIDE EVALUATION CHECKLIST (HIS-FM-013v1)
The mounting medium must not be too thin or too thick as this may disturb the quality of the staining.
Remember to wipe off unwanted tissues before mounting.

Calculation of results

NA

Reference range/Test Interpretation

A well mounted slide must be clean, free from air bubbles or debris and without any mountant dripping from the sides.

REFER TO SLIDE EVALUATION CHECKLIST QC SHEET (HIS-FM-013v1)

Alert/critical values, where appropriate

NA.

Notes, Limitations and Anything Else

a) To prevent the possibility of contaminating the dropper or application stick with excess cells, the dropper should never touch the surface of the cells.
b) A few drops of Xylene can be added to the dispensed mountant to get rid of bubbles.
c) Cover-slips must always be wiped clean before use (with a safety caution) and wipe off excess xylene from the bottom of the slide

Reference

Bancroft J.D. Stevens, A. Theory and Practice of Histological Techniques; Churchill, Livingstone, London, 1982.

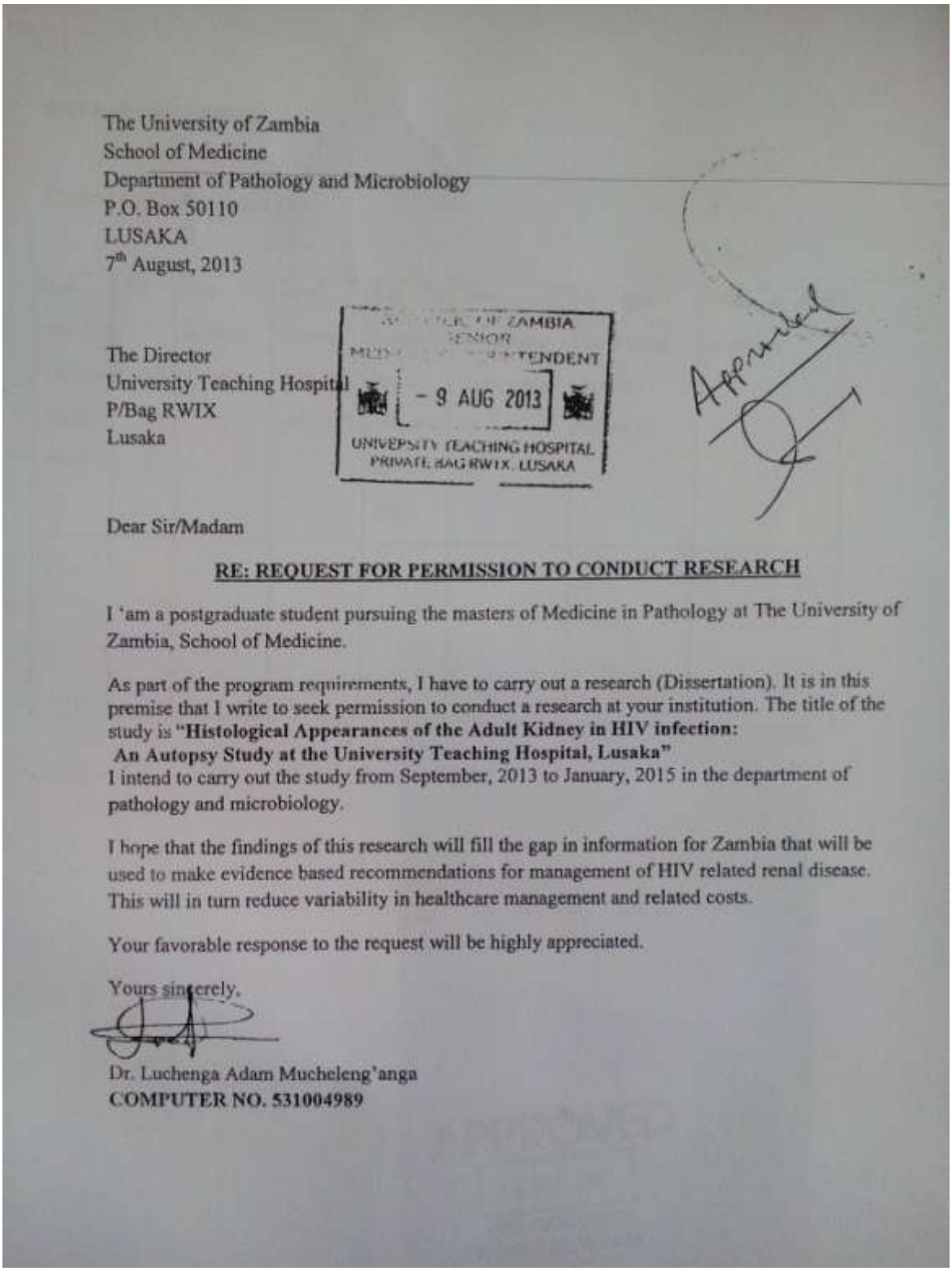
Bancroft, J.D.; Cook, H.C. Manual of Histological Techniques, Churchill, Livingstone, London, 1984.

Bancroft JD, Gamble M, Theory and Practice of Histological Techniques; Churchill, Livingstone, London, 2008.

Cook H.C.; Manual Histological Demonstration Techniques; Butterworth's, 1974.

Ministry of Health Standard operating Procedure for level lll Hospitals (2008 Revision), Lusaka, Zambia.

Appendix 12: Permission letter to do study at UTH from management.



The University of Zambia
School of Medicine
Department of Pathology and Microbiology
P.O. Box 50110
LUSAKA
7th August, 2013

The Director
University Teaching Hospital
P/Bag RWIX
Lusaka

UNIVERSITY TEACHING HOSPITAL
PRIVATE BAG RWIX, LUSAKA
- 9 AUG 2013

Approved

Dear Sir/Madam

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH

I 'am a postgraduate student pursuing the masters of Medicine in Pathology at The University of Zambia, School of Medicine.

As part of the program requirements, I have to carry out a research (Dissertation). It is in this premise that I write to seek permission to conduct a research at your institution. The title of the study is **"Histological Appearances of the Adult Kidney in HIV infection: An Autopsy Study at the University Teaching Hospital, Lusaka"**
I intend to carry out the study from September, 2013 to January, 2015 in the department of pathology and microbiology.

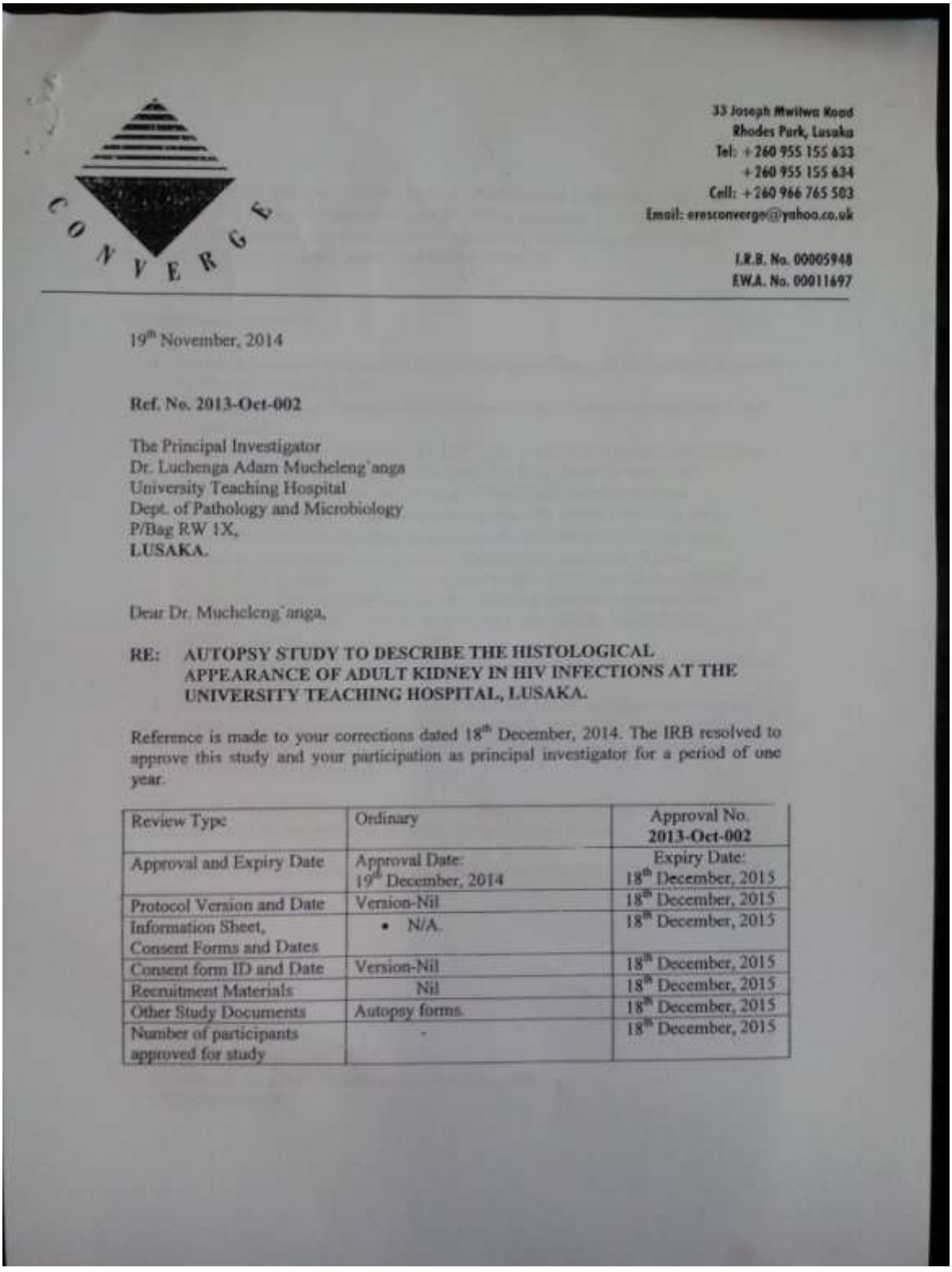
I hope that the findings of this research will fill the gap in information for Zambia that will be used to make evidence based recommendations for management of HIV related renal disease. This will in turn reduce variability in healthcare management and related costs.

Your favorable response to the request will be highly appreciated.

Yours sincerely,

Dr. Luchenga Adam Mucheleng'anga
COMPUTER NO. 531004989

Appendix 13: Letter of Ethical clearance to carry out research.



33 Joseph Mwilwa Road
Rhodes Park, Lusaka
Tel: +260 955 155 633
+260 955 155 634
Cell: +260 966 765 503
Email: eresconverge@yahoo.co.uk

I.R.B. No. 00005948
F.W.A. No. 00011697

19th November, 2014

Ref. No. 2013-Oct-002

The Principal Investigator
Dr. Luchenga Adam Mucheleng'anga
University Teaching Hospital
Dept. of Pathology and Microbiology
P/Bag RW 1X,
LUSAKA.

Dear Dr. Mucheleng'anga,

RE: AUTOPSY STUDY TO DESCRIBE THE HISTOLOGICAL APPEARANCE OF ADULT KIDNEY IN HIV INFECTIONS AT THE UNIVERSITY TEACHING HOSPITAL, LUSAKA.

Reference is made to your corrections dated 18th December, 2014. The IRB resolved to approve this study and your participation as principal investigator for a period of one year.

Review Type	Ordinary	Approval No. **2013-Oct-002**
Approval and Expiry Date	Approval Date: 19th December, 2014	Expiry Date: 18th December, 2015
Protocol Version and Date	Version-Nil	18th December, 2015
Information Sheet, Consent Forms and Dates	• N/A.	18th December, 2015
Consent form ID and Date	Version-Nil	18th December, 2015
Recruitment Materials	Nil	18th December, 2015
Other Study Documents	Autopsy forms.	18th December, 2015
Number of participants approved for study	-	18th December, 2015

Specific conditions will apply to this approval. As Principal Investigator it is your responsibility to ensure that the contents of this letter are adhered to. If these are not adhered to, the approval may be suspended. Should the study be suspended, study sponsors and other regulatory authorities will be informed.

Conditions of Approval

- No participant may be involved in any study procedure prior to the study approval or after the expiration date.
- All unanticipated or Serious Adverse Events (SAEs) must be reported to the IRB within 5 days.
- All protocol modifications must be IRB approved prior to implementation unless they are intended to reduce risk (but must still be reported for approval). Modifications will include any change of investigator/s or site address.
- All protocol deviations must be reported to the IRB within 5 working days.
- All recruitment materials must be approved by the IRB prior to being used.
- Principal investigators are responsible for initiating Continuing Review proceedings. Documents must be received by the IRB at least 30 days before the expiry date. This is for the purpose of facilitating the review process. Any documents received less than 30 days before expiry will be labelled "late submissions" and will incur a penalty.
- Every 6 (six) months a progress report form supplied by ERES IRB must be filled in and submitted to us.
- ERES Converge IRB does not "stamp" approval letters, consent forms or study documents unless requested for in writing. This is because the approval letter clearly indicates the documents approved by the IRB as well as other elements and conditions of approval.

Should you have any questions regarding anything indicated in this letter, please do not hesitate to get in touch with us at the above indicated address.

On behalf of ERES Converge IRB, we would like to wish you all the success as you carry out your study.

Yours faithfully,
ERES CONVERGE IRB

Dr. E. Munalula-Nkandu
BSc (Hons), MSc, MA Bioethics, PgD R/Ethics, PhD
CHAIRPERSON

Appendix 14: Data collection sheet from case notes.

DATA ENTRY SHEETS

Clinical details from case notes

Age (16-30,31-45,46-60,61-75)	Sex (F/M)	Past medical history	Drug history including HAART	CD 4 count (0-100,101-200,201-300)	Urea /Creatinine Electrolytes: Na Cl	Proteinuria on dipstick (Trace,+1 , +2 ,+3)	Cause of death at autopsy

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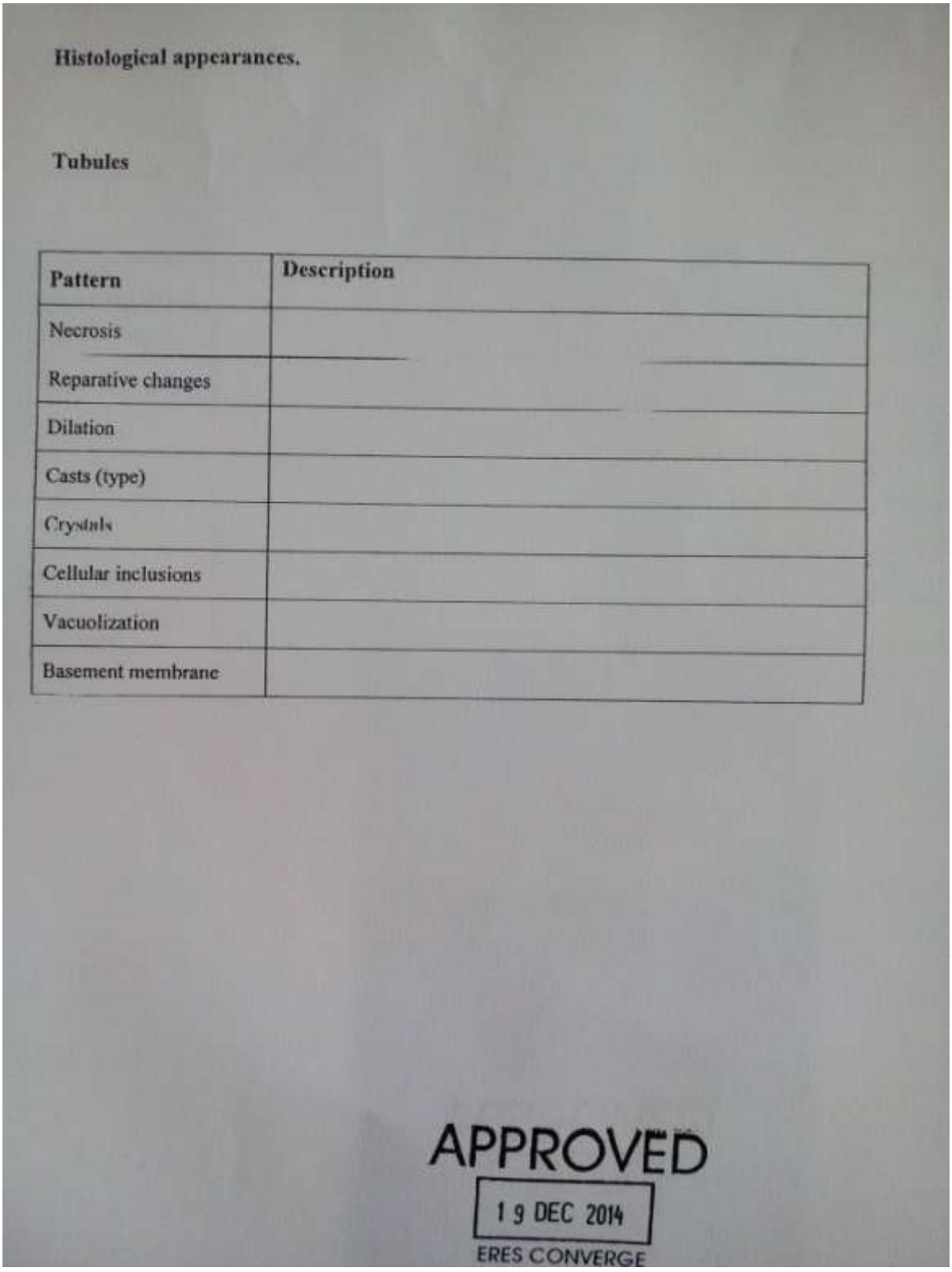
Appendix 15: Histological appearances (Glomerular).

Histological appearances.

Glomerular

Pattern	Description
Number of glomeruli	
Size and cellularity	
Segmental or global changes	
Mesangium	
Leukocytes	
Capillary walls	
Necrosis	
Thrombi	
Adhesions to Bowman capsule	
Deposits (location)	
Crescents (%)	
Sclerosis (distribution and % of involvement of glomerulus)	

Appendix 16: Histological appearances (Tubules).



Histological appearances.

Tubules

Pattern	Description
Necrosis	
Reparative changes	
Dilation	
Casts (type)	
Crystals	
Cellular inclusions	
Vacuolization	
Basement membrane	

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Appendix 17: Histological appearances (Blood vessels).

Histological appearances.

Blood vessels

Intimal thickening (type)	Description
Elastic changes	
Media hypertrophy	
Hyalinosis	
Thrombosis	
Necrosis	
Inflammation	
Juxtaglomerular apparatus	

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Appendix 18: Histological appearances (Interstitium).

Pattern	Right	Left
Oedema		
Inflammation		

Appendix 19: Histological appearances (Neoplasms).

Right	Left

Appendix 20: Histological appearances (infections and other findings).

Infections and other findings

Appendix 21: Definitions (Glomerular compartment).

Focal	Involving <50% of glomeruli
Diffuse	Involving 50% or more of glomeruli
Segmental	Involving part of a glomerular tuft
Global	Involving all of a glomerular tuft
Mesangial hypercellularity	3 or more nuclei in a peripheral mesangial segment
Endocapillary hypercellularity	Increased cellularity internal to the GBM composed of leukocytes, endothelial cells and/or mesangial cells
Extracapillary hypercellularity	Increased cellularity in Bowman's space, i.e. > one layer of parietal or visceral epithelial cells, or monocytes/macrophages
Crescent	Extracapillary hypercellularity other than the epithelial hyperplasia of collapsing variant of FSGS
Fibrinoid necrosis	Lytic destruction of cells and matrix with deposition of acidophilic fibrin-rich material
Sclerosis	Increased collagenous extracellular matrix that is expanding the mesangium, obliterating capillary lumens or forming adhesions to Bowman's capsule
Hyaline	Glassy acidophilic extracellular material
Membranoproliferative	Combined capillary wall thickening and mesangial or endocapillary hypercellularity
Lobular (hypersegmented)	Consolidated expansion of segments that are demarcated by intervening urinary space
Mesangiolysis	Detachment of the paramesangial GBM from the mesangial matrix or lysis of mesangial matrix

Appendix 22: Definitions (Tubulo-interstitial compartment).

Necrosis	a) Frank necrosis: sloughing off of cells b) Flattened, regenerating epithelium
Edema	Increased interstitial space with loose appearance and normal thickness tubular basement membranes
Interstitial inflammation	a) PMNs—Intratubular PMNs forming plugs b) Interstitial or peritubular capillary PMNs c) Lymphocytes with edema. d) Lymphocytes with interstitial fibrosis e) Eosinophils in the interstitium f) Granulomas g) Necrotizing granulomas
Intratubular casts	a) Pigmented b) Polarizable casts c) Nonpolarizable casts
Interstitial fibrosis	a) Diffuse pattern b) Striped c) Patchy/geographic, "jigsaw puzzle" pattern
Reparative changes	Sloughing of epithelial cells in tubular epithelium.

Appendix 23: Definitions (Vascular lesions compartment).

Sclerosis	Intimal fibrosis/medial hypertrophy

“Fibrinoid” necrosis	term used to describe necrosis of wall with chunky, eosinophilic appearance, often containing fibrin and karryorhectic debris
Vasculitis	Transmural inflammation with lymphocytes/PMNs.