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# Effect of antiretroviral drugs on the renal function of HIV seropositive patients attending ISS clinic of Mulago Hospital, Uganda

John N Kateregga<sup>1</sup>; Collins Atuheire<sup>1</sup>; Justine Nalunga<sup>1</sup>; Ndukui G James<sup>1,2\*</sup>

<sup>1</sup>College of Veterinary Medicine, Animal Resources and Biosecurity, Makerere University, Uganda

<sup>2</sup>Department of Medical Laboratory Sciences, Jomo Kenyatta University of Science and Technology, Kenya

\*Corresponding Author(s): **Ndukui G James**

Department of Medical Laboratory Sciences, Jomo Kenyatta University of Science and Technology, Kenya  
 Email: ndukuiga@gmail.com

## Abstract

**Background:** Highly Active Antiretroviral Therapy (HAART) consisting of at least three active drugs against HIV infection has improved HIV/AIDS management since their inception. However, alterations of lipid metabolism due to HAART can result into secondary diabetic and hypertensive renal damage. This study was carried out to assess the effect of antiretroviral drugs on the renal function of HIV seropositive patients by determining the serum levels of Creatinine, Urea and Electrolytes (Na, K, and Cl) of the patients on treatment.

**Methods:** This was a cross sectional study carried out from January to May 2015. In this study, 109 HIV infected patients on HAART attending ISS clinic in Mulago Hospital in Kampala were evaluated for kidney damage. Patients' age, gender and drug combinations were obtained using data collection forms. Venous blood samples were collected and analyzed for serum Creatinine, Urea and Electrolytes levels using the COBAS Integra 6000 chemistry analyzer. Levels of these parameters before initiation of HAART treatment were obtained from the clinical records. The Wilcoxon-Sign test was used to compare biochemical levels before and after HAART initiation since the data was not normally distributed.

**Results:** The Creatinine levels increased slightly after drug initiation in sera of patients on TDF+3TC+LPV/r ( $p=0.06$ ), ABC+3TC+NVP ( $p=0.5$ ) and TDF+3TC+EFV ( $p=0.54$ ) drug combinations but this increase was not statistically significant. The serum levels of urea were generally not indicative of kidney damage since they significantly decreased compared to pre-HAART initiation levels. This is due to the fact that urea levels are affected by a number of factors including diet, state of dehydration and age. Similarly, the Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> serum levels were not indicative of kidney damage.

This study has established that the effect of ARVs on the renal function of HIV seropositive patients was minimal. A more comprehensive study covering the risk factors that may accelerate any effect on the kidney by HAART is recommended.

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**Keywords:** HAART; Renal function; Creatinine; Urea; Electrolytes

**Abbreviations:** AIDS: Acquired Immune Deficiency Syndrome; AKI: Acute Kidney Injury; ART: Antiretroviral Therapy; ARVs: Antiretroviral Drugs; CD4: Cluster of Differentiation 4; CD8: Cluster of Differentiation 8; GFR: Glomerular Filtrate Rate; HAART: Highly Active Antiretroviral Treatment; HIV: Human Immunodeficiency Virus; NNRTI: Non-Nucleoside Reverse Transcriptase Inhibitors; NRTI: Nucleos(t)ide Reverse Transcriptase Inhibitor; NSAIDs: Non-Steroidal Anti-Inflammatory Drugs; PI: Proteinase Inhibitor.

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## Background

Almost 75 million people have the HIV virus infection and about 36 million people have died of HIV [1]. The burden of the epidemic varies considerably between countries and regions although Sub-Saharan Africa remains most severely affected. It has nearly 1 in every 20 adults living with the virus and accounts for 71% of the world wide HIV infection [1]. In Uganda, HIV has become more than an issue with a prevalence of 7.4% and 190,000 deaths due to AIDS [2]. HIV is a retrovirus that infects immune system cells hence destroying and impairing their function. It causes AIDS which develops during the last stages of the infection. The immune system becomes weaker and weaker as the infection progresses and the person becomes more susceptible to infections [3].

Anti-Retroviral drugs (ARVs) are medicines that prevent the reproduction of retroviruses like the Human Immunodeficiency Virus (HIV) which causes AIDS. ARVs do not kill viruses because that could damage the cells infected by the viruses. Instead these drugs block steps in the process of viral reproduction hence slowing down damage to the immune system [4]. Standard Antiretroviral Therapy (ART) suppresses the HIV virus replication and stops the progression of the HIV disease (WHO, 2013). AIDS has become a chronic, manageable disease because of the antiretroviral therapy hence individuals can survive with the virus close to normal life. It is recommended that the first line regimen for adults and adolescents contain two NRTIs plus one NNRTI. The recommended combinations are Zidovudine/Lamivudine (ZDV/3TC) or Tenofovir/Lamivudine (TDF/3TC) plus Niverapine (NVP) or Efavirenz/ EFZ [4]. A triple NRTI regimen is considered as an alternative for first line ART when NNRTI options have more complications and also to preserve the PI class for second line treatment for example in pregnant women with very low CD4 counts say, 250-350 cells/mm<sup>3</sup>. The recommended triple NRTI combinations are Zidovudine + Lamivudine + Abacavir and Zidovudine + Lamivudine + Tenofovir [4].

The entire regimen is recommended for change if treatment failure occurs. The first line choice determines the second line choice. The second line treatment consists of a proteinase inhibitor supported by two new NRTIs. These can be Abacavir/Didanosine (ABC/ddI) or TDF+3TC (or FTC) plus Lopinavir + ritonavir (LPV/r), alternatively ZDV/ ddI or ABC/ ddI or ZDV/3TC\* plus LPV/r alternatively ABC/ddI or TDF/3TC or FTC plus LPV/r [4]. Indinavir, a protease inhibitor has been associated with renal effects. Ritonavir has been associated with renal tubular damage. Also Lamivudine and didanosine are associated with tubular dysfunction [5]. The renal system comprises of the kidneys. It is important in excretion of the products of metabolism, elimination of toxins, and other foreign substances such as drugs and food additives. It controls the composition and volume of the body fluids. The regulatory function of the kidneys maintains the stable environment of the cells necessary for them to perform the various activities. The kidneys function by filtering the plasma and removing substances from the filtrate at variable rates according to the needs of the body [6].

The alterations of lipid metabolism due to HAART can result into an increased prevalence of diabetes which results into hypertension and hence to secondary diabetic and hypertensive renal damage. Since the introduction of HAART, many renal side effects have been observed which includes development of proteinuria to acute renal failure [5]. Patients' renal functions are evaluated before initiation of HAART treatment and can be monitored by measuring the patients' serum Creatinine levels,

Urea levels and Electrolytes levels [7,8].

Highly Active Antiretroviral Therapy (HAART) consisting of at least three drugs active against HIV infection has improved HIV/AIDS management. This is observed in the mortality and morbidity reductions worldwide. However, its use has been associated with a variety of toxicities, including those affecting the kidney. The kidney plays a key role in the metabolism and excretion of antiretroviral drugs. This makes it vulnerable to various injuries including Acute Kidney Injury (AKI), Chronic Kidney Disease (CKD), tubulopathies and end stage renal disease. As the population of HIV infected patients on HAART increases, metabolic disorders associated with age, HIV and HAART are being encountered by clinicians looking after these patients [9].

Antiretroviral therapy can contribute to renal dysfunction by inducing acute tubular necrosis, acute interstitial nephritis, crystal nephropathy and renal tubular disorders [9]. While ARVs are very useful in treatment of HIV/AIDS, they cause damage to essential organs like the kidney. Not many studies have been conducted on their renal effects in Ugandan patients though some have been done elsewhere [10-12]. The current study screened the values of parameters used to assess the level of kidney damage in the serum of HIV/AIDS patients under HAART treatment in the main referral hospital (Mulago) in Uganda.

## Methods and materials

### Study design

This cross-sectional study was carried out to determine the effects of ARVs on the renal function of HIV/AIDS seropositive patients on treatment. Serum levels of Creatinine, Urea, Sodium (Na<sup>+</sup>), Potassium (K<sup>+</sup>) and Chloride (Cl<sup>-</sup>) were measured and compared with WHO recommended levels. The pre-initiation and post initiation levels of these parameters were statistically evaluated for any significant differences at p = 0.05.

### Data and sample collection

The study was conducted at ISS clinic of Mulago Hospital, Uganda. HIV/AIDS seropositive patients of 15 years and above on ART were the population of interest. Both female and male patients were considered. The study included all HIV patients aged 15 years and above on treatment and had consented to participate in the study. HIV positive patients below 15 years were excluded. Those who were not on treatment were excluded. Even those patients who disregarded the consent were excluded. In addition patients with Diabetes, Hypertension and on antibiotics were excluded from the study. Using a sample size formula by Kish [13], for cross-sectional studies, the sample size was estimated to be 55 patients; basing on results of a study in Ghana that indicated that renal disease prevalence in HIV infected patients on ART-treatment is 3.7% [14]. However, 109 patients were selected to participate in the study to generate more compelling data.

Patients were selected during their routine visits weekly for this study which was conducted from January to May 2015. Patients' data (gender, age, ARV combination) was collected using data collection forms and record books. The data was then entered into the laboratory register for patients who were categorized according to HAART combinations used. The patients' serum levels of Creatinine, Urea and Electrolytes before treatment initiation were also obtained from the clinical records. Blood samples were collected intravenously from the antecubital vein on the arm by means of a simple syringe (5ml) and

needle (21-23 gauge). Vacutainer tubes without anticoagulant were labeled with the patient's name, age and laboratory number for easy identification. A tourniquet was then tied around the arm of the patient above the venipuncture site. After palpating and locating the vein, the site on the patient's arm was cleaned with an alcohol swab (70% v/v). The needle was then fit in the syringe and then venipuncture performed with about 4ml of blood withdrawn into a red vacutainer tube. The tourniquet was then removed and using cotton, pressure was applied a little to stop bleeding at venipuncture site.

The samples were then transported to the clinical chemistry laboratory which was 10 meters next to ISS clinic, within an ice box. Since not all the samples were worked on then, some were centrifuged and the serum preserved within cryovials in a refrigerator at 2-8°C. The samples were centrifuged at 13,000 rpm for five minutes and the serum separated from the blood cells carefully. The serum was analyzed and the blood cells discarded. All samples were considered infectious and handled in a way that respects the patient's privacy. Safety measures were taken by wearing appropriate personal protective equipment like gloves, laboratory coat, and closed shoes.

#### Determination of serum urea, creatinine and electrolyte levels

The serum samples were analyzed after calibrating the machine/ analyzer, running controls and standards, adding the right measurements of reagent volumes, centrifugation of samples at the right speed and time. This was done by following standard operating procedures. The analysis was done using the Cobas Integra 6000 Chemistry Analyzer. Initially, orders were created by entering the sequence number, patient's name and selecting the tests to be done into a computer connected to the analyzer. Next, 600 µl of blood sample were pipetted into the sample cups and loaded onto an appropriate rack. The racks with samples were then loaded onto a rack tray and the rack tray placed onto the loading panel of the Cobas 6000 chemistry analyzer. Then the machine was started. Samples were transferred automatically from the sample cup to the module. Serum Creatinine, serum Urea, K, Na, and Cl levels (mmol/L) were then determined and compared to the WHO recommended reference ranges for a valid conclusion. The machine/analyzer operated on the principle of spectrophotometry and calorimetry.

#### Quality control

The analytes were determined following a sequential flow of pre-analytical procedures, analytical procedures and post-analytical procedures. The pre-analytical stage involved; collecting the right samples, avoiding hemolysis, using the right sample containers and labeling with the correct patient information. The analytical phase involved analysis of the test samples. The post-analytical stage involved correct results interpretations and troubleshooting in case of variation in results. This phase also involved filing of patient results at the clinic for their doctors to review. Quality control was ensured by right pipetting, using sterile sample cups for every sample, centrifuging at the right speed and time and preserving serum at the right temperatures.

#### Data analysis

Socio-demographic, serum biochemical and electrolyte data were entered into Microsoft Excel and imported into the Statistical Package for Social Scientists (SPSS) software, version 17.0. The Wilcoxon-Signed rank test was used to determine if there were significant differences (at  $p < 0.05$ ) in levels of the analytes

for the different HAART combinations before initiation of HAART and afterwards.

#### Ethical considerations

The study protocol was approved by the Ethics Review Committee of the School of Biomedical Sciences of Makerere University College of Health Sciences. Permission was sought from the Hospital and Laboratory authorities. The study Participants were given full and adequate oral and written information about the nature, purpose, possible risks, and benefits of the study. The participants were given adequate opportunity to ask questions and allowed time to consider the information provided. The participants signed and dated informed consent that was obtained before conducting this study. The study data were stored in a computer database while maintaining confidentiality. The Patients in this database were identified by the unique enrolment number only. The ethical principles of scientific research as well as related national laws and regulations were strictly adhered to.

#### Results

##### Socio-demographic characteristics of the study subjects

One hundred and nine (109) patients on ART were enrolled for the study and they were categorized according to sex, age and drug combination. The proportion of females was 72.5% and that of males was 27.5% hence the study included more females than males. Furthermore, 19.3% of the patients were aged between 20 and 30 years, 31.2% of the patients were aged between 31 and 41 years. On the other hand, 37.6% of the patients were aged between 42 and 52 years and 11.9% of the patients were greater than 52 years of age as shown in Table 1.

The highest proportion of patients were on ZDV+3TC+NVP drug combination and that is, 46.8% with a higher female proportion of 74.5% as compared to 24.5% of males. ABC+3TC+NVP drug combination had the lowest proportion of patients (Table 2).

##### Creatinine, urea and electrolytes levels

Basing on the WHO approved reference values of 44 to 106mmol/L for serum Creatinine, 5.5% of the patients had high Creatinine levels. All patients (100%) had normal potassium levels considering 3.5 to 5.5mmol/L as the reference values. Considering reference ranges of 2.7 to 6.4 mmol/L levels for urea levels, 26.6% of the patients had low levels and 0.9% of the patients had high urea levels. Considering reference ranges of 135 to 150 mmol/L for Sodium, 3.7% of the patients had low sodium levels. Considering reference ranges of 95 to 110 mmol/L, 0.9% of the patients had high chloride levels as shown in Figure 1.

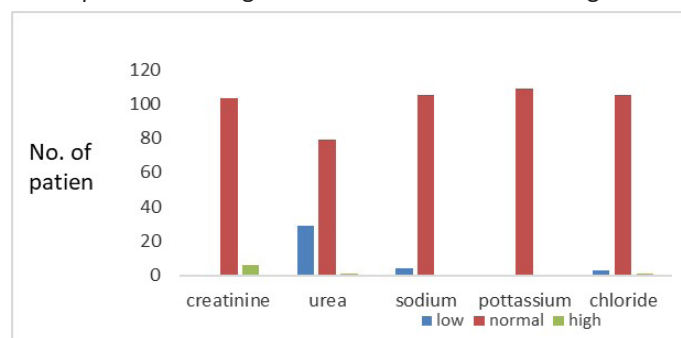


Figure 1: Distribution of Creatinine, Urea and Electrolyte levels in the serum of the patients.

There was an increase in Creatinine levels after HAART initiation compared to the pre-HAART initiation levels but this increase was not statistically significant for patients on TDF+3TC+LPV/r drug combination (p=0.06; Wilcoxon-Sign test), ABC+ 3TC+NVP (p=0.5) and TDF+3TC+EFV (p=0.54). There was a decrease in urea levels for patients on ZDV+3TC+NVP, ZDV+3TC+EFV, TDF+3TC+EFV and TDF+3TC+NVP at post HAART compared to pre HAART and it was significant (p<0.05) as shown in Table 3.

The serum levels for all electrolytes post-HAART initiation were not significantly different compared to the pre-HAART initiation serum levels. This was the case for all drug combinations (p>0.05) except for the serum Sodium levels of those patients on ZDV+3TC+NVP drug combination where the decrease was significant (p=0.007) as shown in Tables 4 and 5.

**Table 1:** Socio-demographic characteristics of study participants.

Parameter		Frequency	Percentage
Gender	Females	79	72.5
	Males	30	27.5
	<b>Total</b>	<b>109</b>	<b>100%</b>
Age	20-30	21	19.3
	31-41	34	31.2
	42-52	41	37.6
	>52	13	11.9
	<b>Total</b>	<b>109</b>	<b>100%</b>

**Table 2:** Gender distribution of study participants according to drug combination.

Parameter		Frequency		Total	Percentage
		Females	Males		
Drug combinations	ZDV+3TC+NVP	38	13	51	46.8
	ZDV+3TC+EFV	11	3	14	12.8
	TDF+3TC+EFV	17	7	24	22.0
	TDF+3TC+NVP	11	4	15	13.8
	TDF+3TC+LPV/r	1	3	4	3.7
	ABC+3TC+NVP	1	0	1	0.9
<b>Total</b>		<b>79</b>	<b>30</b>	<b>109</b>	<b>100</b>

**Table 3:** Serum biochemical values of participants on different drug combinations.

Median (Inter-quartile range) mmol/l						
Combination	Creatinine level			Urea level		
	Before treatment	After treatment	P value	Before treatment	After treatment	P value
ZDV+3TC+NVP	79 (60-94)	77 (72-84)	0.39	4.8 (4.2-5.7)	3.2 (2.6-3.7)	<0.001
ZDV+3TC+EFV	71.5 (64-95)	70.5(67-77)	0.79	5.3(4.6-6.1)	3.75(2.2-4.1)	0.01
TDF+3TC+EFV	71 (58-82.5)	79(69.5-90.0)	0.54	5.35(4.5-5.8)	2.8(2.45-4.00)	<0.001
TDF+3TC+NVP	78 (68-91)	75(66-90)	1.00	4.7 (2.9-6.1)	3.2(2.6-4.1)	0.001
TDF+3TC+LPV/r	67(48.5-95.0)	96(90.5-107.5)	0.06	4.7(3.25-3.75)	3.15(3.05-3.45)	0.63
ABC+3TC+NVP	54	72	0.5	3	3.5	1.0

All data presented as median (interquartile range). P-value <0.05 were considered significant.

**Table 4:** Serum Na<sup>+</sup> and K<sup>+</sup> levels of study participants.

Combination	Median (Inter-quartile range) mmol/l					
	Sodium			Potassium		
	Before	After	P value	Before	After	P value
ZDV+3TC+NVP	144 (138-148)	140 (138-141)	0.007	4.1 (3.8-5.1)	4.2 (4.12-4.51)	0.45
ZDV+3TC+EFV	145 (139-147)	140 (138-142)	0.10	4.25 (3.7-4.6)	4.21 (4.12-4.45)	1.00
TDF+3TC+EFV	141.5 (137.5-148.5)	139.5 (137.5-141)	0.54	4.45 (3.9-5.25)	4.39 (4.16-4.69)	0.84
TDF+3TC+NVP	145 (135-149)	141 (138-142)	0.30	4.2 (4.0-4.8)	4.56 (4.15-4.7)	1.00
TDF+3TC+LPV/r	142.5 (139.5-147.5)	138 (137-138.5)	1.00	4.25 (3.75-5.10)	4.47 (4.40-4.61)	1.00
ABC+3TC+NVP	136	146	0.13	3.9	4.42	1.00

All data presented as median (interquartile range). P value <0.05 were considered significant.

**Table 5:** Serum Cl<sup>-</sup> levels of study participants.

Drug combinations	Median (Inter-quartile range) mmol/l		
	Before treatment	After treatment	p-value
ZDV+3TC+NVP	101 (97-105)	100 (98.8-101.7)	0.58
ZDV+3TC+EFV	103.5 (101-109)	99 (98.2-102.8)	0.58
TDF+3TC+EFV	101.5 (97-107)	102.35 (100.2-103.3)	1.00
TDF+3TC+NVP	103 (99-105)	103.5 (99.8-105.6)	0.30
TDF+3TC+LPV/r	102 (99-105.5)	96.35 (96.35-101.35)	1.00
ABC+3TC+NVP	99	104	1.00

All data presented as median (interquartile range). P values <0.05 were considered significant.

## Discussion

The current study was designed to assess the renal functionality of HIV seropositive patients on treatment by determining the change in serum levels of Creatinine, Urea and Electrolytes after HAART initiation. There was a higher proportion of females, 72.5% recruited into the study compared to males 27.5%. This could be due to a higher prevalence of HIV among females compared to males in Uganda as studies indicate across all regions of the country except in West Nile [15]. Other studies covering sub-Saharan Africa show that women account for more than half the total number of people living with HIV [16]. It could also be due to the finding that women are more likely to seek medical help compared to men [17]. The fact that women are more infected with HIV could probably be as a result of some women being unaware of their male partner's behaviors such as injection-drug use or having sex with other men as these increase the risk for HIV. Women may also be afraid of being left by their partners or even being physically abused if they talk about condom use [18].

Assessment of renal dysfunction can be accomplished by determination of serum Creatinine, Urea, and Electrolyte (Sodium, Potassium and Chloride) levels. This is because these parameters are filtered from the extracellular fluid by the kidney and excreted in urine. Their excretion is relatively constant in the absence of disease. Creatinine is recommended in preference to the measurement of serum Urea because it is a better indicator of over-all renal function (Glomerular filtration rate, GFR) since its levels are less affected than urea levels by age, diet and dehydration [19]. In the present study, most of the serum biochemi-

cal parameter levels did not differ significantly before and after drug initiation. This is in line with a study conducted in Nigeria [14]. Obirikorang and colleagues established that all serum biochemical parameters did not differ significantly between HIV patients on HAART and those not on HAART treatments.

The kidney is one of the reservoir organs of HIV hence the virus can exert a direct pathogenic activity on it [20]. The normal serum biochemical values of patients in this study at baseline could be attributed to samples being taken at an early stage of infection before the virus has caused any significant damage to the kidneys [21]. The largely normal serum biochemical levels of the patients even after HAART initiation could be attributed to the fact that HAART functions to bring down the viral loads and increase CD4 counts hence a decrease in the organ damage rate caused by HIV infection [21].

Furthermore, patients in this study had only slight changes in their serum Creatinine levels and were not significant. This may probably be due to the fact that renal damage by HAART drugs could be exacerbated by other factors such as diabetes mellitus, hypertension, length of HAART treatment and concurrent drug administration yet these were not part of this study [22]. It is also shown that diabetic patients exhibit greater electrolyte disturbances than people on HAART hence diabetes may accelerate renal damage in patients on HAART [23]. Probably, most patients were neither diabetic nor hypertensive hence the minimal effect on kidney function observed in this study. Furthermore, other drugs such as aminoglycoside antibiotics can worsen HAART-initiated renal damage. However, all these con-

founding factors were not part of this study.

In this study, serum Urea levels differed significantly for the patients on ZDV+3TC+NVP, TDF+3TC+EFV, ZDV+3TC+EFV and TDF+3TC+NVP (all p values less than 0.05) drug regimens. Since urea levels were decreasing, they are not indicative of renal impairment. Urea levels are affected by a number of factors such as dehydration, diet and age hence they are inferior to serum Creatinine levels in determining kidney disease and cannot be relied upon solely.

A significant difference in serum Sodium levels was also observed for patients on ZDV+3TC+NVP drug combination ( $p=0.007$ ) in the current study. There was also a decrease in the sodium levels. Low sodium (hyponatremia) may be due to the retention of water or excessive loss of Sodium, a case when loss of salt and water (by vomiting, diarrhea or excessive sweating) is replaced by water only [19]. Since the study had a limited scope, by not considering factors like side effects of some drugs aside HAART, the decrease was not directly indicative of renal failure. Therefore, there is need to carry out a wide scope study that involves more confounding factors that could be used to ascertain the major causes of renal and/ or myocardial malfunctions on patient on HAART.

### Conclusion

The current study showed minimal effect on the renal function of HIV seropositive patients on treatment with antiretroviral drugs. A more comprehensive study taking into account the period on treatment for the patients, effect of concurrent treatment with other medicines, effects in pediatrics and risk factors like diabetes and hypertension should be considered. All these are factors that may accelerate renal damage by HAART.

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### Authors' Contributions

JNK and JN conceptualized the project, performed most of the laboratory experiments and wrote the manuscript. CA assisted in statistically analyzing the data. JGN assisted in drafting and finalizing the manuscript. All authors read and approved the final manuscript.

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