

Full Length Research Paper

# The kidney function trends in human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) patients at the Nylon District Hospital, Douala, Cameroon

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Human immunodeficiency virus (HIV) related kidney disease is one of the leading causes of death and affects predominantly people of black descent. Data is unavailable on the presence of kidney disease amongst HIV positive patients in Cameroon and a high prevalence of the disease depicts a high incidence of HIV associated nephropathy. A cohort study was carried out from May to August 2010 at the Nylon District Hospital Douala to investigate the Kidney function trends amongst seropositive individuals. Kidney function tests like serum urea, serum creatinine, creatinine clearance, proteinuria and urine chemistry was measured amongst 329 participants amongst whom 100 (30.4%) were HIV negative and 229 (69.6%) were HIV positive. The age range of the study population was 18 to 60 years, with mean age of  $35.122 \pm 0.543$ . There were 94(28.6%) males and 235 (71.5%) females. The percentage of HIV seropositivity was higher in females than in males (74.3% vs. 25.7%  $p < 0.05$ ). Although, Serum creatinine, creatinine clearance and proteinuria were significantly higher in the control group than in the HIV infected subjects ( $p < 0.0001$ ,  $p = 0.046$  and  $p = 0.001$ , respectively), these values were not indicative of renal pathology. Considering only HIV positive individuals the mean serum creatinine was significantly higher in the Antiretroviral treatment (ART) naïve group when compared to those who were already on ART. These findings indicate that renal function is not affected by the seropositivity status of individuals.

**Key words:** Kidney function, human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) seropositivity, Cameroon.

## INTRODUCTION

Human immunodeficiency virus (HIV) infection can cause a broad spectrum of clinical manifestations, ranging from an asymptomatic carrier state to severe immunodeficiency. Patients infected with HIV have been described to have an unusual form of renal disease

known as HIV- associated nephropathy (HIVAN). This condition is characterized by nephrotic range proteinuria (a urine protein-to-creatinine ratio greater than 3 or a 24 h urine protein greater than 3 g), rapid progression to renal insufficiency and a morphologic pattern of focal segmental glomerulosclerosis (FSGS) on renal biopsies (Carbone et al., 1989).

HIV 1 infects renal cells and the kidney may be an important long-term reservoir for the virus (Winston et al., 2001). Problems with kidney function in HIV infected people may be due to medications or HIV itself. Kidney disease in HIV Infected people has been associated with more advanced HIV disease, low  $CD_4^+$  cell counts, diabetes, hypertension, acute bacterial infection of the

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**Abbreviations:** Lam, Lamivudine; Stoc, stocrin; Efv, efavirenz; Duo, duovir; Nvp, nevirapine; Trio, triomune; Zi, zidovudine; Triviro-LNS, lamivudine, nevirapine and stavudine.

kidney and sepsis, smoking for over ten years (Miguez-Burbano, 2004) and can present as acute or chronic kidney disease (Roling et al., 2006).

HIVAN is now being recognized as a distinct clinico-pathological entity that presents with proteinuria in the nephrotic range and impairment of renal function. Many antiretroviral agents are eliminated at least partly by the kidneys and require dosage adjustments in patients with reduced glomerular filtration rate (GFR). Side effects due to anti-HIV drugs are many. They include hepatotoxicity and nephrotoxicity (Kamga et al., 2010; Fokunang et al., 2010). Renal disease in HIV may be caused by HIV itself or by medications. The prevalence of renal disease among HIV Infected patients has been reported to be 2 to 10% with about 3.5% from black origin (Gardner et al., 2003). HIVAN is the most common form of renal disease in HIV patients with a  $CD_4^+$  < 200 cells/ $\mu$ l. HIVAN is becoming an increasingly prevalent entity amongst HIV patients (Naicker, 2003) especially those of black descent. Renal complications of HIV infection have been reported since 1984. However, data on the incidence and prognostic significance of renal dysfunction in HIV disease are limited (Gardner et al., 2003). Although, HIVAN has been documented in indigenous African patients (Kimmel, 2003), little is known about the prevalence or risk factors for renal disease in this population (Naicker, 2003).

Although, the infectious disease society of America (IDSA) HIV renal guidelines (Gupta et al., 2005) recommends determining baseline renal function prior to initiation of antiretroviral (ARVs) therapy, many programs in sub-Saharan Africa initiate treatment without this assessment due to resource constraints (Wools-Kaloustian et al., 2007). In Cameroon very little work has been done on the kidney function of HIV positive patients and this test has been eliminated as a routine test in most HIV treatment centres. This study therefore tries to investigate the kidney function trends in seropositive patients, and set out baseline data for clinical management of HIV/AIDS patients.

## METHODS

### Study design and patient enrolment

A cohort study was performed between May to August, 2010, involving patients who visited the Nylon District hospital in Douala for routine HIV test or  $CD_4^+$  counts. Patients who were diabetic or hypertensive were excluded from the study to avoid falsely raised statistics. The ethical clearance was approved by the National Ethics Committee through the Provincial Delegation of Public Health. Patients were schooled on the study and written informed consents were obtained.

After an overnight fast, three millilitres of blood was collected using sterile disposable non-pyrogenic syringes (CATHY YOUNG®<sup>®</sup>, France) into dry vacutainer tubes from each of the participants. Clean 1.5 L containers were handed to the participants to bring 24 h urine the following day. A code number identical to the collection dry tubes and number on the urine bottle were then given to the

patients, to use for collection of samples. The blood samples collected were separated to produce serum using a centrifuge at 3000 rpm for 3 to 5 min and the serum samples frozen for future analysis. The urine samples were then brought to the hospital the following day for laboratory analysis.

### Study site

This study was conducted at the Nylon District Hospital (NDH) in Douala, the economic capital of Cameroon. With the sixth highest HIV prevalence rate (5.5%) in the country (Ministry of Public Health (2007), Douala constituted a favourable site for the study. The hospital in which the study was conducted comes third after Laquintinie Hospital and Douala General Hospital in the care of people living with HIV. The NDH also receives patients from other parts of the country due to its consistent supply of reagents for  $CD_4^+$  counts for HIV infected patients.

### Laboratory methods

Patients were instructed to collect the first morning urine and discard, write down the time to mark the onset of collection, collect for the next 24 h and then take the specimen directly to the laboratory. Urine samples were then thoroughly mixed and measured, and around 3 ml of the urine was poured into appropriately labelled test tubes and corked. The remainder of the urine was used for urine chemistry measurements and the excess discarded. Urine chemistry was done using URIPATH series test strips. The strips were dipped into urine up to the test area for about a second. The colour changes were read by comparing the colours with that provided by the manufacturer.

For urine creatinine measurement, the previously corked urine samples were diluted at 1:100 by mixing 990  $\mu$ l of de-ionised water with 10  $\mu$ l of urine. Creatinine measurements were then done on the diluted samples using Creatinine liquid reagent supplied by Hospitex Diagnostics Ltd. Equal volumes of the creatinine reagent 1 (picric acid) and reagent 2 (NaOH) were mixed in an empty, clean and sterile container. This mixture constituted the working reagent. 1000  $\mu$ l of the working reagent was pipetted into the test tubes, corresponding to the number of samples present. 200  $\mu$ l of the diluted samples were then pipetted into the working reagent and read using a spectrophotometer at 510 nm. Before measurements, the machine was calibrated using a standard solution provided by the company, and quality control sera.

Further to a thorough mixing, the absorbance A1 was read against reagent blank after 30 s and the second reading A2 was recorded exactly 2 min later. The concentration was calculated using the formula:

$$[(A_2 - A_1) \text{ assay} / (A_2 - A_1) \text{ standard}] \times \text{Standard concentration (2 mg/dL)}$$

For serum creatinine measurement, the frozen serum samples were allowed to stand on the bench to attain room temperature. The serum was used for creatinine and urea measurements. 1000  $\mu$ l of working reagent was pipetted into test tubes corresponding to the number of samples present. 200  $\mu$ l of serum was added to the working reagent and read spectrophotometrically (bioCHEMISTRY®) at 510 nm. There was no incubation period for this analysis since the absorbance readings and concentrations were read kinetically. The values obtained were recorded and used to calculate creatinine clearance, alongside urine creatinine values and values for the urine volumes (Reference range: 13 to 20 mg/L).

For serum urea measurement reagents were obtained from Hospitex Diagnostics Ltd. Dissolving the contents of enzymatic

**Table 1.** Sociodemographic characteristics of the study population.

Age (years)	Number (%)	Number (%) of males	Number (%) of females	Number (%) of HIV positive	Number (%) of HIV negative
<=30	121(36.8)	29(8.8)	92(28.0)	63(19.1)	58(17.6)
31-40	121(36.8)	29(8.8)	92(28.0)	101(30.7)	20(6.1)
> 40	87(26.4)	36(10.9)	51(15.5)	65 (19.7)	22(6.6)
Total	329(100)	94(28.8)	235(71.4)	229(69.6)	100(30.4)

reagent 1 into the chromogen buffer constituted reagent 1. Reagent 2 was prepared by adding 100 ml of de-ionised water into the alkaline reagent. 1000 µl of reagent 1 was pipetted into test tubes corresponding to the amount of samples present. 10 µl of serum was then pipetted into the tubes containing reagent 1. The mixture was agitated and stored for 3 min at 37°C. After 3 min, 1000 µl of reagent 2 was added to each of the tubes, agitated and incubated for 5 min at 37°C. The concentrations of the samples were then read using a spectrophotometer at 578 nm against a standard. These procedures were repeated for each batch of analysis along with the analysis of a quality control serum. Serum creatinine concentration was calculated using the formula:

$$(\text{Absorbance sample} / \text{Absorbance standard}) \times \text{Standard concentration}$$

Total CD<sub>4</sub><sup>+</sup> cell counts were performed using the Partec Cyflow® counter (Partec GmbH, Germany, 2006) using the procedure described by the manufacturer. Essentially, 20 µl of whole blood was added to a Partec tube. Thereafter, 20 µl of reagent containing the CD<sub>4</sub>-mAb PE (PE –conjugated monoclonal antibody to human CD<sub>4</sub>) was added to the tube. The tube contents were mixed gently for homogenisation and incubated in the dark at room temperature for 15 min. After incubation, 800 µl of no lyse buffer was added to the tube which was vortexed gently and the mixture analysed using Partec Cyflow® counter and the results printed automatically. Results obtained for the CD<sub>4</sub> counts were grouped as follows: Counts < 200 cells/µL; Counts between 200-<350 cells/µL; Counts between 350-<500 cells/µL; Counts >500 cells/µL (WHO, 2004)

Creatinine clearance (CC) was calculated using values for creatinine's urine concentration (U<sub>Cr</sub>), urine flow rate (V), and creatinine's plasma concentration (P<sub>Cr</sub>). Since the product of urine concentration and urine flow rate yields creatine's excretion rate, creatinine clearance is also said to be its excretion rate (U<sub>Cr</sub>×V) divided by its plasma concentration (Schwartz and Furth, 2007). This is commonly represented mathematically as:

$$C_{Cr} = (U_{Cr} \times V) / P_{Cr}$$

Creatinine clearance was used as a measure of glomerular filtration rate (GFR) and values were grouped as follows: Normal values (males >70 to 140 ml/min, females >60 to 128 ml/min), mild decrease in CC hence, GFR (males >40 to 70 ml/min, females >40 to 60 ml/min), moderate decrease in GFR (>10 to 40 ml/min), severe decrease in GFR (<10 ml/min) such patients are usually on dialysis.

The serological status of the participants was determined using two standard procedures as stipulated by the Ministry of Public Health. An immunochromatographic test was done using Retrocheck and one step anti HIV 1 /2 3.0 Bioline standard diagnostics was used for confirmation. However, for the control group, we performed the testing as prescribed by the old algorithm of HIV testing. Determine™ (Abbot Laboratories, Japan) HIV strips were used to screen for the presence of HIV antibodies. Positive

samples were further screened with ImmunoComb II HIV 1 and 2 Bispot kit (PDS-Organics, Israel). This was the confirmatory test and analysis was performed as recommended by the manufacturers. Positive cases after ImmunoComb were regarded as truly positive and excluded from the control group.

### Statistical analysis

Data were entered into Microsoft Excel, 2003 (Microsoft Corporation Inc, USA) and transported to the software package, SPSS for windows version 11.5 for analysis. The study participants were grouped as: HIV negative individuals, HIV positive patients on ART and ART naïve HIV positive patients. Differences between group means were compared using the student's t-test or analysis of variance (ANOVA). Categorical data were compared using chi-square test. Spearman's rho correlation was used to assess the relationship between non-parametric data. Statistical significance was set at P ≤ 0.05.

## RESULTS

A total of 333 adults, visiting the Nylon district hospital, Douala were enrolled into the study. After analysis, four patients were excluded from the study for uncompleted clinical and biological data, giving a total number of 329 participants at data analysis. 100 (30.4%) patients were HIV negative and 229 (69.6%) were HIV positive. The age range of the study population was 18 to 60 years. Amongst the HIV positive subjects, 45.9%(105) were not yet on antiretroviral therapy while 54.1%( 124) were already on routine ART. The age range of the study population was 18 to 60 years, with mean age of 35.122 ± 0.543. The mean age ± SEM for HIV positive participants was 35.122 ± 0.5340 and 32.101 ± 1.1007 for HIV negative participants. These ages were stratified into 3 groups with 121(36.8%) participants < = 30 years, 121(36.8%) aged between 31 to 40 years and 87(26.4%) > 40 years as shown in Table 1. 94(28.6%) of the study population were males while 235(71.4%) were females. The percentage of HIV seropositivity was higher in females than in males (74.3% vs. 25.7% p < 0.05).

The mean CD<sub>4</sub><sup>+</sup> counts ± SEM of the HIV positive participants in the study population was 322.64 ± 13.45 (SD 204.692) with the CD<sub>4</sub><sup>+</sup> counts grouped as < 200 (n =62, 27.0%), 200 to 350 (n = 86, 37.4%), 351 to 500 (n = 41, 18.3%) and > 500 (n = 40, 17.4%).

Of the 124 subjects on antiretroviral therapy, 4(3.2%) were on Lam + Stoc, 9(7.3%) on Lam + Efv, 13(10.5%) on Duo+Nvp, 33(26.6%) on Trio 30, 56(45.2%) on Trio 40, 2(1.6%) on Triviro, 2(1.6%) on Duo+Efv and 1(0.8%) respectively on, Lam + Nvp + Zi, Triviro + LNS, herbs after having stopped Nvp. For statistical reasons, only 5 antiretroviral regimens were included in data analysis. They are Lam + Stoc, Lam + Efv, Duo + Nvp, Trio 30 and Trio 40.

Of the study population 104(31.6%) had creatinine clearances (CC) < 70 mL/min, while 44(13.4%) had CC values less than 40 mL/min. 73(31.74%) of the HIV infected participants had CC values below 70 mL/min while 29(12.6%) of them had values below 40 mL/min. 14.6% of the subjects had CC values above normal amongst which 30(9.12%) were HIV positive.

Urinalysis results were normal in almost all the participants. However, 38 people (11.55% of the population) had proteinuria values  $\leq$  30 mg/dL, while 21(6.38%) had proteinuria values > 30 mg/dL. Of the 12(3.65%) patients who had haemolysis (considered as from + on the strip), 4(1.22%) had proteinuria greater than or equal to 30 mg/dL, and 2(0.61%) had significant Proteinuria. Leukocytes were found in urine of 4(1.22%) subjects out of which 2(0.61%) had significant proteinuria.

The mean serum creatinine was significantly higher in HIV negative participants than in HIV positive one ( $p < 0.0001$ ) as shown in Table 2. The mean  $\pm$  SEM serum urea in HIV positive participants ( $0.4404 \pm 0.02436$ ) was not significantly different from the control group ( $0.4227 \pm 0.00319$ ) ( $p = 0.635$ ). Creatinine clearance of HIV positive patients was significantly lower compared to the control group ( $p = 0.046$ ) through these values fall within the normal reference range. Urine creatinine values were obtained to assist computation of creatinine clearance. Whereas the mean  $\pm$  SEM proteinuria concentration was significantly higher in HIV positive subjects ( $0.81 \pm 0.078$ ) than in the control group ( $p = 0.001$ ).

Table 3 shows the kidney function tests in HIV infected participants who are and who are not on ART compared with controls. There was also a significant difference in serum creatinine values ( $p < 0.0001$ ) between HIV negative people, HIV positive people on ART and HIV positive people not on ART. HIV positive subjects on ART had the highest mean values for serum urea ( $0.4669 \pm 0.04455$ ) when compared with those who were not yet on ART and the control group. The mean CC difference was not significant when compared with HIV positive patients who were on routine ART, those who were not on ART and the control group ( $p = 0.136$ ). Considering HIV patients who were on ART, those who were not yet on ART and the control group, the highest mean  $\pm$  SEM of urine creatinine was seen in the control group and this difference was statistically significant ( $p < 0.0001$ ). A higher value for proteinuria was seen in HIV positive subjects who were not yet on ART ( $0.86 \pm 0.120$ ) when

compared with the control group and HIV patients on ART and this difference was statistically significant ( $p = 0.003$ ). The mean values  $\pm$  SEM in all these 3 categories fall within the normal range for proteinuria, creatinine clearance and below minimal value for serum creatinine.

Table 4 shows the kidney function tests in HIV patients in the different ART categories. Amongst the 124 patients on ART (54.3%) the difference in creatinine clearance between the different kind of ART was not significant ( $p = 0.513$ ). Looking at the different kinds of ART, there was no statistically significant difference in the mean serum urea values ( $p = 0.942$ ), the mean serum creatinine values ( $p = 0.415$ ), the mean CC value ( $p = 0.448$ ), the mean  $CD_4^+$  value ( $p = 0.462$ ) and the mean protein value ( $p = 0.903$ ), even though patients placed on Lam + Stoc therapy presented the lowest  $CD_4^+$  count, although, Table 5 shows the comparison of kidney function tests in HIV infected men and women (gender) within the various age groups. There was a significant difference in the serum urea of HIV patients aged 31 to 40 who were partitioned according to sex ( $t = 3.504$ ,  $p = 0.001$ ). Within the 31 to 40 Years age group, there was a significant difference in urine creatinine ( $t = 1.999$ ,  $p = 0.048$ ) between males and females. In those greater than 40 years, there was a significant difference in serum creatinine ( $p = 0.038$ ), urine creatinine ( $p = 0.008$ ) and urea/creatinine ratio ( $p = 0.010$ ) between males and females.

Kidney function tests in patients with different  $CD_4^+$  counts are shown in Table 6. Those with  $CD_4^+$  counts < 200 cells/ $\mu$ L had the highest mean  $\pm$  SEM serum creatinine value ( $7.695 \pm 0.332$ ) though this difference was not significant ( $p = 0.086$ ). Serum urea values were highest amongst those with  $CD_4^+$  counts between 350 and 500 ( $0.5683 \pm 0.13137$ ) but when compared with the other groups, the difference was not statistically significant ( $p = 0.098$ ).  $CD_4^+$  count category above 500 cells/ $\mu$ L had had the highest mean urine creatinine value ( $10.984 \times 10^{-2} \pm 0.8318$ ), but this difference was not statistically significant ( $p = 0.196$ ). The mean proteinuria was highest amongst those with  $CD_4^+$  counts > 500 cells/ $\mu$ L, however this difference was not statistically significant ( $p = 0.393$ ). The mean creatinine clearance was highest amongst those with  $CD_4^+$  counts > 500 cells/ $\mu$ L though this difference was not statistically significant ( $p = 0.370$ ).

Table 7 shows the kidney function tests and presence or absence of cotrimoxazole therapy. No significant difference was found between values for serum creatinine with the presence or absence of cotrimoxazole therapy ( $p = 0.208$ ), values for uremia and cotrimoxazole therapy ( $p = 0.106$ ). Those who were not on cotrimoxazole therapy ( $91.754 \pm 4.024$ ) had higher mean values for CC and this difference was statistically significant ( $p = 0.028$ ). Proteinuria was higher amongst those on cotrimoxazole therapy ( $0.86 \pm 0.114$ ) when compared with those who were not on cotrimoxazole therapy and this difference was significant ( $p = 0.016$ ).

**Table 2.** Kidney function tests in HIV positive individuals compared with the control group.

Kidney function tests	Mean $\pm$ SEM for HIV positive subjects (n = 229)	Mean $\pm$ SEM for HIV negative subjects (n = 100)	Statistical analysis
Urea (g/L)	0.440 $\pm$ 0.024	0.423 $\pm$ 0.003	p = 0.635
Serum creatinine (mg/L)	7.170 $\pm$ 0.147	8.019 $\pm$ 0.262	p < 0.0001
Creatinine clearance (mL/min)	83.289 $\pm$ 0.537	95.611 $\pm$ 7.302	p = 0.046
Proteinuria (mg/dL)	0.810 $\pm$ 0.078	0.360 $\pm$ 0.082	p = 0.001
Urine creatinine (mg/L)	9.790 $\pm$ 0.353	12.374 $\pm$ 0.596	p < 0.0001

**Table 3.** Kidney function tests in HIV infected participants who are and who are not on ART compared with controls.

Subject category	Mean value $\pm$ SEM of:			
	Serum urea (g/L)	Serum creatinine (mg/L)	Creatinine clearance (mL/min)	Proteinuria (mg/dL)
HIV negative n = 100	0.423 $\pm$ 0.003	8.190 $\pm$ 0.262	95.611 $\pm$ 7.303	0.36 $\pm$ 0.082
HIV positive on ART n = 124	0.467 $\pm$ 0.045	6.791 $\pm$ 0.169	83.668 $\pm$ 3.495	0.77 $\pm$ 0.104
HIV positive not on ART n = 105	0.408 $\pm$ 0.0052	7.621 $\pm$ 0.244	82.839 $\pm$ 3.702	0.86 $\pm$ 0.120
Statistical analysis	F = 1.118 p = 0.328	F = 10.275 p < 0.0001	F = 2.007 p = 0.136	F = 5.949 p = 0.003
Total n = 329	0.435 $\pm$ 0.0171	7.477 $\pm$ 0.132	86.997 $\pm$ 2.834	0.67 $\pm$ 0.061

**Table 4.** Kidney function tests in HIV patients in the different ART categories.

Kinds of ART (n = 115)	Serum urea (g/L)	Serum creatinine (mg/L)	Creatinine clearance (mL/min)	Proteinuria (mg/dL)	CD <sub>4</sub> <sup>+</sup> counts
Trio 40 (n = 56)	0.517 $\pm$ 0.099	6.470 $\pm$ 0.236	90.205 $\pm$ 4.824	0.73 $\pm$ 0.145	378.59 $\pm$ 26.614
Trio 30 (n = 33)	0.443 $\pm$ 0.017	6.888 $\pm$ 0.265	83.729 $\pm$ 7.665	0.78 $\pm$ 0.219	356.75 $\pm$ 35.259
Lam + Efv (n = 9)	0.398 $\pm$ 0.011	7.722 $\pm$ 0.889	90.500 $\pm$ 18.462	0.56 $\pm$ 0.338	310.89 $\pm$ 35.462
Lam + Stoc (n = 4)	0.445 $\pm$ 0.050	6.850 $\pm$ 0.512	93.575 $\pm$ 15.843	0.50 $\pm$ 0.289	213.50 $\pm$ 101.263
Duo + Nvp (n = 13)	0.430 $\pm$ 0.019	7.038 $\pm$ 0.801	67.969 $\pm$ 8.729	1.00 $\pm$ 0.439	359.77 $\pm$ 34.613
Statistical analysis	p = 0.942	p = 0.415	p = 0.448	p = 0.903	p = 0.462

**Table 5.** Comparison of Kidney function tests in HIV infected men and women within the various age groups.

Age (yrs)	Serum urea (g/L)	Serum creatinine (mg/L)	Creatinine clearance (mL/min)	Proteinuria (mg/dL)	CD <sub>4</sub> <sup>+</sup> count (cells/ $\mu$ l)
<30 (n=63) t = -0.448 p = 0.656	31-	t = 0.851 p=0.398	t = -0.591 p = 0.557	t = -0.667 p =0.501	t = -0.643 p = 0.523
40 (n=101) t = 3.504 p = 0.001		t = 1.413 p=0.161	t = 0.417 p = 0.678	t = -0.372 p = 0.711	t = 0.418 p = 0.677
>40 (n=65) t = -0.749 p = 0.456		t = 2.116 p=0.038	t = 1.325 p = 0.190	t = 0.591 p = 0.557	t = -1.045 p = 0.300

**Table 6.** Kidney function tests in patients with different CD<sub>4</sub><sup>+</sup> counts.

CD <sub>4</sub> <sup>+</sup> count (cells/ $\mu$ L)	N (%)*	Serum urea (g/L)	Creatinine (mg/L)	CC (mL/min)	Proteinuria (mg/dL)
< 200	62(27.0)	0.424 $\pm$ 0.008	7.695 $\pm$ 0.332	77.786 $\pm$ 4.561	0.81 $\pm$ 0.172
200-350	86(37.4)	0.403 $\pm$ 0.005	7.106 $\pm$ 0.217	81.887 $\pm$ 4.641	0.80 $\pm$ 0.116
351-500	41(17.9)	0.568 $\pm$ 0.131	6.569 $\pm$ 0.241	88.350 $\pm$ 5.620	0.60 $\pm$ 0.164
>500	40(17.4)	0.413 $\pm$ 0.013	7.125 $\pm$ 0.396	89.522 $\pm$ 5.300	1.05 $\pm$ 0.202
Statistical analysis		p = 0.098	p = 0.085	p = 0.370	p = 0.393

\*percentage based on total number of HIV positive participants (229).

**Table 7.** Kidney function tests and presence or absence of cotrimoxazole therapy.

Kidney function test	Mean value in patient not on cotrimoxazole therapy (n = 120)	Mean value in patients on cotrimoxazole therapy (n = 109)	Level of Significance
Serum urea(g/L)	0.414 ± 0.003	0.4712 ± 0.046	p = 0.106
Serum creatinine (mg/L)	7.606 ± 0.184	7.261 ± 0.173	p = 0.208
CC (ml/min)	91.745 ± 4.024	78.821 ± 3.356	p = 0.028
Urine creatinine (mg/L)	11.229 ± 0.409	9.415 ± 0.461	p = 0.005
Urea/creatinine ratio	0.061 ± 0.002	0.070 + -0.008	p = 0.186
Proteinuria (mg/dL)	0.56 ± 0.069	0.86 ± 0.114	p = 0.016
CD4 <sup>+</sup> counts (cells/μL)	354.46 ± 23.834	290.96 ± 13.298	p = 0.018

## DISCUSSION

Renal disease has been recognized as a common and intimately associated complication of human immunodeficiency virus (HIV) infection. It is now known that there are several renal syndromes and diseases associated with HIV infection (Cohen and Kimmel, 2007). Although, the incidence of complications is decreasing, HIV infection remains an important risk factor for the development of end stage renal disease (ESRD) (Eggers and Kimmel, 2004).

In this study involving HIV infected outpatients and healthy (HIV negative) controls at the Nylon district hospital, 30.45% of the HIV infected subjects had creatinine clearance below 60 mL/min, indicative of mild decrease in creatinine clearance. This value is higher than the one obtained by Wools-Kaloustian et al. (2007) in a Kenyan cohort of stable HIV infected outpatients, who found out that 11.5% had a CC less than 60 ml/min and 4.8% less than 50 ml/min. This could be explained by the fact that while the conventional formula for calculating creatinine clearance was used in this study, the Kenyan cohort made use of the Cockcroft and Gault formula (The Nephron Information Center, 2010) for determining GFR.

The higher number of subjects with decreased creatinine clearance in this study could also be attributed to dietary status, weight loss as a result of the disease and the fact that we were dealing with both ART naïve patients and patients who were already on therapy. Creatinine clearance can be modified by intake of meat and protein, muscle mass, medications, and intercurrent catabolic illness. These modifying factors for GFR and creatinine clearance are especially pertinent in HIV infected patients living in Sub-Saharan Africa (Schwartz and Furth, 2007).

Cotrimoxazole therapy could also be responsible for the decreased counts amongst HIV positive patients, since there was a significant difference in the creatinine clearance of people who were on cotrimoxazole therapy compared to those who were not on it. Kidney disease has been linked to *cidofovir* (*Vistide*), *indinavir* or *cotrimoxazole* (*Seprin/Bactrim*) therapy, systemic chemotherapy for lymphoma, acute bacterial infection of the kidneys and sepsis (Eggers and Kimmel, 2004).

There was a significant difference in the CC values of HIV infected subjects when compared with healthy controls. This finding is in accordance with the findings of Rao (1991) who first described an AIDS-associated nephropathy. Kelley (1997) reported that one of the causes of kidney disease is viral infections (hepatitis B and C and HIV).

There was no significant difference in the creatinine clearance values of HIV patients who were on ART when compared with those who were not yet on ART. Schwartz et al. (2005) showed that HAART most likely reduced the AIDS growth rate as well as the progression to HIV positive ESRD and the mortality rate of HIV positive ESRD patients. Other studies (Szczech et al., 2002; Kimmel, 2003) suggested that HAART is associated with decreased prevalence of renal disease and amelioration of progression in the general population and in HIV infected patients with chronic renal disease. This finding however is not in accordance with the findings of Kanai and Hanabusa (2005) who reported cases of kidney disease after the introduction of Tenofovir containing HAART. Other drugs (Indinavir, Efavirenz and Keletra) have also been incriminated as the cause of kidney disease in HIV positive individuals; however this was not the case in our study. Though, we found a reduced mean value for patients taking Nevirapine and Duovir, when compared to other HAART regimens, the difference was not significant. Highly active antiretroviral treatment may be associated with an improved renal outcome and even reversal of kidney disease in some patients (Ray et al., 2004); that was probably the case in our study.

Significant proteinuria was seen in 8.3% of the HIV infected participants. This value is greater than the 6.2% seen in the Kenyan cohort study (Wools-Kaloustian et al., 2007) and the 6% found in South Africa (Han et al., 2006). It is however smaller than the 13.5% reported by Gardner et al. (2003) who worked with seropositive women, and Andia et al. (2005) who reported a prevalence of 20% in Uganda. Gardner et al. (2003) included women who were hypertensives in their study population, which was one of the exclusion criteria during this work. Many other factors could have however contributed for the increase in prevalence in our findings, for example severe bacterial infection of the kidneys,

transient and/or orthostatic proteinuria, fever, cotrimoxazole therapy (Kelley, 1997) evident by the significant difference in proteinuria between those who were on cotrimoxazole therapy and those who were not.

Leukocytes was found in the urine samples of 2 patients who had significant proteinuria; this is evident of bacterial infection, which may not necessarily lead to a reduction in renal function except it is left untreated. Up to 30% of HIV positive individuals may have protein in their urine (Atta et al., 2005). Given the prevalence of proteinuria in Western cohorts of 14 to 32% (Schwartz and Furth, 2007) as opposed to the 8.3% found in this study, the thought therefore arises that kidney disease may not be as prevalent in Cameroon as it is in Western communities.

Serum creatinine is inversely proportional to creatinine clearance, though in our study only two patients had serum creatinine values greater than 15 mg/L. A similar finding was reported by Andia et al. (2005) in which only 3 patients in the study population had a serum creatinine value > 20 mg/dL. This also could be further explained by the fact that serum creatinine values become indicative of renal disease only after approximately 50% of the kidneys have been damaged.

In our study, serum urea levels were apparently not affected by sex, cotrimoxazole therapy, serological status, CD<sub>4</sub><sup>+</sup> count and type of ART. Though, urea can at best be a rough guide to renal function, it will ordinarily not be significantly raised until GFR is decreased by at least 50%. Looking at males and females between 31 and 40 years, we found a significant difference in their mean serum urea; this may be due to catabolism or excess urea production in males when compared to females.

A decrease in CD<sub>4</sub><sup>+</sup> counts apparently did not have an effect on our study population. Though, patients with CD<sub>4</sub><sup>+</sup> counts < 200 cells/μL had the lowest mean CC when compared to the others, the decrease was not statistically significant. A CD<sub>4</sub><sup>+</sup> count < 200 cells/μL is often associated with significant proteinuria and thus, kidney disease in HIV positive individuals, but there was no such association in our study. A similar result was found in an Iranian cohort (Afhami, 2007). This is probably due to the fact that majority of our patients with these counts were not yet on ART, evident by the mean CD<sub>4</sub><sup>+</sup> count of HIV positive patients who are not yet on ART.

Many studies have suggested a dramatically heightened susceptibility to the development of renal disease in patients of African descent who become infected with HIV (Cantor, 1991; Rao, 1991). In most of these studies depicting a high prevalence of HIV related renal diseases; the samples were obtained from AIDS patients, some of whom already had signs of kidney disease and from autopsy studies. This is probably one of the reasons why there was a discrepancy between our results and the results obtained from other cohorts. It is

possible however, that environmental factors may also contribute to the epidemiology of renal disease burden in HIV infected patients of African descent (Wools-Kaloustian et al., 2007).

These findings indicate a need for further study of renal epidemiology in HIV infected populations, in order to identify potential genetic as well as environmental factors associated with kidney disease (Schwartz and Furth, 2007). Also, the rapid nature with which HIV/AIDS develops in patients in Africa may not allow for renal disease to develop before death (Pepper et al., 2004), thus, the low prevalence of renal disease seen in our population. Indinavir, one of the nephrotoxic drugs taken by HIV positive patients is only administered in our community after toxicity has been proven with other drugs in a patient. This greatly reduces the probability of a patient developing kidney disease due to medications.

Tenofovir, another ART known to be nephrotoxic is not administered to HIV positive patients in our community thus leading to a marked decrease in the exposure of our patients and thereby reducing the risk of developing kidney disease. This however does not imply that kidney disease is not present in our community. Our results clearly indicate the presence of a reduction in kidney function in HIV positive patients when compared to healthy individuals and thus, an indication for better evaluation, long-term follow-up and further studies using biopsies and ultrasound to specify the kind of pathology in these patients.

## Conclusion

Creatinine clearance and thus GFR is reduced in HIV/AIDS patients when compared to negative controls. Although, proteinuria was present in some HIV/AIDS patients in our study population, this parameter alone is not indicative of HIVAN. Kidney disease in our study population is not caused by the kind of HAART taken by the patients. Rather, HAART may instead improve patient outcome by reducing patient's susceptibility to kidney disease. Cotrimoxazole therapy though beneficial, affects GFR and should be administered with care to these patients if not eliminated from their drug regimen.

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