East African Medical Journal Vol. 97 No. 8 August 2020
RENAL ULTRASONOGRAPHIC FINDINGS RELATIVE TO CD4+ CELL COUNT IN HIV POSITIVE ADULTS IN SOUTH NIGERIA

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ABSTRACT

***Objectives*: This study evaluated the renal sonographic changes in HIV positive patients in Port-Harcourt, south south Nigeria; to determine the relationship, if any, to CD4+ cell count in the study population.**

***Design*: A prospective descriptive study.**

***Setting*: University of Port Harcourt Teaching Hospital (UPTH), Port Harcourt, Nigeria.**

***Results*: There were 260 patients (80 males and 180 females), aged 22 to 75 years with a mean age of 36.0 ± 8.7years. Females had significantly higher CD4+ cell count than males (427±304 cells/mm^3 vs. 344.7±266.8 cells/mm^3, p=0.036). Renal sonographic abnormalities were commoner in patients with very low CD4+ cell counts (<200 cells/mm^3). Loss of corticomedullary echo differentiation was commoner with reducing CD4+ cell count.**

***Conclusion*: Renal sonographic features in HIV patients in the study area are characterized by increased renal parenchymal echogenicity, loss of corticomedullary differentiation and increased right renal cortical thickness with decreasing CD4+ cell count. Thus, the use of renal ultrasound in evaluation of HIV positive patients is advocated as early detection and management of renal abnormalities will reduce renal morbidity associated with HIV/AIDS.**

INTRODUCTION

Human Immunodeficiency Viral infection and Acquired Immune Deficiency Syndrome (HIV/AIDS) is a public health problem worldwide. In the sub-Saharan Africa, 24.9 million people are HIV positive (1). In Nigeria, three million, two hundred thousand persons are infected in Nigeria (2).

Every system in the human body may be affected by HIV/AIDS, and imaging plays a pivotal role in demonstrating the complex disease processes that result from HIV infection. Neurologic, respiratory, and gastrointestinal complications have all been extensively described in the medical literature; however, renal manifestations and, in particular, their imaging characteristics, are less well described (3).

Ultrasonography is a cheap and widely available method of assessing HIV related renal disease and it is also non-invasive and easy to perform (4). This modality is especially useful in developing countries where facilities for definitive pathological diagnosis may not always be available. Following the report of Rao *et al* (5) in 1984, many studies have been done on renal changes in HIV infection (2,4,6).

Cluster of differentiation-4 (CD4+) is a glycoprotein expressed on the surface of some T-helper cells, monocytes and dendritic cells. It is a member of the immunoglobulin super family. It functions as a co-receptor that assists the T-cell receptor (TCR) with an antigen presenting cell (7).

The HIV-1 uses CD4+ to gain entry into the host's T-cell by binding to the viral envelop glyco protein called gp120. HIV infection thus leads to progressive reduction in the number of T-cells expressing CD4+ due to viral replication (6).

The number of CD4+ cells in a cubic mm of blood is called the CD4+ cell count and is used to determine when to begin treatment in HIV infection (7). Normal range is 500-1200 cell/mm^3 with values less than 200 being diagnosed as AIDS. CD4+ cell count is also used to determine the efficacy of treatment.

The purpose of this study is to assess renal parameters by ultrasonography in patients with HIV/AIDS in Port-Harcourt, South-South Nigeria and to determine the relationship, if any, between these parameters and CD4+ cell count. This is necessary in view of the high prevalence of HIV/AIDS in our society.

MATERIALS AND METHODS

This was a prospective, descriptive and non-interventional cross-sectional study in HIV positive adult patients at UPTH, South-South Nigeria.

Study location: This study was carried out in the Radiology Department of UPTH.

Recruitment: Study subjects were recruited from the HIV clinic of UPTH.

Inclusion criteria

HIV positive adults (aged 18 and above).

Exclusion criteria

- Patients with history of diabetes mellitus, hypertension and heart failure.
- Sickle cell patients.
- Patients with malignancies unrelated to HIV.
- Pregnant patients.
- Patients with history of conditions that affect CD4+ cell count like steroid therapy and anti-tuberculous therapy.

The patients' blood pressure (BP) and random blood sugar (RBS) were measured with Accosons mercury sphygmomanometer and One Touch® Ultra® glucometer respectively to exclude hypertension and diabetes mellitus.

METHODOLOGY

The study group consisted of 260 consecutive HIV positive patients. Demographic data such as age, sex, weight, height and duration of illness were obtained and documented using structured interview form.

a) Body mass index (BMI) was calculated using the formula: weight (kg)/height $(m)^2$. BMI < 18.5 kg/m^2 was regarded as underweight, 18.5 - 24.9kg/m^2 as normal weight, 25 - 29.9kg/m^2 and >30kg/m^2 as overweight and obese respectively.

b) The duration of HIV diagnosis was categorized into <2 years, 2-5 years and >5 years duration.

c) The classification of CD4+ cell counts was done according to World Health Organization's (WHO) classification of CD4+ cell count in adult HIV infected patients (8) as follows:

i) CD4+ cell counts >500 $cells/mm^3$ = none or non-significant class.

ii) 350 - 499 $cells/mm^3$ = mild.

iii) 200 - 349 $cells/mm^3$ = advanced.

iv) CD4+ cell count <200 $cells/mm^3$ = severe category.

Ultrasound technique:

Gray scale ultrasonography of the kidneys was done on all patients by radiologists, using an ALOKA SSD_3500 machine with a 3.5 - 5 MHz curvilinear probe. The examination was done with patient in supine, right and left anterior oblique positions or prone. Longitudinal and transverse views of the kidney were obtained.

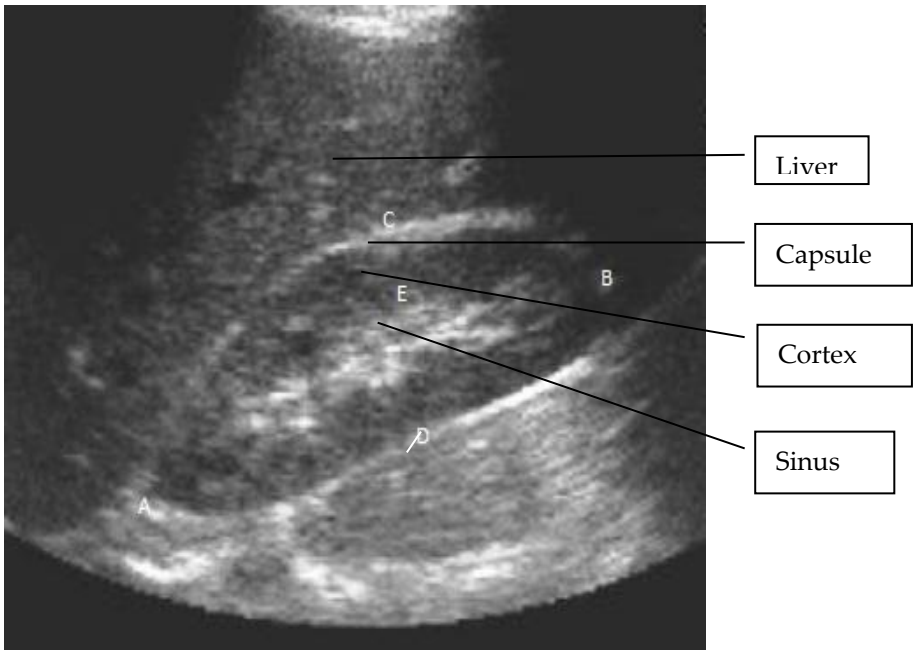


Figure 1. Longitudinal ultrasound image of the right kidney

KEY:
AB: renal length
CD: renal width
CE: cortical thickness

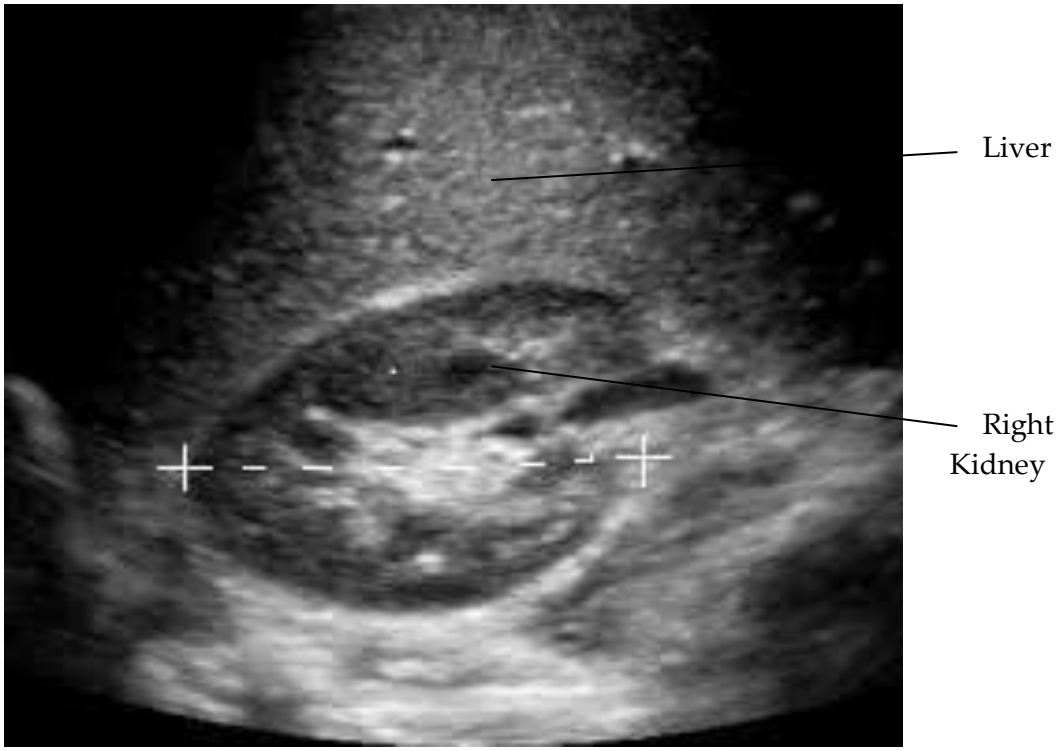


Figure 2. Transverse ultrasound image of the right kidney showing measurement of the transverse diameter

Kidney parameters on ultrasound

a) Kidney length: Using bipolar measurement, kidney length of 9.0 – 11.9cm was regarded as normal, < 9.0cm as small and >12cm as large (renomegaly) (9) fig 1.

b) Renal width (cm) was measured as the maximum distance between the anterior and posterior margins at the mid third of the kidney on a longitudinal scan. (fig 1)

c) Transverse diameter (cm) was taken as the widest diameter on the transverse scan. (fig 2)

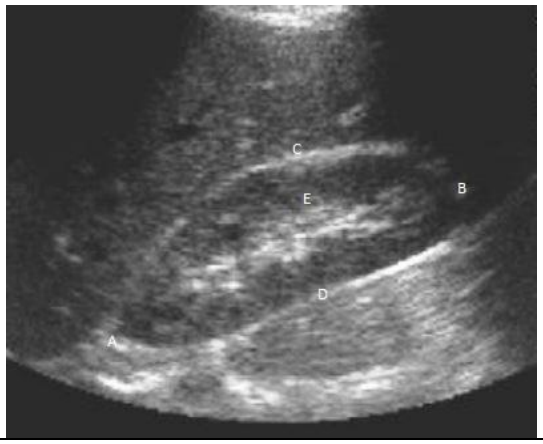
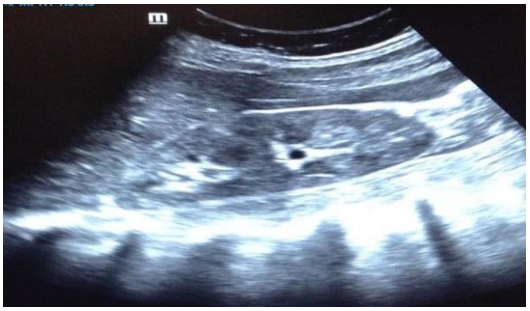
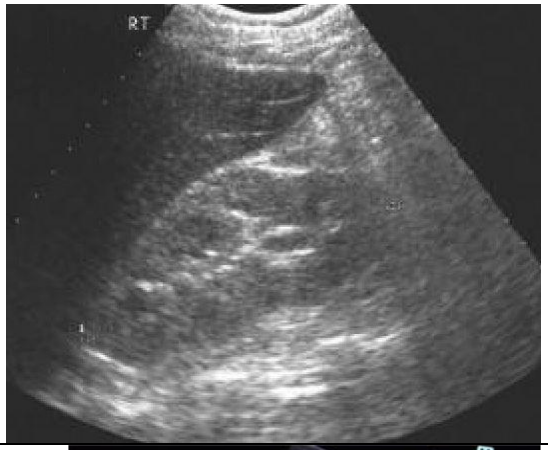
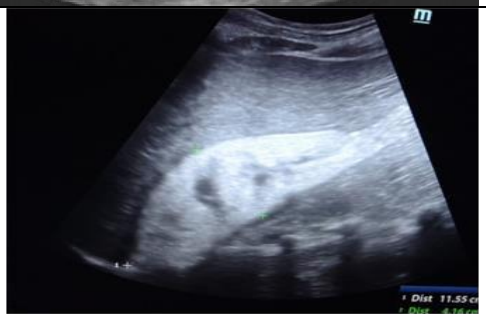
d) Cortical thickness (cm) was measured on the longitudinal scan at the level of the mid kidney at right angle to the cortex (10), from the cortex to the sinus. (fig 1)

e) Corticomedullary differentiation was assessed as visualization of renal pyramid/cortex distinct from renal sinus and noted to be maintained or lost.

f) The degree of renal echogenicity was graded into 4 categories (15). See table 1.

Table 1

Gradation of renal echogenicity

Grade of renal echogenicity	**Radiological characteristics**	**Example**
Grade 0	renal cortex is less echogenic than the liver	
Grade 1	renal cortex and liver are of the same echogenicity	
Grade 2	renal cortex is more echogenic than the liver	
Grade 3	renal cortex and renal sinus are of equal echogenicity	

CD4+ cell count estimation:

The CD4+ count was evaluated by a hematologist using the Partec® cyflow counter-1, a single –platform, three parameter (SSC plus two-colour fluorescence) desktop volumetric flow cytometer.

The reagents and protocols for CD4 T-lymphocytes count were obtained from Partec®.

Procedure

Reagent preparation and handling are as par manufacturer's protocol.

20 µl of well-mixed EDTA anticoagulated whole blood was placed in a test tube and 10 µ l of CD4 mAb PE and 10 µl of CD45 PE-CyS were added and incubated for 15mins at room temperature in the dark.

400 µl of no lyse dilution buffer 1 was added, mixed gently

400µ of buffer 2 was added and mixed gently and then the sample tube attached to the cyflow counter for automated counting.

Data acquisition and analysis were performed in real time and displayed on the screen.

Study period: This study spanned a period of one year from November 2014 to October 2015.

Ethical approval was obtained from the research and ethics committee of UPTH Informed written consent was obtained from all participants.

Data analysis: Statistical package for social sciences for windows (SPSS Inc USA) version 20.0 was used to analyze the data. Categorical data were presented as frequencies and percentages, while continuous variables were presented as means ± standard deviation. Tables and graphs were used where appropriate.

Pearson's correlation coefficient (r) was used to assess the relationship between the renal sonographic findings and CD4+ cell count while chi-square test was used for comparing parametric proportion. The decision rule was based on alpha (p). A value of 0.05 or less was considered significant.

RESULTS

A total number of two hundred and sixty (260) eligible HIV positive adults were recruited during the study period. Their ages ranged from 22 to 75years with a mean age of 36.0 ± 8.7years. The mean age of males was 38.7 ± 8.8years while that of females was 34.8 ± 8.2years. The age group with the highest frequency of subjects was the 4th decade (30-39 years) made up of 35 males and 84 females. There were 80 (30.8%) males and 180 (69.2%) females with a male to female ratio of 1: 2.25 (figure 3).

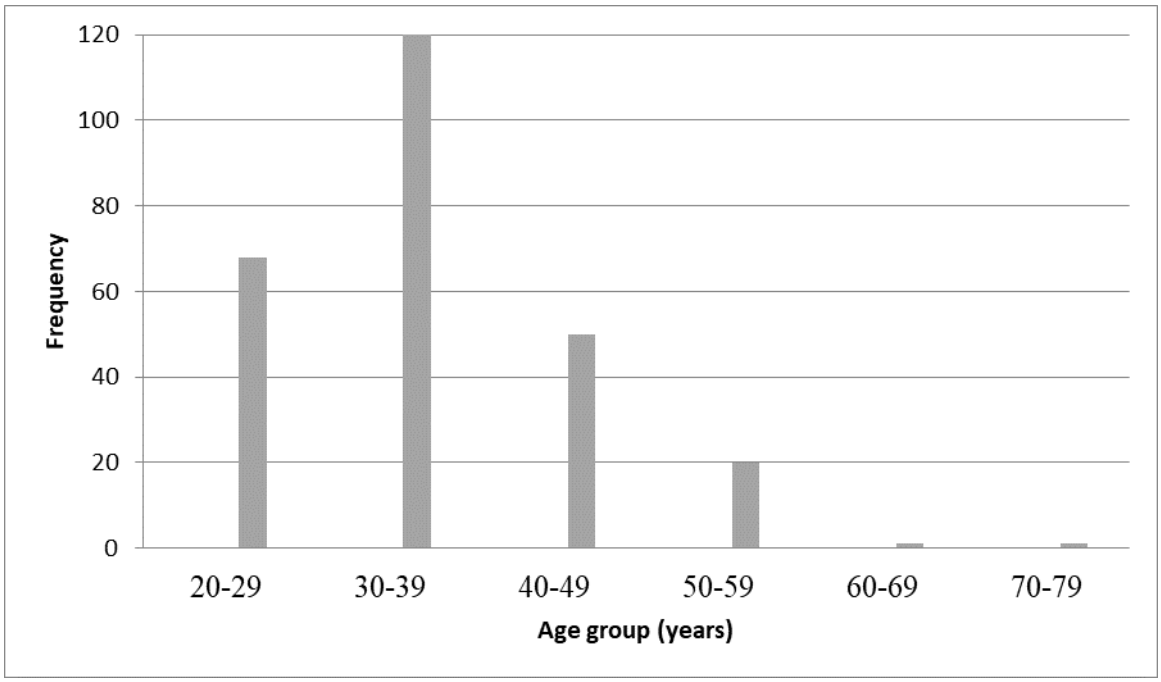


Figure 3: Bar chart showing age distribution of the study population

The summary of demographic characteristics of subjects is presented in table 2.

Table 2

Demographic characters of subjects

Demographic characteristics	**All patients**	**Male**	**Female**
Total number of patients	260	80	180
Mean age (in years)	36.0±8.7	38.7±8.8	34.8±8.2
Mean Body Mass Index BMI (in Kg/m^2)	24.8±4.12	23.88±3.17	25.20±4.3
Mean CD4+ cell count (in $cells/mm^3$)	402±295.4	344.7±266.8	427.7±304.5

One hundred and three (39.6%) subjects had recent infection (<2years) while 60.4% had been infected for more than 2 years (figure 4).

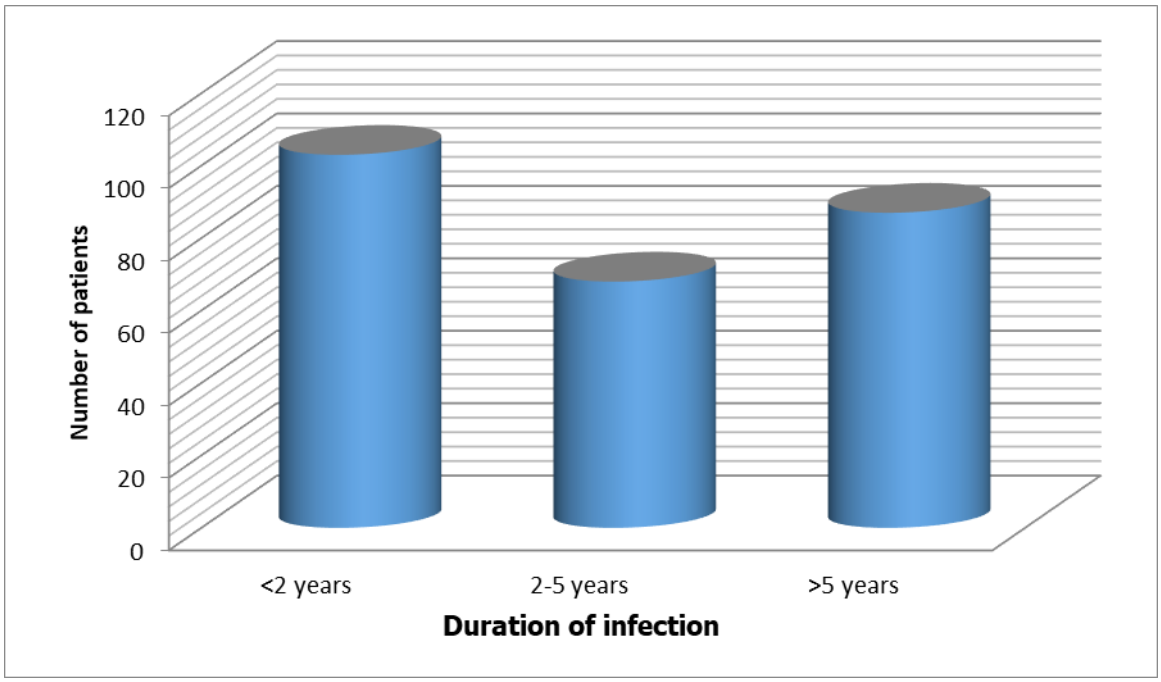


Figure 4: Bar chart showing duration of HIV infection

Renal sonographic findings were normal in 64% and abnormal in 36% of patients. The measured renal dimensions is as noted in table 3.

Table 3

Summary of renal ultrasound measurements

Ultrasound findings	**Right Kidney**	**Left Kidney**
Length (cm)	9.88±1.07	10.35±1.08
Small sized kidneys (%)	15	7.7
Normal sized kidneys (%)	81.2	85.3
Large sized kidneys (%)	3.8	2.7
Renal width (cm)	4.06±0.59	4.4±0.66
Renal transverse diameter (cm)	5.44±4.55	5.41±0.7
Renal cortical thickness (cm)	1.06±0.37	1.71±0.44

Increase in renal echogenicity was the most frequent finding. This increase in echogenicity is demonstrated in figure 5.

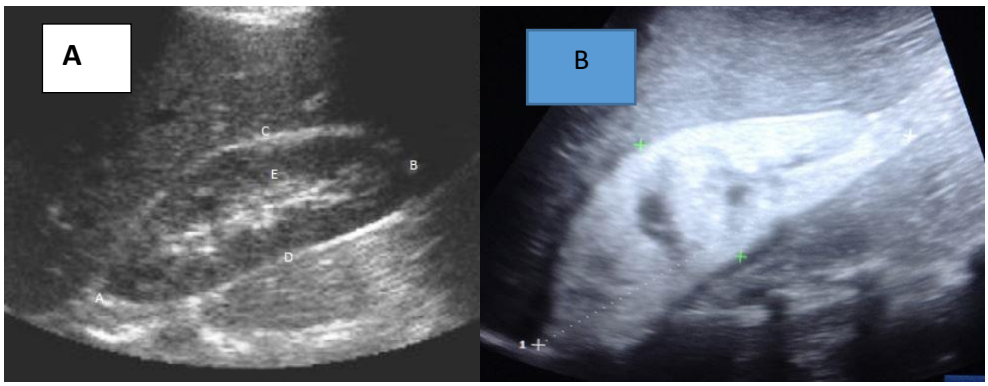


Figure 5: Shows a gray scale ultrasound image of a left kidney with normal echogenicity [A] and echogenic left kidney in a 42-year-old HIV positive man [B]

Of the 260 patients studied, 183(70.4%) had normal renal echogenicity (grade 0), while 46(17.7%), 19(7.3%) and 12(4.6%) were in the grades 1, 2 and 3 respectively (Figure 6). There was a significant linear negative correlation between the renal echogenicity and the CD4+ cell count (r = 0.196 p = 0.205) that is, as CD4+ count reduced, the renal echogenicity increased.

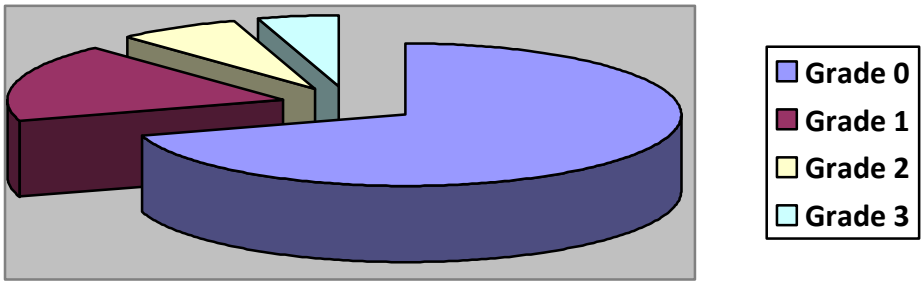


Figure 6: Pie chart showing renal parenchymal echogenicity

The mean CD4+ cell count of the study population was 402.1 ± 295.4 cells/mm^3 with a range of

12 – 1581 cells/mm^3. Females had significantly higher CD4+ cell counts than males (427.7 ±

304.5 cells/mm^3 vs. 344.7 ± 266.8 cells/mm^3, p = 0.036).

Compared to males, more females had CD4+ cell count within normal range (27.5% vs. 37.8%). The distribution of subjects in different CD4+ cell category (10) and gender are shown in the table 4 and figure 7 respectively.

Table 4

Summary of CD4+ cell count

CD4+ Cell categories	Number of patients
Not significant (>500 cells/mm^3)	90
Mild category (350-499 cells/mm^3)	45
Advanced category (200- 349 cells/mm^3)	44
Severe category (<200 cells/mm^3)	81

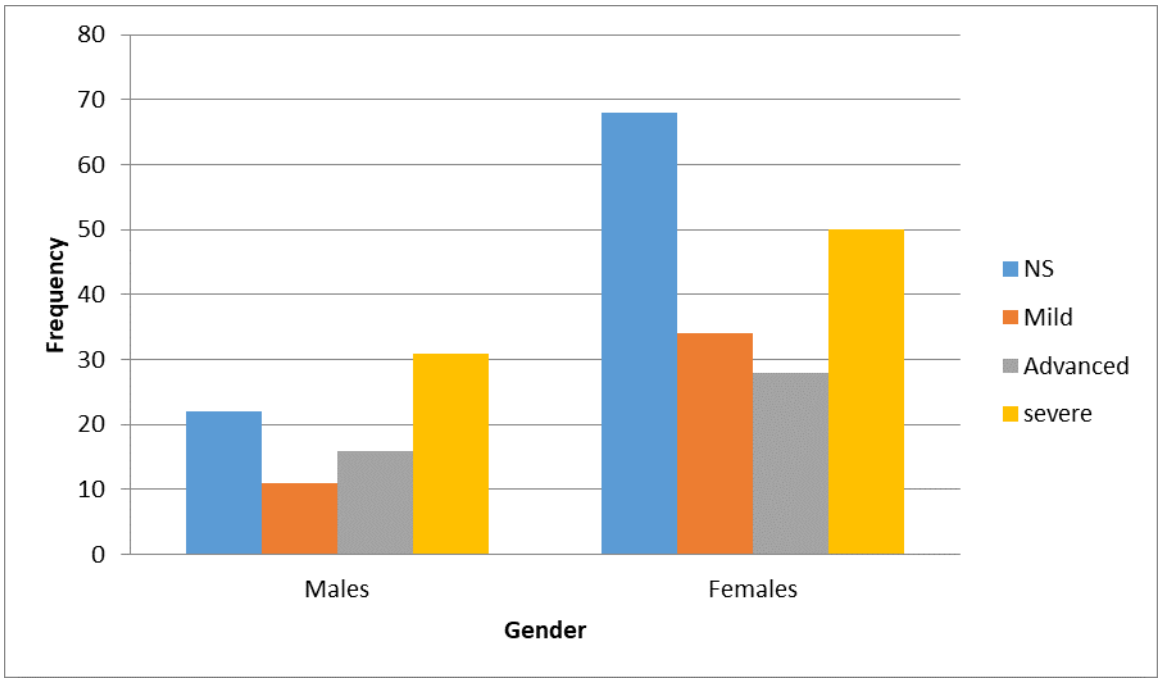


Figure 7: Bar chart showing distribution of males and female in each CD4+ cells category

Table 5

Correlation between variables and cd4+ cell count

Variable	Correlation coefficient(r)	p-value
Age	0.095	0.127
BMI	0.288	0.001*
Right renal length	-0.079	0.205
Right renal width	-0.188	0.202
Right renal transverse diameter	-0.023	0.715
Right renal cortical thickness	-0.174	0.005*
Left renal length	-0.015	0.812
Left renal width	0.046	0.525
Left renal transverse diameter	0.074	0.233
Left renal cortical thickness	-0.038	0.541
Corticomedullary differentiation	-0.155	0.002*
Renal parenchymal echogenicity	0.196	0.001*

* *Significant $p \leq 0.05$*

DISCUSSION

The mean age of presentation of patients in this study was 36.04 years (SD ± 8.74) and patients in the 4th decade of life constituted the largest population of patients. About 80% of patients in this study were between 20 and 50 years and this age range is both the economic productive group and the sexually active group of the Nigerian society (11). This is the reason of the strain of HIV/AIDS on the socio-economic fabric of the society (2). The mean age and the age distribution of the study group is in keeping with findings by other researchers who recorded ages between 34.6 - 38.02 years for HIV infected adults (4,9,12). The mean age of HIV infected patients is however, expected to rise with increase in availability of highly active antiretroviral therapy (HAART) and increasing survival among HIV patients (9).

In this study, females were more affected than males (69% vs. 31%). Female preponderance is in keeping with previous studies done in our locality (4,9,12,) and is because vaginal sex which is more practiced in our society places the women 2-3 times at greater risk of acquiring HIV than their male counterparts (13). It however differs from findings by Di Fiori (14) in California, USA where males constituted the majority in the study group. This difference is probably due to the smaller sample size of the study in California and difference in locality or the changing epidemiology of HIV infection, as HIV/AIDS was commoner in homosexual males in the USA.

HIV transmission is still high in Nigeria and the country has the second highest number of new infection reported each year (2). This study corroborates this fact as 66% of patients had been infected for less than 5 years.

HIV infection leads to progressive weight loss due to calorie deficit (15). The mean body mass index (BMI) in the study group was 24.4 kg/m^2 which is in the normal range. Normal BMI was also noted in a study by Adeyekun (5) in which a mean BMI value of 22.06 kg/m^2 was recorded. A lower value was however observed by Emem *et al* (12) in which the mean BMI was 18.5kg/m^2 which is in the underweight BMI range. This is likely due to the fact that their study population had deterioration of renal function at the time of study which leads to progressive weight loss (15) Most of the patients in this study had recent infection and were on HAART which modifies the disease progression (16).

The CD4+ cell count is used as a marker of the immune status in HIV-positive patients. It is used to monitor, follow up and determine the severity of illness (7). HIV infection leads to depletion of CD4+ cells (16). The normal value of CD4+ cell count is between 500-1500 per cubic millimeter of blood (17). The mean CD4+ cell count in this study was 402±295.4 cells/mm^3. This was in the mild CD4+ cell count category. The range of CD4+ cell count was from 12 cells/mm^3 to 1581cells/mm^3. The mean CD4+ cell count varies from a previous study in Benin Nigeria (9) in which less CD4+ cell count value (161-270cell/mm^3) was reported. The higher mean CD4+ cell count obtained in this study is probably due to the fact that most of the patients were recently diagnosed/ infected. There is also now easier accessibility of highly active antiretroviral therapy (HAART) to patients than was previously obtainable. HAART has been shown to modify disease progression in HIV/AIDS patients (16).

While 35% of patients had normal CD4+cell count values, 65% had abnormal values and 31% of patients fell into the severe CD4+ cell count category. The proportion of patients in each category differs from those by Chakraborty (18) in which 18% and 82% had normal and abnormal CD4+ cell counts with 24% being in the severe CD4+ cell count category. This

is likely because of the wider availability of HAART.

Female patients in this study had significantly higher CD4+ cell counts compared to males (427.7cells/mm^3 vs. 344.7cells/mm^3) in agreement with previous findings in Benin (8). Females generally have higher CD4+ cell counts (18) and usually have higher health seeking tendencies than men (9).

The mean renal length in this study was 9.88 ± 1.07cm and 10.35 ± 1.08cm for the right and left kidneys respectively. The longer length observed for the left kidney has been observed previously (5,9) and is due to the fact that there is more space for its growth. There was no correlation between the kidney lengths and the CD4+ cell count as was observed by Wyatt (19). It also differs with findings of Ulu (20) in which negative correlation of renal length with CD4+ cell count was noted. This may be due to the fact that both paediatric and adult patients were included in their study population.

The normal renal width and transverse diameter are 4-5cm and 4-6cm (21). These parameters are not routinely assessed sonographically in relation to renal diseases. In this study, the mean width, and transverse diameter of the right kidney was 4.06cm ± 0.59cm and 5.44cm ± 4.55cm while values of 4.44 ± 0.66cm and 5.41 ± 0.7cm were recorded on the left side. The renal width is in keeping with findings in Benin Nigeria (5) where values of 4.3 ± 0.59cm and 4.52 ± 0.63cm were obtained on the right and left kidneys respectively. In this study, the dimensions of the renal width and transverse diameter were within normal limits implying that these parameters are not affected in HIV. There was no correlation between the kidney width and transverse diameter with the CD4+ cell count as was previously observed by Wyatt (19).

Though the effect of HIV/AIDS on renal cortical thickness has not been fully described in literature, the effect of other chronic medical conditions on renal cortical thickness is however well documented. Renal cortical thickness has been shown to be a better indicator of chronic renal conditions than other renal measurements (22). The mean cortical thickness of the right kidney was 1.60 ± 0.37cm (0.9-3.30cm) and on the left, it was 1.71 ± 0.44cm with no significant gender variation. The left kidney showed a thicker cortex ($p = 0.001$) and this may be because the left kidney is usually bigger than the right kidney (21). There was significant linear negative correlation between the cortical thickness of the right kidney and the CD4+ cell count ($r = -0.174$, $p= 0.005$). No documented report was seen on correlation between renal cortical thickness and CD4+ cell count. There was however no correlation between the left kidney cortical thickness and the CD4+ cell count. This may be because the left kidney has more space to increase cranio-caudally than the right.

Increase in renal parenchymal echogenicity was observed in 29.6% of the HIV patients in this study. This was comparable to findings in previous studies (5,9,). However, the proportion of patients with increase in renal echogenicity is smaller than values recorded by Di Fiori's (14) and Ulu's (20) (91% and 77.7% respectively). The cause of increase in renal echogenicity is not clearly understood but glomerular changes, tubular dilatation and rising creatinine levels have been postulated (23). The discrepancy in these figures is probably due to the fact that patients with conditions like diabetes and hypertension which could also result in increased parenchymal echogenicity were not excluded in the studies by Di Fiori (14) and Ulu (20). The study population in the former also had deterioration of renal function at the time of scanning (14,19). There was a significant linear negative correlation between the renal echogenicity and the CD4+ cell count ($r= -0.196$, $p= 0.001$)

and this finding is in agreement with some previous studies (9,20,).

With increasing parenchymal cortical echogenicity in the setting of chronic renal diseases, there is increased likelihood of loss in corticomedullary echo differentiation. It has been observed that there is increase in renal cortical echoes in HIV (23) with loss of corticomedullary echo differentiation. In this study, loss of corticomedullary echo-differentiation was commoner in subjects with very low CD4+ cell count.

CONCLUSION

This study found normal renal dimensions and parenchymal echogenicity in most of the HIV-positive patients studied. There was significant negative correlation of renal cortical echogenicity, right renal cortical thickness and renal cortico-medullary differentiation with the CD4+ cell count. Among the renal parameters evaluated, renal parenchymal echogenicity correlates best with CD4+ cell count and should be assessed and monitored on follow up scans.

RECOMMENDATION

Renal ultrasound evaluation is recommended for HIV positive patients with CD4+ cell count of ≤ 349 cells/mm^3.

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