Kidney tissue characterization using magnetic resonance in HIV infected individuals undergoing kidney biopsy.

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Table of contents Pages

List of Abbreviations

ADC ..... Apparent diffusion coefficient

AKI.....Acute kidney injury

ART..... Antiretroviral therapy

ATN..... Acute tubular necrosis

BMI..... Body mass index

BOLD..... Blood oxygen level dependent

CKD..... Chronic kidney disease

CUBIC..... Cape Universities body imaging centre

DTI..... Diffusion tensor imaging

eGFR..... estimated glomerular filtration rate.

FA ..... Fractional anisotropy

HIV/AIDS.....Human Immunodeficiency virus/ Acquired Immune deficiency.

HIVAN..... HIV associated nephropathy.

HREC..... Human research ethics committee

IQR..... Interquartile range

KDIGO..... Kidney disease improving global outcomes.

KRT..... Kidney replacement therapy

LTFup ..... Lost to follow up.

MD..... Mean Diffusion

MRI..... Magnetic resonance imaging

MRS..... Magnetic resonance spectroscopy

PWH..... People with HIV

RD..... Radial Diffusion

SSA..... Sub Saharan Africa

TB..... Tuberculosis

UPCR..... Urine protein/ creatinine ratio

USA..... United states of America

Magnetic Resonance Imaging (MRI) specific definitions

ADC: Apparent diffusion coefficient

It is a quantitative measure of the magnitude of diffusion of water molecules within a tissue and is commonly clinically calculated using MRI with Diffusion weighted imaging. ADC values tend to decrease in chronic kidney disease, depending on the degree of renal impairment.

BOLD: Blood oxygen level dependent

This is a type of MR imaging that measures the proportion of oxygenated hemoglobin in an organ mirroring blood flow and therefore function. It is based on the fact that metabolic activity uses oxygen and so blood in the vicinity of metabolically active tissues has different proportions of oxygenated to deoxygenated hemoglobin. These have different magnetic properties which are used to image the function of a tissue.

DTI: Diffusion tensor imaging

DTI is an advanced imaging technique used to assess the structural integrity of the microfiber of tissues as well as diffusion in the said tissue in the body by calculating the parameters of apparent diffusion coefficient (ADC) and fractional anisotropy (FA).

DWI: Diffusion weight imaging

It is a form of magnetic resonance imaging based on measuring the random Brownian motion of water molecules within a voxel of tissue and is calculated using ADC. The signal intensity is high if water molecules are restricted in their motion, which can be caused by cell membranes or, in the case of free fluid, by high viscosity. The MR signal intensity is low if water molecules can diffuse freely.

FA: Fractional onisotropy

Fractional anisotropy measures direction of fluid flow; measurements used range from 0 to 1: zero indicating flow in all directions, and 1 supporting full flow in one specific direction. Kidney medulla tends to have higher FA values than cortex.

MD: Mean diffusion

Mean diffusion indicates extent of diffusion by water molecules across a membrane, thereby signifying membrane permeability. MD corelates with inflammation and/or infection

MRS: Magnetic resonance spectroscopy

MRS measures the metabolic components of tissues and quantifies the fatty acid content depicted with proton spectra, which measure lipid peaks. Proton MRS has been correlated with the infection/inflammation.

RD: Radial diffusion

It is part of diffusion anisotropy and measures flow of fluid/ diffusion along radial/ perpendicular direction. In the kidneys, it is along the tubules.

T1/ T2 mapping

T1-weighted MRI enhances the signal of the fatty tissue and suppresses the signal of the water. T2-weighted MRI enhances the signal of the water. Consideration of all the information provided by these modalities is conducive to MRI image analysis and diagnosis. These different methods are used to detect different structures or chemicals in a particular organ. The T2-weighted sequence is especially helpful in characterizing cysts and intraparenchymal abscesses and in evaluating hydronephrosis. Furthermore, the T2-weighted sequence is helpful in detecting solid lesions.

List of Tables

List of Figures

Kidney tissue characterization using magnetic resonance in HIV infected individuals undergoing kidney biopsy.

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ABSTRACT

BACKGROUND: Kidney disease is a common complication of human immunodeficiency virus (HIV) infection. Kidney biopsy is the gold standard for assessing causation, but being invasive, it carries increased risk in advanced chronic kidney disease (CKD). Magnetic resonance imaging (MRI) is noninvasive and may serve as an alternative to kidney biopsy in a subset of patients.

OBJECTIVES: The primary aim of this study was to compare the kidney biopsy Sethi chronicity score and MRI findings.

METHODS: This was a prospective pilot study, conducted at Groote Schuur Hospital, Cape Town and the Cape Universities Body Imaging Centre (CUBIC). People with HIV (PWH) >18 years with a clinical indication for kidney biopsy were included. Demographics and other clinical data were documented. Uncontrasted MRI scans were performed between 1-7 days prior to kidney biopsy that included diffusion tensor imaging (DTI), and proton (H^1) spectroscopy. The kidney biopsy Sethi chronicity score was compared to MRI-DTI using linear regression modeling as well as with H^1 spectroscopy.

RESULTS: Sixteen participants were included in the study. Thirty-one percent were female, 47% hypertensive, 6% diabetic, 44% had current tuberculosis, and 56 % were receiving antiretroviral therapy (ART). The most common histology observed on kidney biopsy were granulomatous interstitial nephritis (31%), hypertensive changes (31%) and HIV associated nephropathy (HIVAN) (25%). Mixed pathology was observed in 25% of participants. There was significant negative correlation with Sethi chronicity score and MRI-DTI findings. Spectroscopy showed an element of cell degradation and inflammation in all participants with highest lipid peaks in participants with HIVAN, and pauci-immune crescentic glomerulonephritis.

CONCLUSION:

In this small pilot study Sethi chronicity scores negatively correlated with MRI-DTI findings suggesting that this may potentially be a useful tool to assess chronicity. Further research is required to corroborate these findings and include BOLD sequence and T1 and T2 parametric mapping.

Introduction

Globally there is an estimated 37.7 million people living with HIV (PWH), with more than 1.5 million diagnosed annually. In 2020 Sub-Saharan Africa (SSA) had the highest disease burden accounting for almost 60% of new HIV infections[1].

Kidney disease is a common complication of HIV/AIDS. The spectrum of disease includes HIV associated nephropathy (HIVAN), opportunistic infections particularly tuberculosis (TB), drug reactions as well as non-communicable diseases including diabetes and hypertension.[2] In a systematic review and meta-analysis of chronic kidney disease (CKD) in adults with HIV, the overall CKD prevalence globally was 6.4% with the highest prevalence in SSA (7.9%).[3] HIVAN progresses rapidly to kidney failure in the absence of antiretroviral therapy (ART) and is the 3rd leading cause of kidney failure among blacks aged 20- 64 years in the United States of America (USA).[4]

In two large early biopsy series from Cape Town (1989) and Johannesburg (2014), South Africa, HIVAN was found to be the most common pathology observed in 57.3% and 43.4% of cases respectively.[5,6] In comparison, a study from the USA by Kudose et al (2020), demonstrated that immune complex glomerulonephritis (GN) and diabetic nephropathy were more common than HIVAN. Fortunately, recent data describes a downward trend in HIVAN in both the USA and South Africa. [7,8,9]

Ultrasound is neither sensitive nor specific to diagnose kidney failure. Small echogenic kidneys on ultrasound can often assist with the diagnosis of CKD. However, kidney sizes, in the setting of HIVAN are often preserved or enlarged therefore making this diagnosis on ultrasound more challenging. Kidney biopsy is the gold standard for determining the aetiology of kidney disease.[10] However, it is an invasive procedure and carries risk especially in patients with advanced disease. [10]

Due to high clinical demands, there are often delays in referral for tertiary assessment for kidney disease compounded by very high occupancy rates for inpatient evaluation. Magnetic resonance imaging (MRI) is a noninvasive technique that can be offered as an outpatient. Its use in patients with kidney disease was previously limited by potential toxicity of gadolinium and movement artifacts from abdominal organs. Recent technical developments using uncontrasted MRI to assess kidney anatomy and tissue characteristics has changed the prospects of this modality, making MRI suitable for the assessment of both acute kidney injury (AKI) and CKD. It has high temporal and special resolution, excellent soft tissue contrast, without ionizing radiation. It offers the potential of detailed ultrastructural characterization of the kidneys and has the possibility of offering a 'virtual histology', if imaging biomarkers such as diffusion tensor imaging (DTI), diffusion weighted imaging (DWI), blood oxygen level dependent (BOLD) MRI and T1/T2 mapping can be correlated to histological indices. Several uncontrasted MRI techniques have been piloted for the study of kidney disease, including DTI, BOLD, proton (H1) spectroscopy and T1 and T2 parametric mapping.[11,12,13]

DWI is a technique that evaluates the displacement of the water molecules in a tissue. DWI is particularly sensitive to alterations in renal interstitium such as in renal fibrosis, cellular infiltration, and can be used to assess changes in renal perfusion and water handling in

tubules. DTI however although is a DWI technique, it can be used to assess microstructural orientation within the kidney such as fractional anisotropy (FA). The medulla has higher degree of anisotropy compared to the cortex, presumably due to predominant water motion along tubular and vascular bundles of pyramids and so is a very useful tool to evaluate the degree of disruption to tubule microstructure. DTI can also be used to calculate the mean diffusivity (DWI ADC equivalent) which reflects an overall measure of water diffusion and microcirculation in the tissue. DTI is particularly suitable for evaluating the tubules in the nephron and hence used in this preferentially in this study.

Mass spectrometry is a rapidly evolving imaging modality that has been successfully used to assist with understanding the evolution of CKD and aid in identification of serum and urinary biomarkers for the diagnosis of CKD.[11] MRI also has the potential to prognosticate kidney disease. This could serve to alleviate the need for a kidney biopsy and to determine those who have reached end stage kidney disease. However, several gaps still exist in our knowledge. The diagnostic role of uncontrasted MRI sequences needs to be clarified. Spectrometry could potentially be used to determine tissue pathology and diagnosis as well monitoring disease progression while DTI can determine the chronicity of disease pathology although further studies are required to ascertain this.

The primary aim of this study was to compare the Sethi chronicity score on kidney biopsy, with the MRI biomarker DTI to correlate/compare the tissue chronicity seen. It also aimed to evaluate whether MRI spectroscopy can be used as a tool to correlate with kidney histology. The secondary aim was to review mortality of the cohort as well as kidney function over a 2-year period.

METHODOLOGY

This prospective pilot study was conducted in the Division of Nephrology at Groote Schuur Hospital, University of Cape Town and the Cape Universities Body Imaging Centre (CUBIC). The study was approved by the University of Cape Town Faculty of Health Sciences Human Research Ethics Committee (HREC REF: 550/2016). All the data collected was anonymized to maintain privacy of the participants.

The study population was PWH, who were > 18 years and admitted for a kidney biopsy to determine the cause of their kidney disease. Participants were excluded if informed consent was not obtained and/or if there was a contraindication to MRI or kidney biopsy.

Demographic and clinical data were documented including age, sex, ethnicity, and presence of comorbidities including diabetes, hypertension, and tuberculosis (TB). The diagnosis of TB was based on the use of anti-TB medication or a positive diagnostic test. The following tests were also performed: serum creatinine (µmol/l) and estimated glomerular filtration rate (eGFR) (mL/min/1.73m^2), urine dipsticks, urine protein/creatinine ratio (g/mmol), CD4 count (cells/µl), use of ART, and kidney ultrasound findings.

A percutaneous needle biopsy was performed under ultrasound guidance. Two cores were obtained and sent to anatomical pathology for light and electron microscopy, and immunoperoxidase staining. Specimens were analyzed by a nephropathologist in conjunction

with the nephrology team.

An uncontrasted MRI scan was done between day (1-7) prior to the kidney biopsy to avoid changing the echotexture of the kidney. All participants were well hydrated prior to undergoing MRI. MRI involves four sequences: diffusion weighted imaging (DWI), diffusion tensor imaging (DTI), blood oxygen level dependent (BOLD) and T1 and T2 mapping. In this study, MRI of the kidneys was performed with DTI and proton magnetic resonance spectroscopy (MRS). In addition, native T1 and T2 mapping of the kidneys was undertaken but could not be analyzed as the images were not electrocardiographic vector gated at time of acquisition. BOLD imaging was also performed but has not been included in this analysis.

Kidney biopsy analysis

The spectrum of kidney pathology was classified using the KDGO classification for HIV related kidney disease.[14] The biopsies were analyzed using the Sethi chronicity scoring system to assist in determining the chronicity index. (Table 1), The total score was a combination of 4 different chronicity scores namely glomerulosclerosis (GS), interstitial fibrosis (IF), tubular atrophy (TA), and arteriosclerosis.[15] GS was defined as global or segmental collapse of glomerular capillary walls and consolidation of the glomerular tuft by extracellular matrix, causing capillary luminal obliteration and was scored from 0 to 3. TA was defined as the shrinkage of tubules with variable thickening of the tubular basement membrane and flattening of the tubular epithelium and scored from 0 to 3. IF was defined as the accumulation of fibrous tissue between the tubules and scored from 0 to 3. Arteriosclerosis was defined as fibrous thickening and /or hyalinosis of the intima and scored from 0 to 1. All the scores were added to form the total kidney chronicity score (see Tables 1 and 2), which was then used to grade the severity of chronic lesions. The total Sethi chronicity score was compared to the MRI DTI score. [15]

Table 1: Grading of chronic lesions on kidney biopsy utilizing the Sethi chronicity score.

Tissue compartment	Score			
	0	1	2	3
Glomerulosclerosis	<10%	10 – 25%	26 – 50%	>50%
Interstitial Fibrosis	<10%	10 – 25%	26 – 50%	>50%
Tubular Atrophy	<10%	10- 25%	26-50%	>50%
Arteriosclerosis	Intimal thickening <thickness of media	Intimal thickening ≥thickness of media		

Table 2: Grades of chronic changes based on total kidney chronicity score (0 to 10)

Grade	Total chronicity score
Minimal chronic changes	0-1
Mild chronic changes	2 – 4
Moderate chronic changes	5 – 7
Severe chronic changes	≥8

Magnetic Resonance Imaging analysis

MRI of the kidney was done using two of the sequences, DTI and MRS as follows:

a. Diffusion tensor imaging analysis (DTI)

DTI assesses structural integrity of the microfiber of tissues and has several components of interest. Fractional anisotropy (FA) measures direction of fluid flow; measurements used range from 0 to 1: zero indicating flow in all directions, and 1 supporting full flow in one specific direction. Mean diffusion (MD) indicates extent of diffusion by water molecules across a membrane, thereby signifying membrane permeability. MD corelates with inflammation and/or infection.[16] DTI analysis was done using AFNI version 22 (https://afni.nimh.nih.gov/), TORTOISE version 3 (https://tortoise.nibib.nih.gov/tortoise) , and 3D Slicer version 4.11 (https://www.slicer.org/). DW and structural T2 weighted turbo spin echo (TSE) image volumes were converted from DICOM into NIfTI format. DW volumes were pre-processed to reduce artefacts caused by eddy currents, susceptibility distortion, and motion. The DW volumes were spatially co-registered to the structural TSE image volume. A threshold mask was created to remove the surrounding abdominal tissue. Finally, volumes were used to calculate the diffusion tensor and other parameters of interest including mean diffusivity (MD), fractional anisotropy (FA), and radial diffusivity (RD). 3D Slicer was then used to further refine the mask to extract the MD, RD, and FA from the following sections: left and right whole kidneys, left and right medulla, and left and right cortex.

b. Magnetic resonance spectroscopy (MRS)

MRS measures the metabolic components of tissues and quantifies the fatty acid content depicted with proton spectra, which measure lipid peaks. Proton MRS has been correlated with the infection/inflammation.[17] The MRS analysis was conducted using LCModel version 6.3 (https://doi.org/10.1002/mrm.1910300604). LCModel was used to quantify the proton MRS spectra taken from a voxel in each subject's kidney. LCMgui is a graphical user interface that performs the functions of LCModel. For each patient, a water suppressed single voxel spectroscopy (SVS) file was selected for analysis using LCMgui. The full spectrum was analysed from 8 ppm down to -1 ppm (to ensure metabolites near 0 ppm were not missed). Water scaling was done. Unsuppressed water SVS file was then selected to be used as a scale factor to estimate the metabolite concentrations. LCModel does not have a specific kidney profile and the liver profile was utilised to approximate kidney tissue. The liver profile spectra contain lipids, choline, glycogen, and a water signal which is similar to that of the kidney.

Statistical analysis

Data obtained from the patients were entered into an Excel spreadsheet and analyzed using Statistica 14 (TIBCO Software Inc.) to calculate mean (S.D) and median (IQR) and perform linear regression comparing Sethi Score with DTI findings. Frequency distribution was presented with percentages for categorical data. Spearman correlation coefficient was used to compare an association between MRI DTI scores and histopathological findings. Statistical value was done with level of significance at $p<0.05$.

RESULTS

Table 3: Baseline characteristics and histology findings of all participants undergoing a kidney biopsy and magnetic resonance imaging (MRI).

Clinical Variable	**n=16**
Demographics	
Age (years), Median (IOR)	34.5 (33 -41)
Ethnicity (%)	4 (25%) Mixed race; 12 (75%) Black
5ex – Males (%)	11 (69%)
BMI (kg/m^2), Median (IQR)	21.4 (18.5-26.2)
Hypertension (%)	7 (44%)
Diabetes (%)	1 (6%)
Active Tuberculosis (%)	5 (31%)
ART (%)	10 (63%)
Smoking (%)	6 (38%)
Illicit drug use (Methamphetamines (%)	2 (13%)
Biochemical Evaluation	
Proteinuria on dipstick (mean)	2+
UPCR (g/mmol), Median (IQR)	0.4 (0.2- 0.9)
CD4 Count (cells/µL), Median (IQR)	85.5 (46- 330)
Creatinine (µmol/l), Median (IQR)	694.5 (180 -1468)
Ultrasound measurements	
Right kidney (cm), Median (IQR) Left kidney (cm), Median (IQR)	12 (12-13.9) 13 (12.5-13.3)
Histology	
Hypertension * Granulomatous Interstitial Nephritis HIVAN Others	5 (31%) 5 (31%) 4 (25%) 2 (13%)

Key: BMI; Body mass index, ART; Antiretroviral therapy, UPCR; Urine protein creatinine ratio, HIVAN; HIV associated nephropathy, IQR: Interquortile range

**2 patients had malignant hypertension*

A total of 16 participants were enrolled in the study. Table 3 describes the demographics of the participants. The median age was 34.5 years, Interquartile range (IQR) (33-41), 11/16 (69%) were males, 7/16 (47%) were hypertensive, 1/16 (6%) was diabetic, 5/16 (44%) had current TB, and 10/16 (56 %) were on ART. The median baseline creatinine was 694.5μmol/l, IQR (180- 1468). The median CD4 count was 85.5cells/μL, IQR (46-330), and median hemoglobin was 7.5g/dl, IQR (6.7- 8.9). All participants had at least a mean of 2+ proteinuria.

Table 4 describes the biopsy findings of the participants. These were varied; however, the most common findings were hypertensive nephrosclerosis in 5/16 (31%), granulomatous interstitial nephritis also in 5/16 (31%) and HIVAN in 4/16 (25%). At least 5/16 (31%) of the participants had dual pathology. The highest Sethi chronicity scores were noted in participants with pauci-immune glomerulonephritis (GN) and HIVAN with a score of 8/10. Hypertension had consistently higher Sethi chronicity scores of >5 in 80% of participants. Acute tubular necrosis (ATN) and pyelonephritis showed the least fibrosis with a Sethi chronicity score of 2.

All patients completed the MRI examination. Five participants had inadequate MRI images to determine DTI scores and as such were discarded leaving a total of 11 for analysis. There was a consistent trend for negative correlation between diffusion of water molecules and chronicity score based on the DTI-MRI and Sethi chronicity score. Three of the correlations reached a statistical significance at $p< 0.05$ (Table 4 and Figures 1-3). Hypertension (2), pauci-immune GN (1) and HIVAN (1) had the highest Sethi chronicity scores and the lowest MD scores.

Table 4: Sethi Chronicity score and MRI-DTI score compared to kidney biopsy findings in each participant.

	Kidney biopsy histology	Sethi Chronicity score	MRI DTI of left kidney		
			MD	FA	RD
1.	Post infectious glomerulonephritis	5	0,0182	0,0022	0,0166
2.	Hypertension, mild lymphocytic infiltrate	7	0,023	0,002	0,0211
3.	Granulomatous interstitial nephritis, severe granulomas	4	0,0277	0,0023	0,0253
4.	Mesangial-capillary glomerulonephritis with crescents*	6			
5.	Light chain disease, granulomatous interstitial nephritis	4	0,0243	0,0026	0,0218
6.	Acute tubular necrosis, pyelonephritis*	2			
7.	Granulomatous interstitial nephritis *	4			

8.	HIVAN	4	0,0146	0,0016	0,0131
9.	Malignant hypertension	7	0,0105	0,0014	0,0092
10.	Mild interstitial fibrosis	4	0,0259	0,0038	0,0203
11.	HIVAN	2	0,0307	0,0033	0,0276
12.	Hypertension, granulomatous interstitial nephritis	2	0,0243	0,0029	0,0217
13.	HIVAN	8	0,0176	0,0019	0,0159
14.	Pauci immune glomerulonephritis with crescents*	8			
15.	Malignant hypertension*	6			
16.	HIVAN	5	0,019	0,0034	0,0162

Key; MRI: Magnetic resonance Imaging, DTI: Diffusion tensor imaging, MD: mean diffusivity, FA: fractional anisotropy, RD: radial diffusivity, HIVAN: HIV associated nephropathy.

** In these participants, the DTI scores could not be determined*

Figure 1: A scatterplot demonstrating the mean diffusion of water molecules in the left kidney compared to the Sethi chronicity score (r=-0.6054, p<0.05)

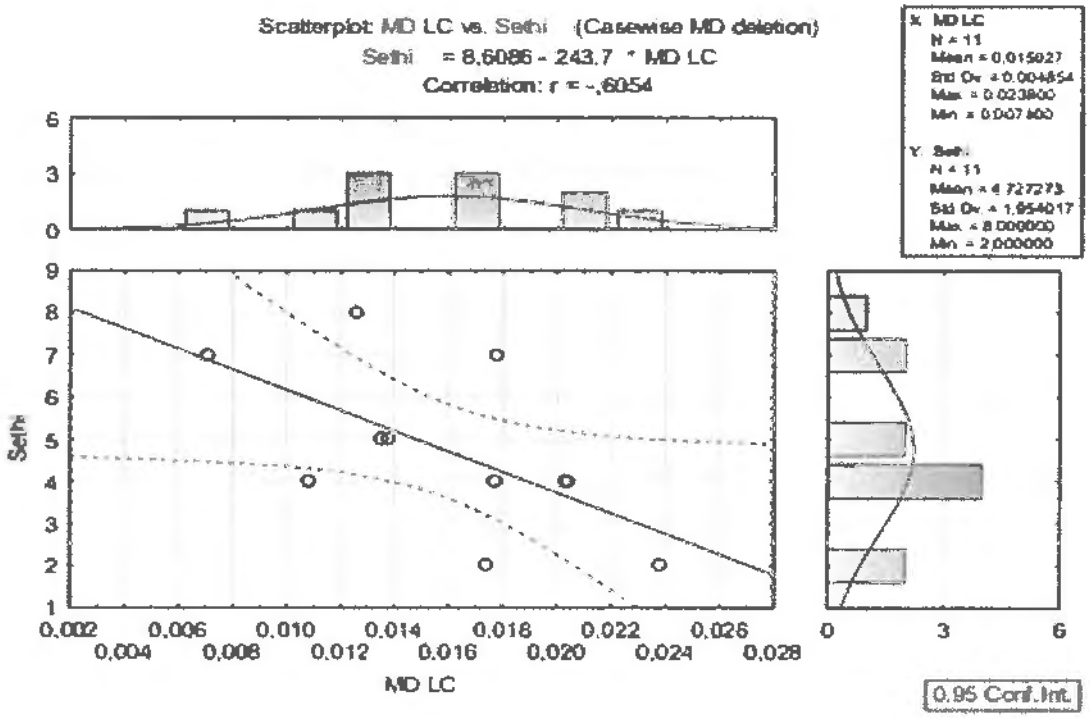


Figure2: A scatterplot demonstrating the radial diffusion of the medulla compared to Sethi chronicity score (r=-.0,6063, p<0.05)

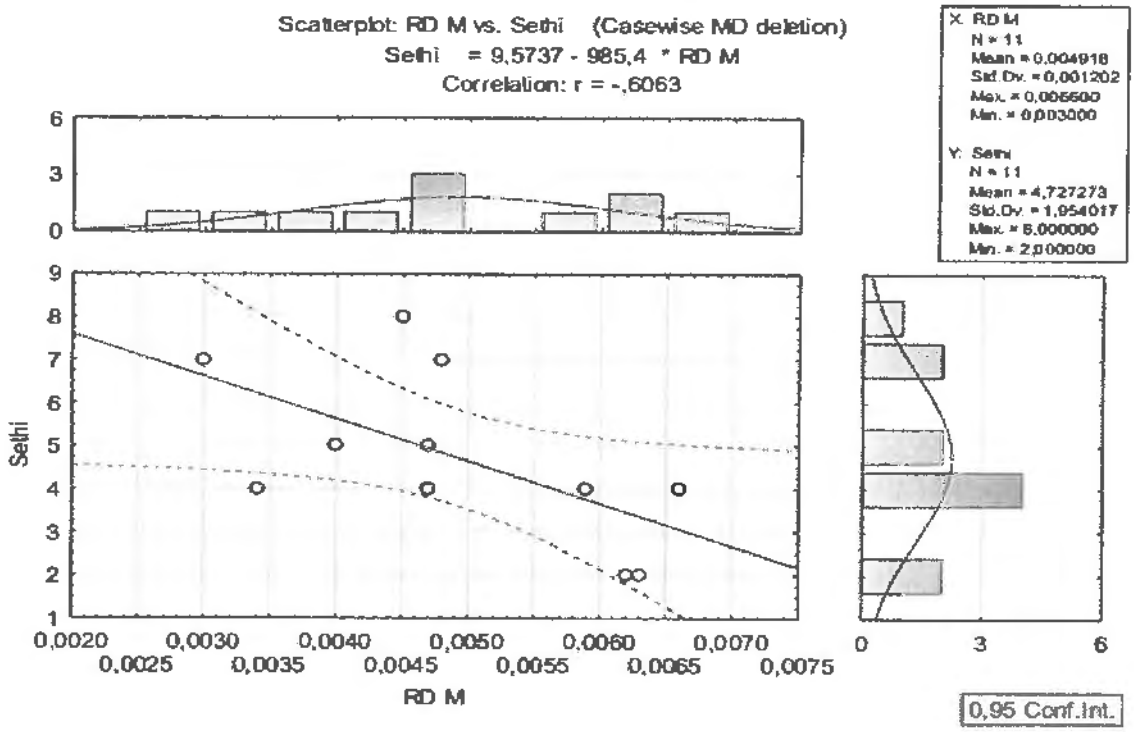
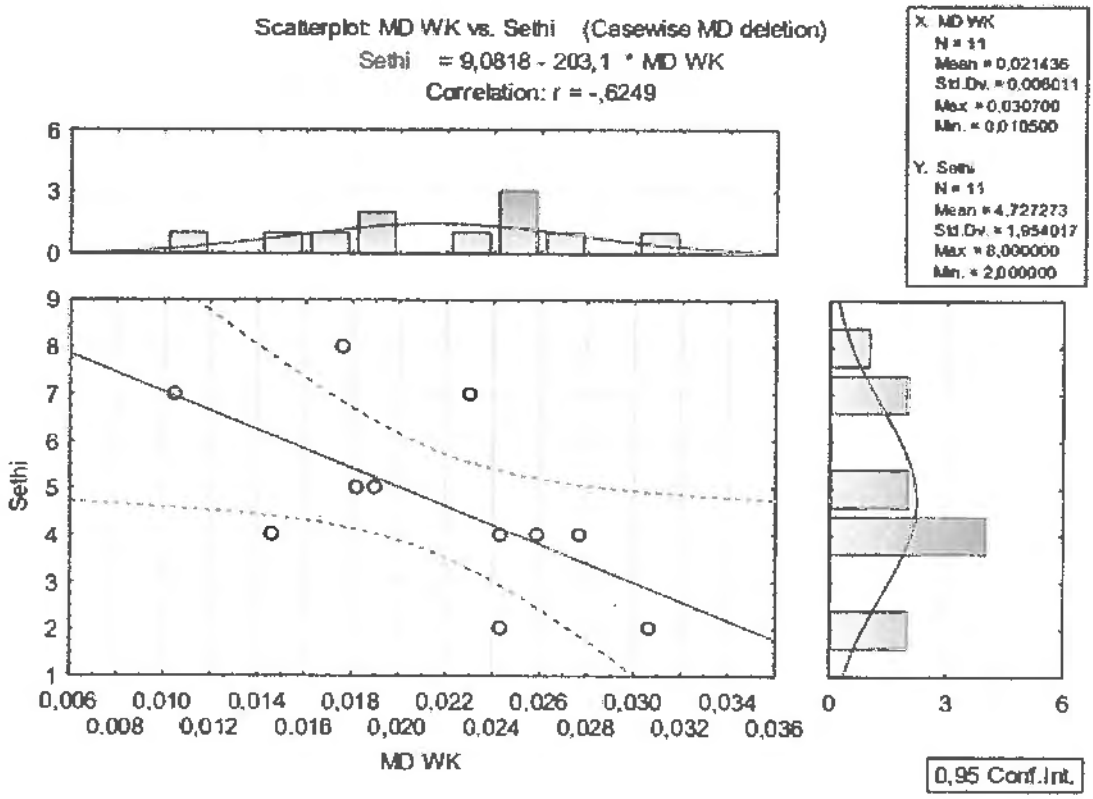


Figure 3: A Scatterplot demonstrating the mean diffusion of whole kidney compared to Sethi chronicity score (r=-0.6249, p<0.05)



Spectroscopy

MRS showed varied peaks depending on the underlying pathology consistent with inflammation, cell degradation and necrosis in all the participants. Eighty percent of the participants showed a lipid peak at 1.3 parts per minute (ppm). The highest lipid peak was seen in participants with ATN and pyelonephritis, followed by mesangiocapillary GN, pauci-immune crescentic GN and HIVAN, while the lowest lipid peak was seen in patient with granulomatous interstitial nephritis. Three (3) of the five (5) participants with granulomatous interstitial nephritis, showed lipid peaks between 1.6 to 1.8ppm. Consistent water peaks were shown at 4.7 ppm with area under the curve ranging from 5.0ppm to 4.4ppm in all but 1 of the samples.

A small choline peak was only noted in 3 out of 5 patients who had hypertension identified on kidney biopsy, while a myoinositol peak was seen in 1 of the participants with hypertension. Other peaks were of GABA, Glutamate, lactate and alanine. Figures 4- 6 show spectrometry peaks seen in participants with hypertension, granulomatous interstitial nephritis and HIVAN.

Figure 4: Spectroscopy peaks seen in a participant with hypertension.

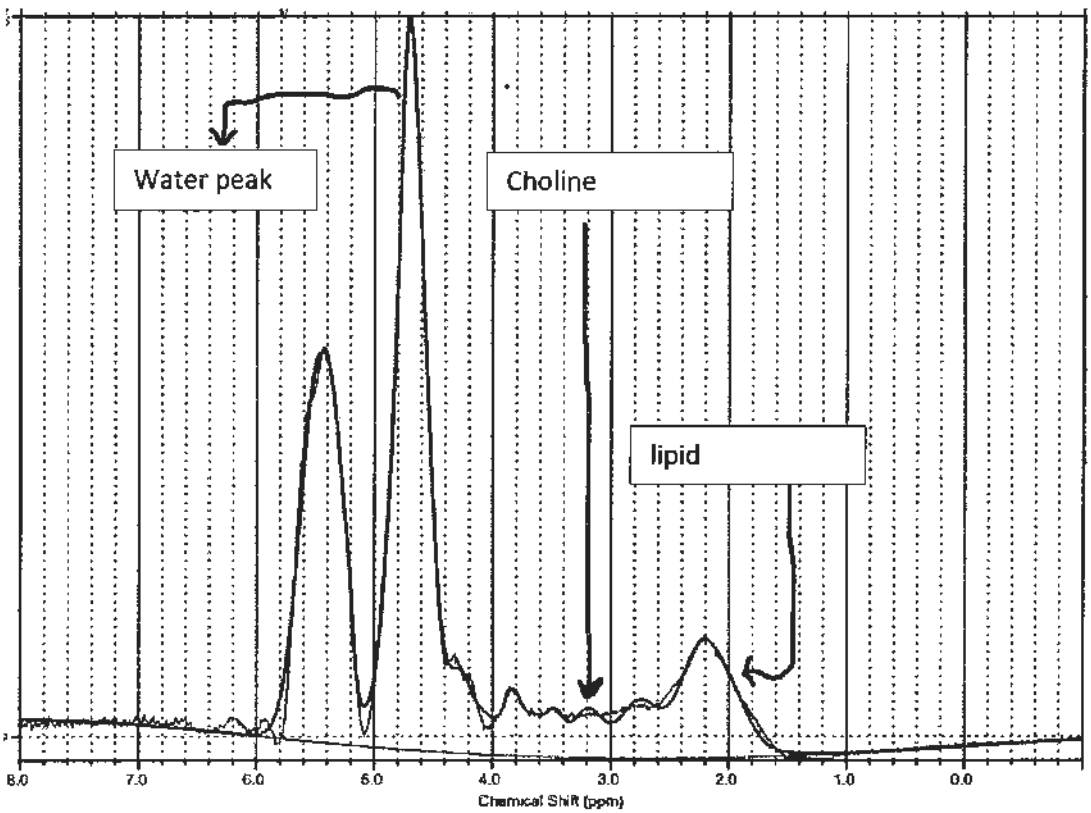


Figure 5: Spectroscopy peaks seen in granulomatous interstitial nephritis.

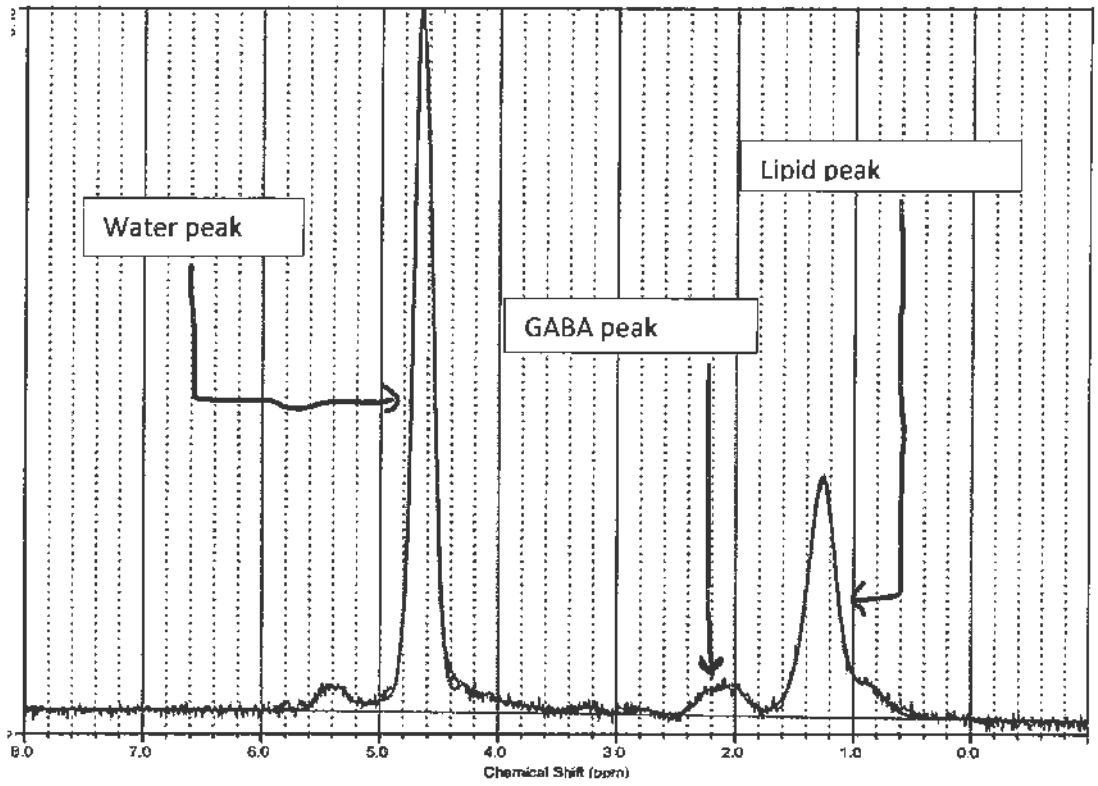
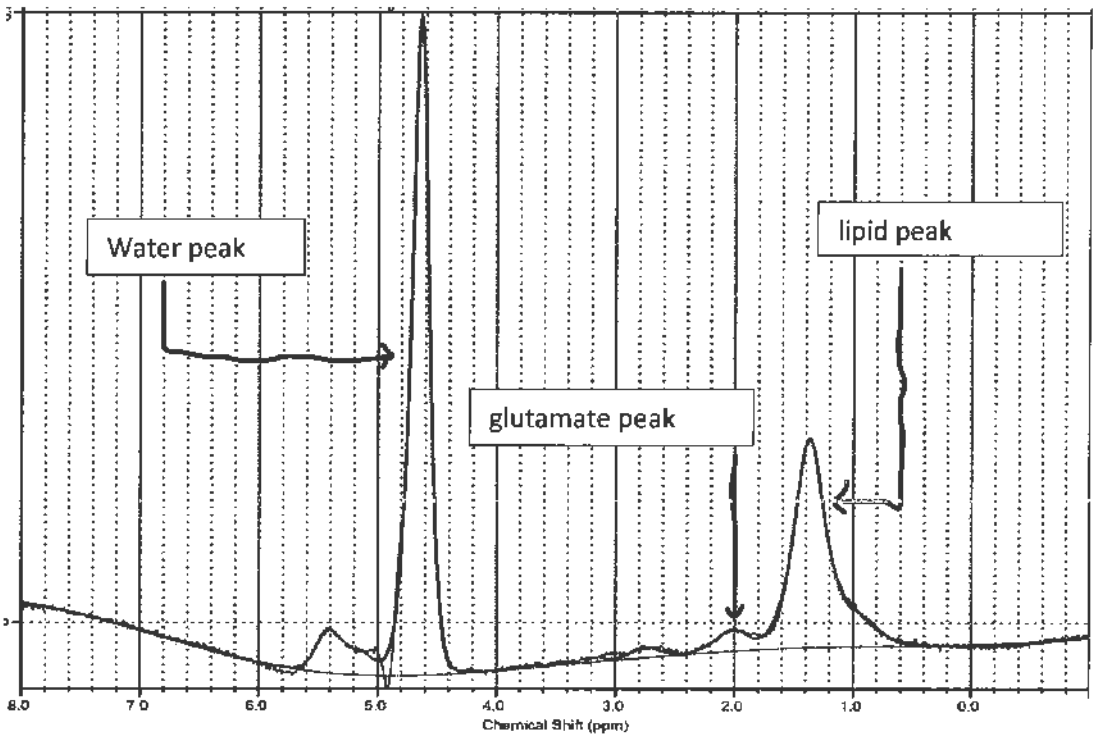


Figure 6: Spectroscopy peaks seen in HIV associated Nephropathy.



Long-term outcomes

Participants were followed over a two-year period (Table 4). Thirty eight percent (38%) of participants were lost to follow up. At the end of the follow up period, 44% of the participants had demised. Of the participants that demised, 71% had a Sethi chronicity score of at least 5.

Three of the participants showed worsening of kidney function over the review period leading to mortality in 2 participants.

Table 5: Outcomes of kidney function and mortality over a 2-year period.

	Baseline Creatinine (µmol/l)	Creatinine µmol/l (0-6months)	Creatinine µmol/l (6 – 12months)	Creatinine µmol/l (1 to 2yrs)	Creatinine µmol/l (>2yrs)	Sethi Score	Outcome	Cause of death
1.	130	80	89	307	560	5	Alive	
2.	1227	696	518	-	-	7	Demised	Kidney Failure
3.	1130	82	82	88	81	4	Alive	
4.	177	146	93	146	-	6	Alive	
5.	1708	85	90	96	LTFup	4	Alive	
6.	913	132	52	LFTup		2	Alive	
7.	497	463	-	-	-	4	Demised	Advanced HIV, kidney failure
8.	178	-	244	-	-	4	Demised	Advance HIV
9.	1468	374	249	-	-	7	Demised	Kidney failure, Sepsis
10.	1547	78	LTFup			4	Alive	
11.	808	654	LTFup			2	Alive	
12.	180	167	LTFup			2	Alive	
13.	1668	976	-	-	-	8	Demised	Kidney failure
14.	296	283	175	698	1262	8	Demised	Kidney failure
15.	581	140	LFTup			6	Alive	
16.	213	76	92	353	915	5	Demised	Disseminated TB, kidney failure

Key: LTFup: Lost to follow up, TB: Tuberculosis, HIV: Human Immunodeficiency virus

Discussion

The main aim of this prospective, pilot study in PWH was to compare the degree of chronicity established on kidney biopsy using the Sethi chronicity score[15] and the MRI-DTI biomarker. It also aimed to review kidney histology and compare with spectroscopy. The study contributes to the limited data published on MRI of the kidney to date. The major finding of the study was a consistent negative correlation between Sethi chronicity scores and MRI-DTI. The other prominent finding was that lipid peaks were observed in the spectroscopy scans in all participants. This finding supports inflammation, cell degradation and necrosis. In addition, there was a choline peak observed in 3/5 cases with hypertension. Lastly, we noted that all the participants who had a Sethi score of more than 7 demised, with kidney failure contributing to, or directly responsible for mortality.

In the setting of HIV associated kidney disease it can often be difficult to determine whether a patient has advanced CKD compared to an acute reversible cause of kidney injury. Reduced kidney sizes are often associated with advanced irreversible CKD. However, in the case of kidney failure due to HIVAN, the size is often increased thereby limiting the usefulness of size to assist with prognosis. Although kidney biopsy is the gold standard to assess pathology, the use of MRI could potentially become a useful strategy to avoid kidney biopsy in patients with advanced kidney disease. Further larger studies need to be performed to confirm the association with chronicity on histology with MRI sequences. The Sethi chronicity score provides a standardized way of assessing chronicity thereby assisting with prognostication. Having a standard technique to assess chronicity would be useful to provide comparable results in future studies. There have been some earlier, small studies that correlate Sethi chronicity scoring on kidney biopsy with prognosis and progression of kidney disease. Srivastava *et al.*, compared the Sethi chronicity score determined from the kidney biopsy to assess chronicity and disease progression. They found that the presence of inflammation in a fibrosed interstitium was associated with a higher risk of subsequent kidney disease progression.[18] Our study, however, took this one step further and compared the Sethi chronicity Score with the MRI-DTI score.

MRI -DTI

MRI-DTI is an advanced imaging technique used to estimate the diffusion of tissue in the body by calculating the parameters of apparent diffusion coefficient (ADC) and fractional anisotropy (FA). In the setting of fibrosis in the kidney there is reduced diffusion and less permeability across the cell membrane resulting in distorted water flow in the parenchyma as shown in the observed low DTI scores. Three specific correlations (mean diffusion left kidney, radial diffusion of medulla and mean diffusion whole kidney) reached statistical significance ($p<0.05$) when correlated to the Sethi chronicity score using linear regression. This was independent of the underlying pathology. The consistent negative correlation signifies reduced permeability of the cell wall and reduced diffusion across the kidney tubules as fibrosis worsens.

The highest Sethi chronicity scores/lowest DTI scores were observed in hypertension, HIVAN and pauci-immune GN. Five out of six (5/6) of the participants with high Sethi scores, also had

the worst kidney function (Table 5). Furthermore, of those that demised, 7/16 (44%), 5/7 (71%) of them had a Sethi score of at least 5. Nassar *et al.*, evaluated DTI to assess its use as an early predictor of kidney fibrosis and disease progression. Results demonstrated that cortical FA values were significantly higher and negatively correlated to eGFR, while cortical ADC negatively correlated to percentage of sclerotic glomeruli, atrophic tubules, and interstitial fibrosis. Furthermore, medullary ADC negatively correlated to tubular atrophy. Multiple linear regression analysis was also performed and revealed that the mean cortex ADC was significantly decreased by 0.199 mg/dL for patients with >50% glomerulosclerosis on the kidney biopsy.[19] These results are similar to our study in that DTI showed a negative correlation to kidney histology. Although chronicity on kidney biopsy was assessed in the study by Nassar *et al.*, it was not done using the Sethi chronicity scoring system but both studies confirmed that DTI could potentially be used as a tool to evaluate kidney disease and assessment of CKD progression, especially using the medullary FA values. This could potentially alleviate the need for a kidney biopsy.

Spectroscopy

MRS measures the metabolic components of tissues and quantifies the fatty acid content depicted with proton spectra, which measure lipid peaks. In proton MRS, presence of lipid peaks has been positively correlated with presence of infection and/ or inflammation. There are other peaks which can be picked on spectroscopy such as GABA, Glutamate, alanine, lactate and N-acetylaspartate (NAA) which resonate differently and signify different disease process. This has been noted in several MRI studies on the brain. Spectroscopy findings in our study demonstrated an element of cell damage, degradation, and inflammation in all patients. MRS was varied in each participant with different peaks being identified with differing pathologies. Choline peaks were only seen in hypertension. The presence of this peak in MRS indicates high cellularity or cell destruction and has been used in MRS as a tumour marker or associated with malignancy in MRS studies on Brain, kidney, liver and breast[20]. However, it could also represent an infective process. Whether this finding in this study indicates that hypertension is a chronic condition generating inflammation for a sustained period as compared to the other pathologies remains unclear and will need further investigation in the future. There are limited studies on spectroscopy in the kidney. Merchant *et al.* summarised that MS is a rapidly evolving discipline and could be used to aid with understanding the evolution of CKD as well as to aid in the identification of serum and urinary biomarkers for diagnosis of CKD.[17] In another experimental study using multiparametric MRI to evaluate CKD, Schley *et al.*, demonstrated a strong potential of MRI parameters to determine kidney tissue characteristics, and that T2 relaxation time is a promising MRI parameter to quantitatively assess kidney fibrosis.[21] Therefore, further studies should include all the other MRI parameters to assist in further understanding and evaluating CKD progression in comparison with histological findings.

Strengths

One of the strengths of this study was that we used a validated chronicity scoring system for histology. The Sethi chronicity score could potentially be used as a standardised grading tool in CKD for future studies which would allow for a better comparison between studies. Another strength is the fact that we included spectroscopy in the evaluation of kidney tissue which showed different peaks in the different histological diagnoses. Our findings add to limited published data on MRI spectroscopy.

Limitations

The study had several limitations. First, the sample size was small limiting the power of the study to make definitive conclusions. Secondly, the participants were not electrocardiographic vector gated during the MRI, this resulted in the loss of BOLD and the T1&T2 images. These imaging techniques could have provided more information on the correlation of the Sethi score for fibrosis and MRI findings. Thirdly, some of the MRI images were of poor quality and could not be used for analysis. A number of factors could have contributed to the poor quality including poor breath holding causing movement artifacts, intra-operator variability and training in this specific abdominal MRI technique or possibly the timing of the scan itself in relations to meals taken by the participant. In the end, this led to further reduction in sample size for statistical analysis. Lastly, the study was done at a tertiary hospital and only on the referred patients to the team thereby introducing sample bias on the data analyzed.

Conclusion

Our preliminary observations suggests that MRI may be a useful way to assess fibrosis as a marker of CKD progression in both people with and without HIV. Both MRI-DTI and spectroscopy, could potentially be validated as a noninvasive marker of CKD in patients with HIV infection and as a prognosticating tool for those who have progressed to CKD stage 5. In the long term, multiparametric MRI could potentially substitute the need for a kidney biopsy to determine the diagnosis and evaluation of CKD, thus preserving already impaired kidney tissue from additional iatrogenic damage as well as reducing hospital stay and financial costs to both the facility and the patient. There is need for a future study with a larger sample size, addressing the challenges and limitations raised in this study to validate these findings and in turn make MRI a clinically viable option to kidney biopsy.

References

1. AIDSinfo | UNAIDS. https://aidsinfo.unaids.org/.
2. Swanepoel, C. R. *et al.* kidney disease in the setting of HIV infection: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* **93**, 545–559 (2018).
3. Ekrikpo, U. E. *et al.* chronic kidney disease in the global adult HIV-infected population: A systematic review and meta-analysis. *PLoS One* **13**, e0195443 (2018).
4. Collins, A. J. *et al.* United States Renal Data System 2008 Annual Data Report. *Am J Kidney Dis* **53**, (2009).
5. Wearne, N., Swanepoel, C. R., Boulle, A., Duffield, M. S. & Rayner, B. L. The spectrum of renal histologies seen in HIV with outcomes, prognostic indicators and clinical correlations. *Nephrology Dialysis Transplantation* **27**, 4109–4118 (2012).
6. Vermeulen, A. *et al.* Patterns of renal disease: A 30-year renal biopsy study at Chris Hani Baragwanath Academic Hospital, Soweto, Johannesburg, South Africa. *S Afr Med J* **109**, 486–492 (2019).
7. Kudose, S. *et al.* The spectrum of kidney biopsy findings in HIV-infected patients in the modern era. *Kidney Int* **97**, 1006–1016 (2020).
8. Diana, N. E., Davies, M., Mosiane, P., Vermeulen, A. & Naicker, S. Clinicopathological correlation of kidney disease in HIV infection pre- and post-ART rollout. *PLoS One* **17**, (2022).
9. Bookholane, H. *et al.* Predictors and Prognosis of HIV-Associated Nephropathy on Kidney Biopsy in South Africa. *Kidney Int Rep* **5**, 1799–1804 (2020).
10. Pettitt, R. M., Brumbaugh, A. P., Gartman, M. F. & Jackson, A. M. Chronic Kidney Disease: Detection and Evaluation. *Osteopathic Family Physician* **12**, 14–19 (2020).
11. Merchant, M. L. Mass spectrometry in chronic kidney disease research. *Adv Chronic Kidney Dis* **17**, 455–468 (2010).
12. Selby, N. M. *et al.* Magnetic resonance imaging biomarkers for chronic kidney disease: a position paper from the European Cooperation in Science and Technology Action PARENCHIMA. *Nephrology Dialysis Transplantation* **33**, ii4–ii14 (2018).
13. Borrelli, P. *et al.* Diffusion Tensor Imaging of the Kidney: Design and Evaluation of a Reliable Processing Pipeline. *Sci Rep* **9**, (2019).
14. Swanepoel, C. R. *et al.* Kidney disease in the setting of HIV infection: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* **93**, 545–559 (2018).
15. Sethi, S. *et al.* A proposal for standardized grading of chronic changes in native kidney biopsy specimens. *Kidney Int* **91**, 787–789 (2017).
16. Caroli, A. *et al.* Diffusion-weighted magnetic resonance imaging to assess diffuse renal pathology: a systematic review and statement paper. *Nephrol Dial Transplant* **33**, ii29–ii40 (2018).

17. Merchant, M. L. Mass spectrometry in chronic kidney disease research. *Adv Chronic Kidney Dis* **17**, 455–468 (2010).

18. Srivastava, A. *et al.* The prognostic value of histopathologic lesions in native kidney biopsy specimens: Results from the Boston kidney biopsy cohort study. *Journal of the American Society of Nephrology* **29**, 2213–2224 (2018).

19. Nassar, M. K. *et al.* Diffusion Tensor Imaging in early prediction of renal fibrosis in patients with renal disease: Functional and histopathological correlations. *Int J Clin Pract* **75**, (2021).

20. Xu, L. *et al.* 3.0 T proton magnetic resonance spectroscopy of the liver: quantification of choline. *World J Gastroenterol* **19**, 1472–1477 (2013).

21. Schley, G. *et al.* Multiparametric magnetic resonance imaging of experimental chronic kidney disease: A quantitative correlation study with histology. *PLoS One* **13**, (2018).

Appendices

1. Human Research Ethics Committee Approval Form
2. Questionnaire
3. Consent Form
4. Groote Schuur Hospital approval
5. Supplementary Baseline characteristics
6. Spectroscopy graphs

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

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Observatory 7925
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Website: www.health.uct.ac.za/fhs/research/humanethics/forms

9 September 2016

HREC REF: 550/2016

Prof B Rayner
Nephrology and Hypertension
Medicine
E13, NGSH

Dear Prof Rayner

PROJECT TITLE: THE UTILITY OF USING MAGNETIC RESONANCE OR ULTRASOUND IMAGING COMPARED TO RENAL BIOPSY IN HIV +VE PATIENTS UNDERGOING RENAL BIOPSY (MMed-candidate D Band)

Thank you for your response submitted to the Faculty of Health Sciences Human Research Ethics Committee received on 5 September 2016.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th September 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The HREC acknowledge that the MMED student D Band will also be involved in this study.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

QUESTIONNAIRE

THE UTILITY OF USING MAGNETIC RESONANCE OR ULTRASOUND IMAGING COMPARED TO RENAL BIOPSY IN HIV +VE PATIENTS UNDERGOING RENAL BIOPSY.

Date |__|__|/|__|__|/|__|__|	Number_____	Code _____

N°	QUESTIONS	CODES
	SECTION 1 : DEMOGRAPHICS	
1.1	**Âge**	
1.2	**Sex:** Male Female	
1.3	**Ethnicity:**	
1.4	**Weight**	
	SECTION 2 : MEDICAL HISTORY	
2.1	**Background history:** Hypertension Yes No Diabetes Yes No Previous TB Yes No Current TB Yes No Pacemaker In Yes No Length of HIV Infection CD4 Count	
	SECTION 3 : CURRENTLY	
3.1	**Proteinuria Yes No**	
	UPCR :	
3.2	CD4 Count : Viral Load:	
3.3	Creatinine:	
3.4	GFR:	
	SECTION 4 : DRUG HISTORY	
	Are you taking ARVs? Yes No	
4.1	Tenofovir Yes No	
4.2	Rifampicin Yes No	

4.3	Cotrimoxazole	Yes	No		
4.4	NSAIDS	Yes	No		
4.5	If Yes to any drugs above, How long have you been taking?				
	SECTION 5 : SOCIAL HISTORY				
5.1	Smoking	Yes	No	Pack hx	
5.2	Ethanol Use	Yes	No		
5.3	Recreational Drugs	Yes	No		
5.31	**If Yes to drugs:**				
	Tik	Yes	No		
	Cocaine	Yes	No		
	Heroin	Yes	No		
	Mandrax	Yes	No		

PATIENT INFORMED CONSENT:

The utility of using magnetic resonance or ultrasound imaging compared to renal biopsy in HIV+ve patients undergoing kidney biopsy

INTRODUCTION

You are invited to take part in this research protocol. Your doctor has already decided that you need to undergo a kidney biopsy to determine the cause of your HIV related kidney problems. The consent for this procedure is separate from the research protocol. Information will be given to you that will help you to decide whether you would like be part of the research or not. This informed consent might contain words or procedures that you do not understand so it is important to ask questions or discuss it with your family before you agree. Your participation is completely voluntary and you future health care will not be compromised by your decision not to participate.

REASONS FOR THE STUDY

HIV+ve patients frequently develop kidney problems and need to undergo a kidney biopsy. This procedure, though generally safe, is associated with complications like bleeding from the kidney. In addition, it requires the services of several specialists to perform and interpret. Magnetic resonance imaging (MRI) of the kidneys has recently been developed and can be safely performed in patients with kidney problems. It is like a special X-ray and there are no risks associated with the procedure. The results of the MRI will be correlated with the kidney biopsy to determine cause and prognosis of your kidney problem. For future patients we may be able to avoid doing a kidney biopsy.

WHY ARE YOU BEING ASKED TO TAKE PART IN THE STUDY?

It has already been decided by a specialist that you require a kidney biopsy to determine the cause and prognosis of your HIV related kidney disease.

WHAT IS THE DURATION OF THE STUDY AND WHAT PROCEDURES WILL BE PERFORMED?

If you agree to be a participant, you will undergo a MRI procedure on the MRI research unit at the University of Cape Town. This is a safe and non-invasive procedure similar to an X-ray but lasts about 20 minutes.

AM I GOING TO BE INCONVENIENCED IN ANY WAY DURING THE STUDY?

There will be minimal inconvenience. You will need to lie still on the MRI machine for about 20 minutes. It is possible you may feel claustrophobic or closed in. Your hospital stay will not be prolonged and there will be no cost to you.

WHAT WILL HAPPEN IF YOU DECIDE NOT TO BE INVOLVED IN THE STUDY?

Your participation is completely voluntary and if you decide not to participate your future health care will not be affected.

ARE THERE ANY RISKS OR DISCOMFORT EXPECTED IN THE STUDY?

.

There are no risks and there will be the discomfort of lying in the MRI machine. If you suffer from claustrophobia, it may not be a good idea to participate. If you have a pacemaker, you must not participate.

ARE THERE ANY BENEFITS FOR YOU DURING AND AFTER THE STUDY?

.

There will be no benefits to you, but your participation may help future patients.

HAS THE STUDY RECEIVED ANY ETHICAL APPROVAL?

The Research Ethics Committee of the University of Cape Town (UCT).

DO I HAVE ANY RESTRICTIONS OR WARNINGS ON MY ACTIVITY?

No

INSURANCE AND FINANCIAL ARRANGEMENTS

Study doctors are covered by insurance. UCT has a no-fault insurance policy for trial related injuries which states:

"UCT undertakes that in the event of you suffering any significant deterioration in health or well-being, or from any unexpected sensitivity or toxicity, that is caused by your participation in the study, it will provide immediate medical care. UCT has appropriate insurance cover to provide prompt payment of compensation for any trial-related injury according to the guidelines outlined by the Association of the British Pharmaceutical Industry, ABPI 1991. Broadly speaking, the ABPI guidelines recommend that the insured company (UCT), without legal commitment, should compensate you without you having to prove that UCT is at fault. An injury is considered trial-related if, and to the extent that, it is caused by study activities. You must notify the study doctor immediately of any side effects and/or injuries during the trial, whether they are research-related or other related complications.

UCT reserves the right not to provide compensation if, and to the extent that, your injury came about because you chose not to follow the instructions that you were given while you were taking part in the study. Your right in law to claim compensation for injury where you prove negligence is not affected. Copies of these guidelines are available on request."

SOURCES OF ANY ADDITIONAL INFORMATION

Professor Brian Rayner, Dr Nicola Wearne and Professor Ntobeko Ntusi are supervising the study. Do not hesitate to contact us for additional information on (021) 404 3318.

CONFIDENTIALITY

Confidentiality of patient's records will be paramount. All files will be locked away and kept private. Patients will be allocated a code (study number). Investigators involved in the study will be the only ones aware of the link between the hospital records and the study code. We will not in way share information about you except to those involved in the research. The information recorded will be analysed, combined and published anonymously in scientific journals. The Research Committee of University of Cape Town may review the results as a regulatory body.

A clinical protocol of the research was submitted and approved by an ethics committee which is registered by the National Health Research Ethics Council and the University of Cape Town Research Ethics Committee. The research structure ethical principles are in accordance with the World Medical Association Declaration of Helsinki 2013 and Guidelines on Clinical Trials and Ethics in Health Research published by Department of Health.

CERTIFICATE OF CONSENT

I have read the information letter, or it has been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to be a participant in this study.

Print Name of Participant _____

Signature of Participant _____

Date _____

If illiterate

I have witnessed the accurate reading of the consent form to the potential participant, and the individual has had the opportunity to ask questions. I confirm that the individual has given consent freely.

Print name of witness _____ **Thumb print of witness** _____

Signature of witness _____

Date _____

Statement by the researcher/person taking consent.

I have accurately read out the information sheet to the potential participant, and to the best of my ability made sure that the participant understands the study that is going to be done. I confirm that the participant was given an opportunity to ask questions about the study. All questions asked by the participant have been answered correctly and to the best of my ability. I confirm that the individual has not been coerced into giving consent and the consent has been given freely and voluntarily.

A copy of the informed consent has been provided to the participant.

Print Name of Researcher/ person taking the consent _____

Signature of Researcher/ person taking the consent _____

Date_____

Day/ Month/ Year

NB: PLEASE KEEP THIS FORM ON A SAFE AND SECURE PLACE

Western Cape Government

GROOTE SCHUUR HOSPITAL
Enquiries: Dr Bernadette Eick
E-mail : Bernadette.Eick@westerncape.gov.za

Prof Ntusi and Prof Rayner
Department of Medicine

E-mail: ntobeko.ntusi@uct.ac.za / brian.rayner@uct.ac.za

Dear Prof Ntusi and Prof Rayner

RESEARCH PROJECT: The utility of using magnetic resonance or ultrasound imaging compared to renal biopsy in HIV positive patients undergoing renal biopsy.

Your recent letter to the hospital refers.

You are granted permission to proceed with your research, which is valid until **30 June 2019.**

Please note the following:

a) Your research may not interfere with normal patient care.
b) Hospital staff may not be asked to assist with the research.
c) No additional costs to the hospital should be incurred i.e. Lab, consumables or stationary.
d) **No patient folders may be removed from the premises or be inaccessible.**
e) Please provide the research assistant/field worker with a copy of this letter as verification of approval.
f) Confidentiality must be maintained at all times.
g) **Should you at any time require photographs of your subjects, please obtain the necessary indemnity forms from our Public Relations Office (E45 OMB or ext. 2187/2188).**
h) Should you require additional research time beyond the stipulated expiry date, please apply for an extension.
i) Please discuss the study with the HOD before commencing.
j) Please introduce yourself to the person in charge of an area before commencing.
k) On completion of your research, please forward any recommendations/findings that can be beneficial to use to take further action that may inform redevelopment of future policy / review guidelines.
l) **Kindly submit a copy of the publication or report to this office on completion of the research.**

I would like to wish you every success with the project.

Yours sincerely

DR BERNADETTE EICK
CHIEF OPERATIONAL OFFICER
Date: 24/04/2019

C.C. Mr. L. Naidoo
Dr Aziz

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Table 6: Supplementary full baseline characteristics for all participants

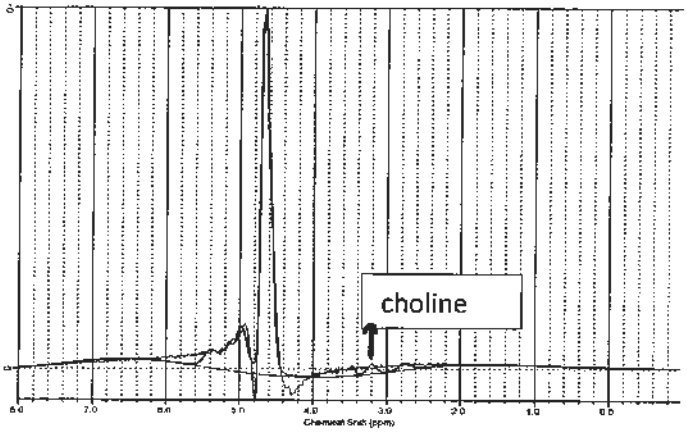
Study	*Age (yrs)*	*Ethnicity*	*Sex*	*BMI (kg/m²)*	*HPT*	*Diabetes*	*Active TB*	*ART*	*Cd4 count (cells/μL)*	*Creat (μmol/l)*	*Kidney Sizes Right /Left (cm)*
1	38	Mixed race	Female	36,26	Yes	Yes	No	No	540	118	13.6 / 13.7
2	34	Black	Male	19,88	No	No	No	Yes	170	1227	13.5 / 13.1
3	34	Black	Male	19,00	No	No	Yes	Yes	46	1130	13.2 / 12.7
4	32	Black	Male	24,99	Yes	No	No	Yes	401	153	14.0 / 14.0
5	41	Black	Male	22,89	No	No	No	Yes	101	1708	11.3 / 12.0
6	28	Black	Female	17,71	No	No	No	Yes	12	913	12.8 / 12.4
7	33	Mixed race	Female	27,58	Yes	No	Yes	No	330	497	15.4 / 15.4
8	30	Black	Male	21,22	No	No	No	No	6	178	12.9 / 13.2
9	57	Mixed race	Female	21,30	Yes	No	No	Yes	300	1468	10.5 / 11.1
10	41	Black	Male	25,00	No	No	Yes	Yes	57	1547	14.5 / 13.3
11	33	Black	Male	21,04	No	No	Yes	Yes	42	808	12.5 / 13.3
12	42	Black	Male	31,13	Yes	No	No	Yes	99	180	12.0 / 12.5
13	34	Black	Male	21,37	No	No	Yes	No	74	1663	12.0 / 12.5
14	40	Black	Male	19,37	Yes	No	No	No	65	283	13.9 / 13.3
15	35	Mixed race	Male	16,83	Yes	No	No	No	351	581	10.1 / 9.5
16	40	Black	Female	14,32	No	No	Yes	Yes	51	213	12.3 / 13.0

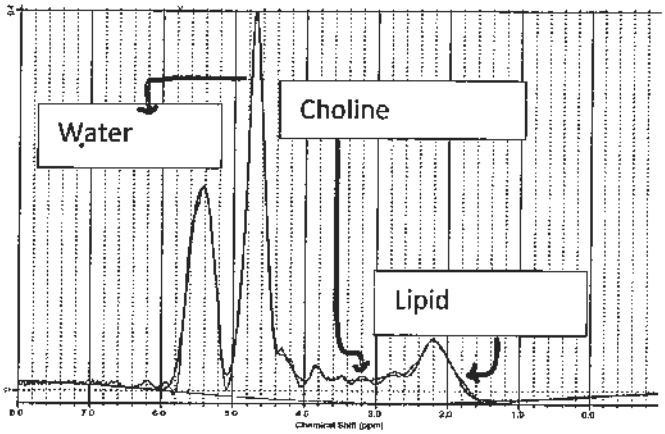
Key: BMI; Body mass index, HPT; Hypertension, TB; Tuberculosis, ART; Antiretroviral therapy, Creat,; Creatinine

Spectroscopy graphs as seen in different kidney biopsy histologies

A) Hypertension

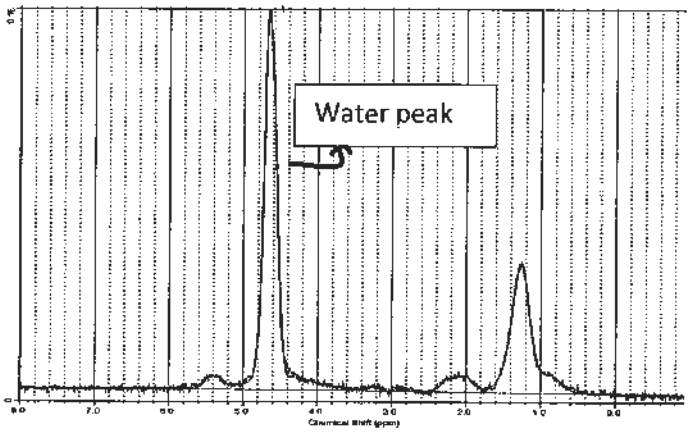
(figure 7)





(figure 8)

(figure 9)



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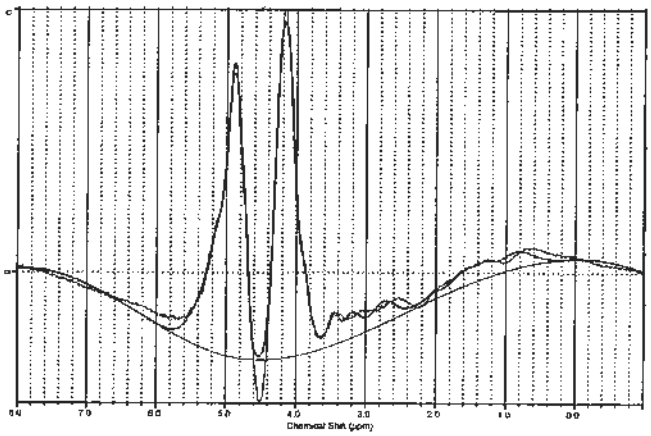

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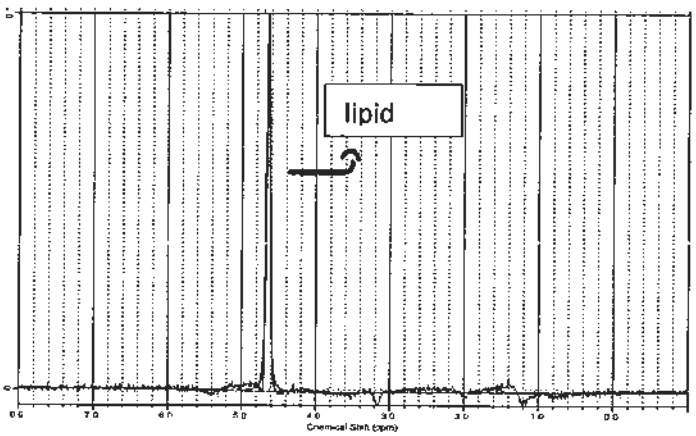
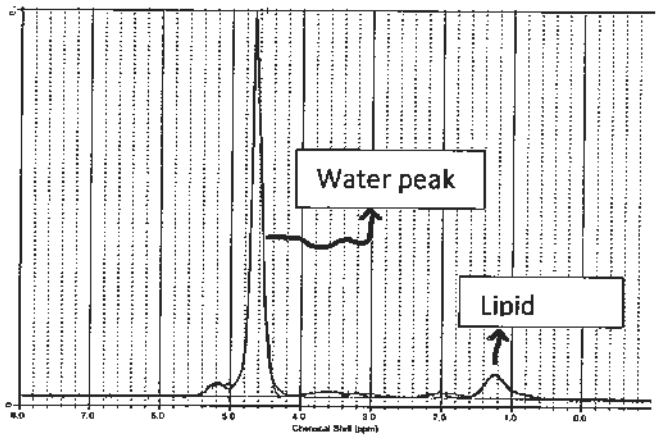


B) Granulomatous Interstitial Nephritis

(figure 12)



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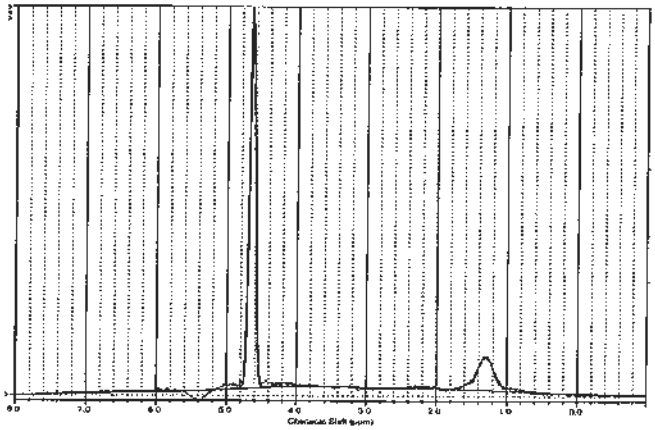

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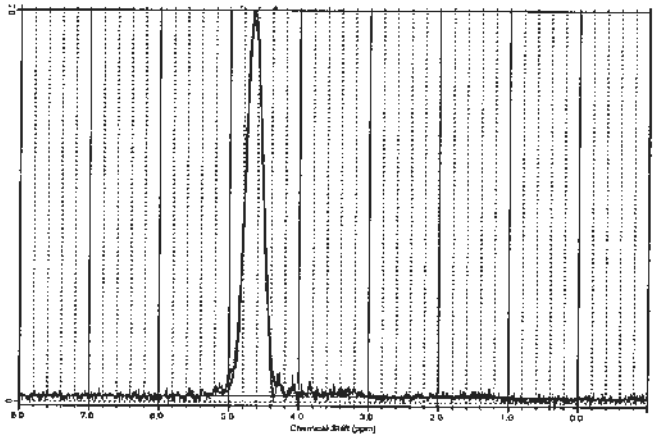
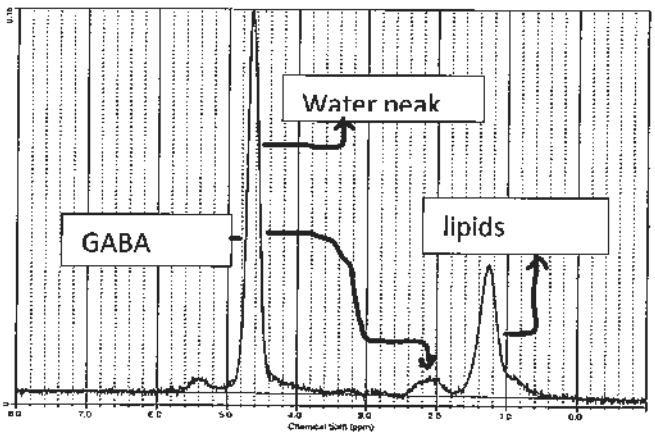
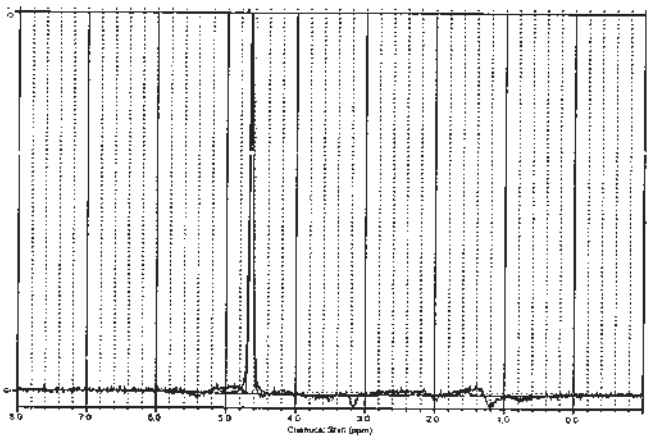


(figure 15)

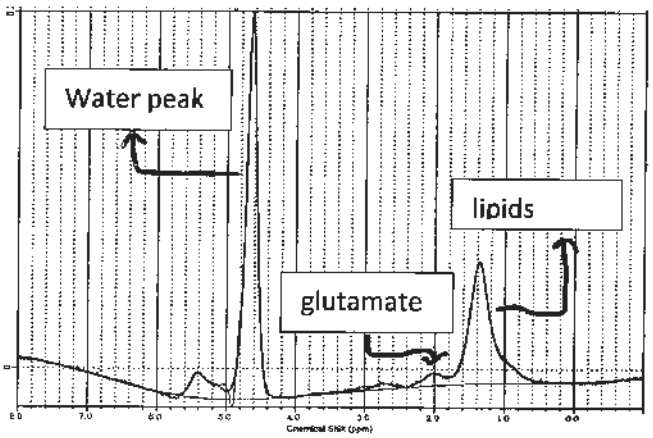


(figure 16)

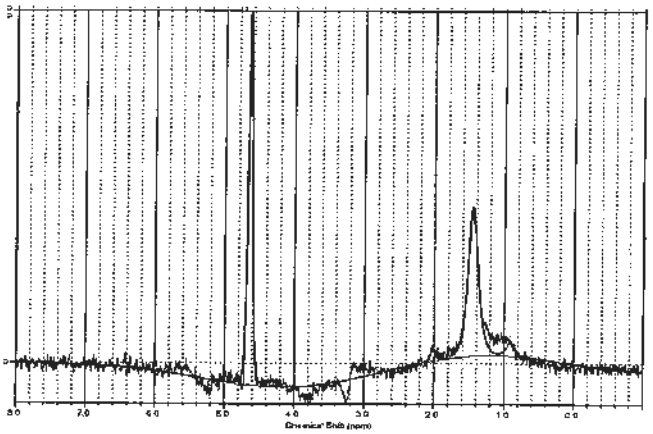


C) HIVAN

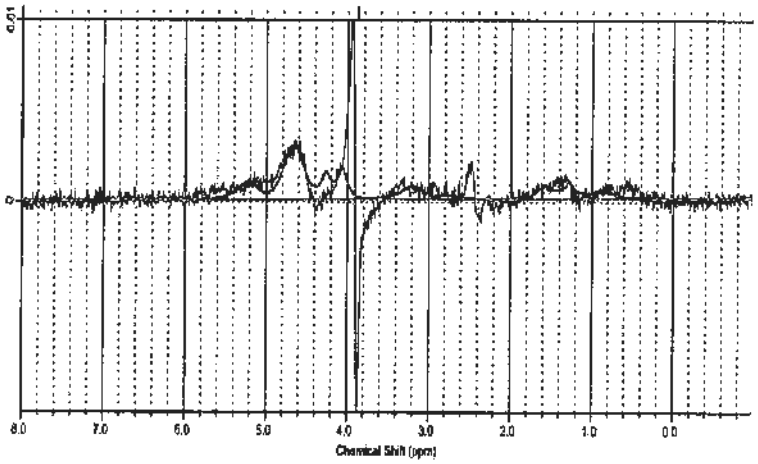
(figure17)



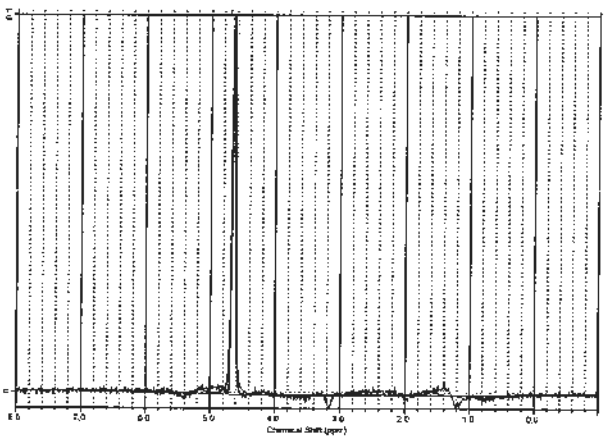
(figure18)



(figure 19)

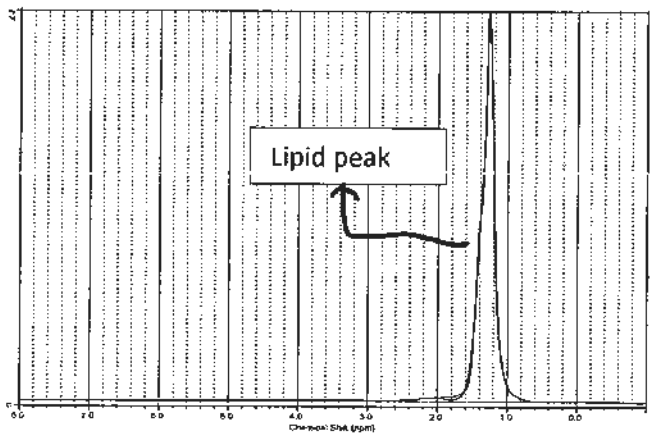


(figure 20)

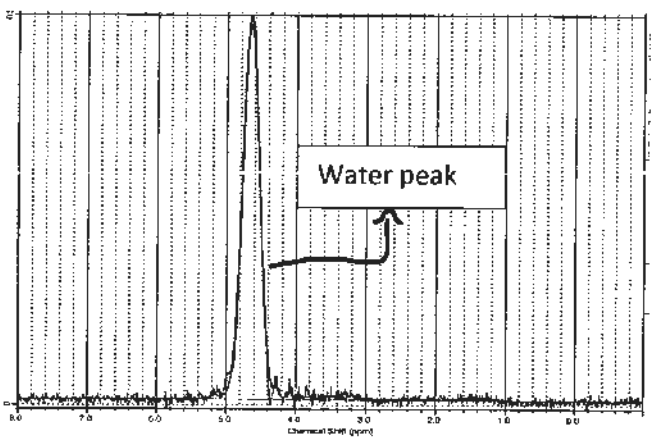


D) Acute Tubular Necrosis/ Pyelonephritis

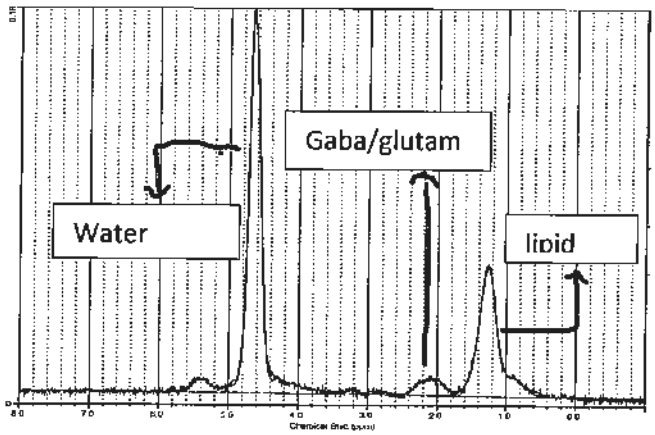
(figure 21)



(figure 22)

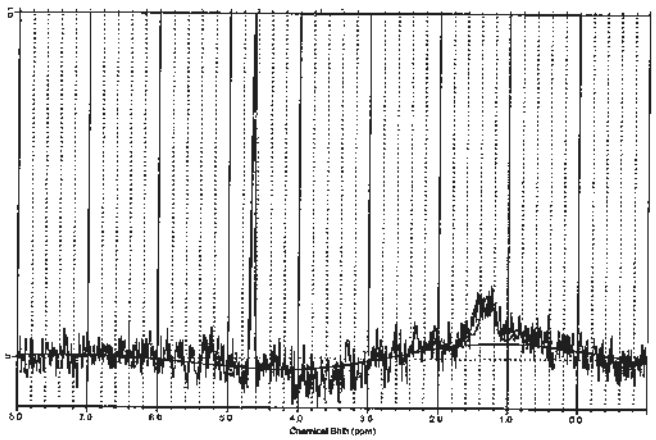


(Figure 23)

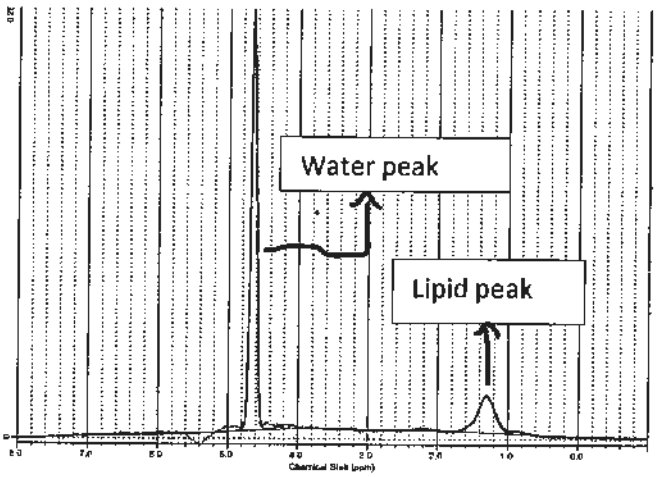


E) Post Infectious Glomerulonephritis

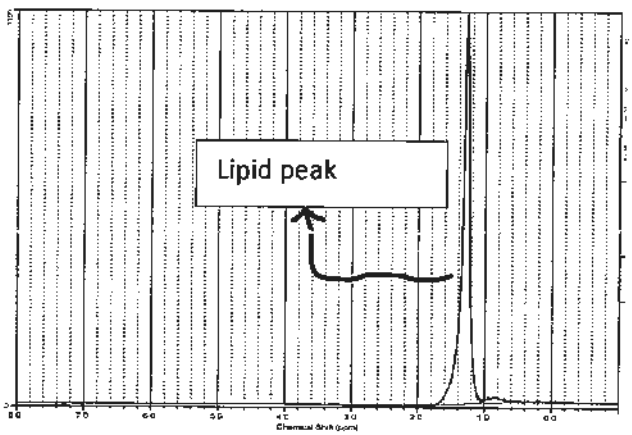
(figure24)



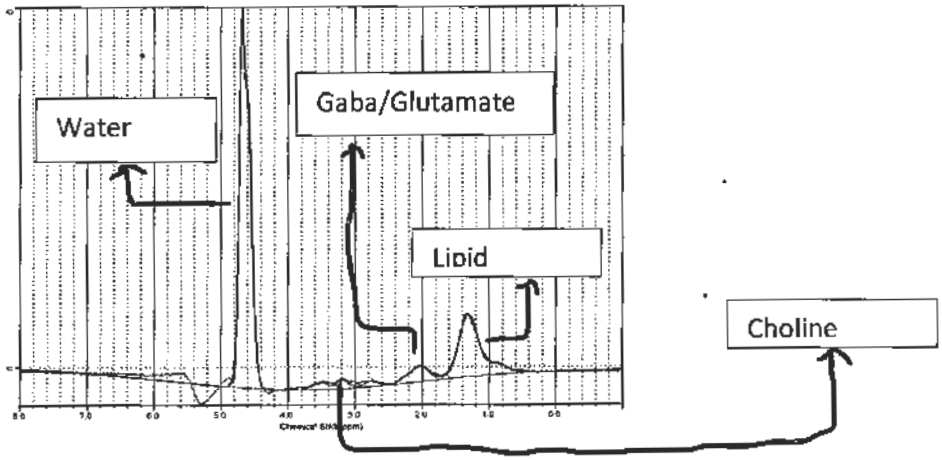
(figure 25)



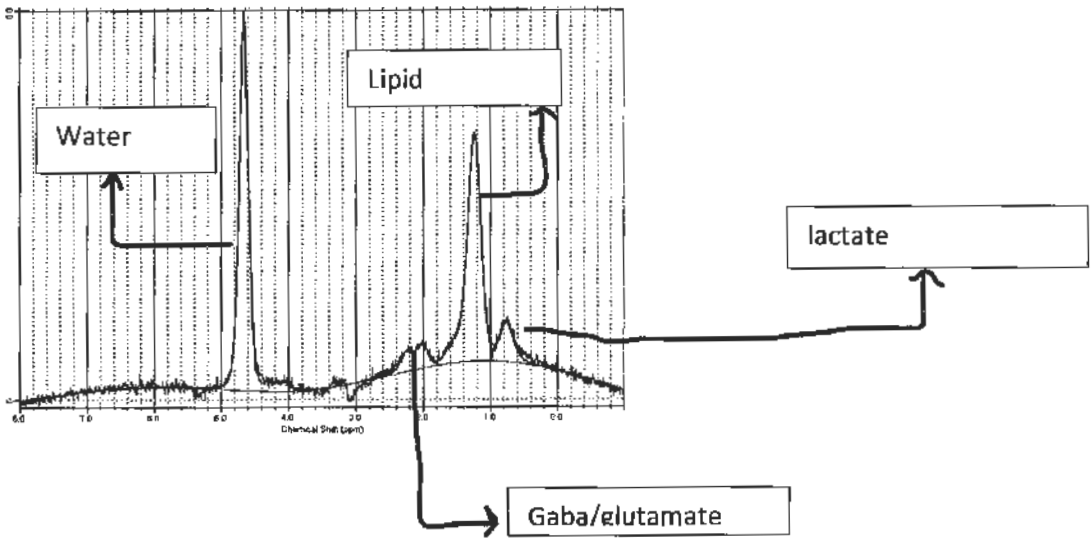
F) *Mesangial Capillary Glomerulonephritis,* (figure 26)



G) *Mild Interstitial Fibrosis* - (figure 27)



H) *Pauci-immune Crescentic nephritis* - (figure 28)



I) Light chain disease – (figure 29)