Correlation of CD4+ Count, Viral Load, Clinical Findings with Transabdominal Ultrasound Findings in Adult Patients Living with HIV/AIDS in Nigeria

Uzoamaka Rufina Ebubedike[1]*, Kanayo Gerald Obieje[1], Kenneth Chukwudi Ezeh[1], and Simeon Achunam Nwabueze[2]

[1]Department of Radiology, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. [2]Department of Community Medicine, Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria.

ABSTRACT

Background: The Human Immuno deficiency Virus (HIV) is a cytopathic retrovirus that causes Acquired Immune Deficiency Syndrome(AIDS) over time. Ultrasonography is a cost effective, safe, accessible and non-invasive means of demonstrating the abdominal manifestations of HIV/AIDS. $CD4^+$ count and viral load are the two surrogate markers widely used to monitor HIV disease progression. **Objective:** The aim was to determine the $CD4^+$ count, viral load and clinical findings in adult patients living with HIV/AIDS, then correlate these with their transabdominal ultrasound findings. **Materials and Methods:** A prospective cross-sectional study involving 210 adult patients living with HIV/AIDS was conducted over a period of 18months and their ultrasound findings correlated with their clinical findings, $CD4^+$ count and viral load. Data processing and statistical analysis were done using IBM SPSS (IBM Statistical Package for the Social Sciences), version 21.0. (IBM corporation, Armonk, NY, U.S.A, 2011). **Results:** Patients with Advanced HIV Disease (AHD), high viral load as well as Moderate and severe clinical stages, had the highest proportion of abnormal ultrasound findings (28.6%, 25.7% and 28.6% respectively). Thus, decrease in $CD4^+$count, increase in viral load result in more abnormal ultrasound findings and worse clinical stage. **Conclusion:** Most transabdominal ultrasound findings showed statistically significant correlation with $CD4^+$ count, viral load and clinical findings. Ultrasonography could be employed as a useful tool in the evaluation of patients with low $CD4^+$ count and high viral load.

Keywords: $CD4^+$ count, Clinical findings, HIV/AIDS, Viral loading, Transabdominal Ultrasound findings.

INTRODUCTION

The Human Immunodeficiency Virus(HIV) is a group of retroviruses that cause a chronic infection that leads to immunosuppression due to decline of CD4 count in affected persons.[1,2] HIV with its unique pathogenesis involving the suppression of immune system reduces

OPEN ACCESS

***Correspondence**:
Email: sur.ebubedike@unizik.edu.ng
Tel: +2348035389501

***Specialty Section**:
This article was submitted to Clinical, a section of TJMR

Article Metrics:
Submitted: April. 29, 2023
Accepted: Aug 30, 2024
Published: 15 Sept, 2024

***Citation**:
Ebubedike U R, Obieje K G, Ezeh K C, and Nwabueze S A. Correlation of CD4+ Count, Viral Load, Clinical Findings with Transabdominal Ultrasound Findings in Adult Patients Living with HIV/AIDS in Nigeria. Trop J Med Res. 2024:23(1);28-37.
10.5281/zenodo.10011724

***Journal Metrics**:
ISSN: p- 1119-0388, e- 2505-0338
Website: www.tjmr.org.ng
E-mail: info.tjmr@gmail.com

***Publisher**: cPrint*

Access Code

www.tjmr.org.ng

the body's ability to combat opportunistic infections in infected persons.[3,4] As a result, various organs of the body may be affected, leading to a variety of clinical presentations. Since any system could be affected, radiology becomes invaluable in the demonstration of these systemic involvement. Abdominal manifestations can be demonstrated by various imaging modalities, but ultrasonography is relatively cheap, accessible and uses non-ionizing radiation. The findings that may be seen on transabdominal ultrasonography include normal findings, multiple lymphadenopathy, splenomegaly, renal abnormalities (nephropathy, cortical renal cysts and hydronephrosis), gastrointestinal tract abnormalities (thickened bowel loops, appendicitis), splenic abscesses, pancreatic abnormalities (pancreatitis) and ascites.[5] Others are splenic infiltration, hepatomegaly, hepatic infiltration, gallbladder and biliary duct abnormalities.[6] The World Health Organisation (WHO)system for adults, sorts adult patients with HIV into four hierarchical clinical stages ranging from stage 1(asymptomatic) to stage 4 (AIDS), with the stage 2 and 3 also known as the mild and advanced symptomatic stages respectively.[7]

Gastrointestinal (GI) manifestations of HIV disease include diarrhoea, dyspepsia, nausea, vomiting, heart burn, constipation, abdominal pain, flatulence, etc. Progressive immunocompromised status is associated with increasing prevalence of GI symptoms.[8]

HIV Ribonucleic Acid (RNA) viral load and $CD4^+$ T lymphocyte ($CD4^+$) cell count are the two surrogate markers that have provided prognostic information on HIV progression and on response to therapy for decades.[9] The $CD4^+$T-cells are "helper" cells that secrete soluble molecules (cytokines) that help B cells (a type of white blood cell of the lymphocyte subtype) produce antibodies and also help macrophages destroy phagocytosed microbes, thus playing a major role in the body's immunity. This CD4+ helper cells are destroyed by HIV infection.[10] $CD4^+$ count primarily serves to assess when to initiate prophylaxis against several opportunistic infections. While it is useful in monitoring response to ART, $CD4^+$ count is , by itself, insufficient in evaluating response to therapy.[9] A normal $CD4^+$count ranges from 500 – 1600 cells per microlitre or cubic millimetre of blood. [11]

The HIV-1 viral load measurement indicates the number of copies of HIV-1 RNA per millilitre of plasma. Viral load monitoring provides an early and more accurate indication of treatment response. [9,12] Higher plasma viral loads are associated with more rapid declines in $CD4^+$ cells and with increased risk of progression to AIDS as well as higher risk of HIV transmission.[9] A viral load of 100,000 copies per millilitre is high.[13] A low viral load is a sign of the virus copying itself in low amounts in the body. HIV viral load is typically undetectable below levels of 40 – 75 copies per millilitre. The goal of ART is to lower the viral load below the detectable level.[9,11]

The lower the $CD4^+$ count, the higher the viral load and in turn the worse the immunodeficiency. The purpose of this study was to determine the $CD4^+$count, viral load and clinical findings in adult patients living with HIV/AIDS and correlate these with their transabdominal ultrasound findings

MATERIALS AND METHODS

This was a prospective cross-sectional study of adult patients with HIV/AIDS who were referred from the HIV care clinic of NAUTH, Nnewi to the Ultrasound Unit of the Radiology Department of the hospital for abdominal ultrasonography, conducted over an 18-month period, between January, 2019 to July 2020.

Inclusion and Exclusion Criteria

The study population comprised adult patients aged 18 to 65 years who were found to be positive on serological testing, not yet on HAART and who have had or were to do viral load and $CD4^+$ count tests, referred from the HIV Care Clinic of NAUTH, Nnewi to the Ultrasound Unit of the Radiology Department of the hospital for abdominal ultrasonography and who gave informed consent. Children < 18 years as well as the elderly (> 65 years) were excluded from this study. Patients already on HAART were also excluded, since HAART may affect abdominal organs in the same way as HIV infection like liver enlargement, acute pancreatitis, especially drugs such as Lopinavir, Ritonavir, Didanosine, Stavudine, Nevirapine and

Tenofovir [14] as well as other chemotherapeutic agents such as pentamidine and trimethoprim-sulfamethaxazole.[15]

Participant Recruitment

Consecutive sampling method was used in this study. All the participants who met the inclusion criteria were recruited consecutively until the sample size was achieved. The choice of this sampling method hinged on the fact that this study was time bound, another reason being limited number of HAART naïve patient available for recruitment, as a few numbers of these new patients were recruited daily with an average of twenty patients per month. In addition, since the current practice is immediate commencement of treatment as soon as the patients are tested positive for the virus, these HAART naïve patients were recruited into the study and scanned on the day of enrolment, as well as had their blood samples collected for $CD4^+$ count and viral load testing just before they were commenced on HAART. Collaboration was made with a consultant physician in the HIV Care Clinic which facilitated initial assessment and easy referral of subjects from the HIV Care Clinic to the Ultrasound Unit of the Radiology Department.

Data Collection

Relevant clinical history was obtained from each patient in the Radiology Department of the hospital through brief history taking and general/systemic examination, prior to ultrasound examination. Other clinical findings as well as $CD4^+$ count and viral load results of the subjects were extracted from their case files/folders.

The ultrasound examinations were performed in fasting states (i.e. after at least 12 hours overnight fast). Ultrasound scan was done on patients who had not eaten on morning of scan. This was to reduce bowel gas shadows and distend the gall bladder.

Abdominal ultrasound scan examinations were performed using ALOKA Prosound SSD-3500SX (ALOKA Inc, Japan 2008) ultrasound machine, fitted with curvilinear probe of variable transducer frequencies ranging from 2.5 to 5 MHz and linear probe with transducer frequency of 7.5 MHz, located in the Radiology Department of the hospital.The high frequency 7.5 MHz linear transducer was used to study superficial organs and structures such as appendix, anterior abdominal wall and gallbladder, while the 2.5 to 5 MHz curvilinear transducer was used to study the deeper abdominal organs and structures, using standard ultrasound scanning procedures and techniques.

Abnormality of any of the abdominal organs/structures was documented and images taken.

The following measurements were used to evaluate the abdominal organs /structures;

Lymphadenopathy was regarded as demonstrable lymph nodes with diameter larger than 1cm, [16], hepatomegaly was regarded as craniocaudal span of the liver ,measuring more than 15cm at the right mid-clavicular lines,[17], splenomegaly was regarded as spleen larger than 12cm at its longest axis. [18]

Thickened gallbladder wall meant dimensions >3mm at the anterior wall.[19] Pancreatic enlargement meant head antero-posterior (AP) dimension >3.5cm, body AP dimension >3.0cm and tail AP dimension >2.0cm.[20] Renomegaly was regarded as long axis of kidney >12cm. Thickened bowel wall meant wall thickness of fluid-filled bowel loops >4mm. Biliary dilatation was regarded as intrahepatic biliary duct luminal diameter >2mm (or if >40% of the diameter of the adjacent portal venous branch or extrahepatic biliary duct luminal diameter >5mm for patients <50years of age (or if > one - tenth of the age of the subject in millimetres). [20] All ultrasound examinations were done by the Radiologists.

METHODOLOGY OF CD4+ COUNT ESTIMATION

Equipment/Materials Partec Cyflow counter R (manufactured in Germany), tubes containing EDTA (Ethylenediaminetetraacetic acid) anticoagulant, needle and syringe, tourniquet and cotton swab.

Procedure About 20mls of blood was collected from the patient by sterile venipuncture into tubes containing EDTA anticoagulant. Twenty millilitres of the patient's blood were mixed with 20mls of partec antibody and the mixture incubated in the dark (as the mixture is photosensitive) for about 15 minutes, to allow antigen-antibody reaction. Then the sample tube containing the mixture was attached to the partecCyflow counter for automated counting

of the CD4+ T-cells. The Cyflow counter is a fully equipped, portable, ultracompact desktop flow cytometer dedicated principally for CD4 counting. The instrument uses fluorescent-labelled anti-CD4 monoclonal antibodies to capture CD4+ T-cells from whole blood and allows automated counting. CD4+ count estimation was routinely done every three months. The CD4+ count estimation was done in the HIV care laboratory of NAUTH, Nnewi.

METHODOLOGY OF HIV-1 VIRAL LOAD ESTIMATION

Equipment/Materials

COBAS Ampli Prep Instrument, COBAS Taq Man Analyzer or COBAS TaqMan 48 Analyzer.

Procedure

Eight millilitres of blood was collected from the patient via venepuncture. The blood samples to be run were stored in the designated freezer at a temperature of - 200 C. Samples for assaying were selected on a first come, first serve basis as arranged in the sample box. Plasma only derived from blood sample collected using EDTA anticoagulant was used for this assay. A minimum of 1050 microlitre of plasma was used for the assay. The HIV RNA plasma was quantified by nucleic acid amplification technologies such as Polymerase Chain Reaction (PCR). The COBAS AmpliPrep / COBAS TaqMan HIV-1 Test used PCR technology to achieve maximum sensitivity and dynamic range for the quantitative detection of HIV-1 RNA in EDTA. Titre results were reported in copies/ml. Viral load estimation were routinely done every 6 months, at 12 months and then every 12months thereafter if the person is established on ART. [12] The pre-treatment viral load and CD4+ count values of not more than one month duration, was collected from the laboratory or the patient's case files/folders. If the viral load or CD4 + count values, were more than three months old, the tests were rejected so as to ensure relevance to the patient's present transabdominal ultrasound findings. The viral load tests were done in the PCR laboratory of NAUTH, Nnewi. The CD4+ count and viral load results of the subjects were extracted from their case files/folders at the Medical Records Department through the help of a well-trained research assistant, following due authorisation.

Patients did not pay for the Viral load and $CD4^+$ count tests as the cost of tests are all sponsored by UNAIDS. In addition, the ultrasound examination fees were waived for the recruited patients after due authorization was gotten from the hospital.

Method of Data Analysis

Data was analysed using IBM SPSS (IBM Statistical Package for the Social Sciences), version 20.0. (IBM corporation, Armonk, NY, U.S.A, 2011). Categorical variables were expressed as percentages. Chi-square statistical analysis of strength of association between $CD4^+$ count, Viral load, Clinical findings and Transabdominal ultrasound findings was carried out. All continuous variables were reported as mean values plus or minus Standard deviation (SD) with Student t-test analysis for comparison. Two-tailed p values < 0.05 was considered statistically significant.

RESULTS

A total of 210 adult patients with HIV/AIDS who met the inclusion criteria were studied. This comprised 88 (42%) males and 122 (58%) females, with a female-to-male ratio of 1.4:1. The age range of the participants was 18 – 65 years with a mean age of 39.10 $\pm$ 10.97 years. The predominant age group was 38 – 47 years (31.4%), followed closely by the age group 28 – 37 years (30%). The predominant age group for females was 28 – 37 years (20.5%) while that of males was 38 – 47 years (11.9%), (Table 1). The age and gender distribution as well as mean values of CD4+ count and median values of Viral loads of adult patients with HIV/AIDS are shown.

Table 1: The age and gender distribution as well as mean values of $CD4^+$ count and median values of Viral loads of adult patients with HIV/AIDS

Age (years)	Gender		Total (%)
	Male (%)	Female (%)	
18-27	15 (7.1)	17 (8.1)	32 (15.2)
28-37	20 (9.5)	42 (20.5)	62 (30.0)
38-47	25 (11.9)	41 (19.5)	66 (31.4)
48-57	21 (10.0)	16 (7.6)	37 (17.6)
58-67	7 (3.3)	6 (2.9)	13 (6.2)
$CD4^+$			
Mean ±STD	323.37±255.49	332.87±246.93	328.89± 249.99
Minimum	17	16	
Maximum	1260	1294	
Viral loads			
Median	400	75.5	154.5
Minimum	0	0	
Maximum	690,525	707,543	

Frequency distribution of the CD4 count and Viral load classification of adult patients with HIV/AIDS is shown in Table 2. The highest frequencies were in the severe CD4 class(34.76%), <10 copies/ml viral load class(34.3%) and the clinical stage 1(39.5%).

Table 2: Frequency distribution of the CD4 count and Viral load classification of adult patients with HIV/AIDS.

Characteristics of patients with HIV/AIDS	Frequency (%)
CD4 Classification	
Not Significant (500 and above)	39 (18.57)
Mild (350-499)	48 (22.86)
Advanced (200-349)	50 (23.81)
Severe (<200)	73 (34.76)
Viral Load Classification	
<10 copies/ml	72 (34.3)
10-100,000 copies/ml	71 (33.8)
>100, 000 copies/ml	67 (31.9)
Clinical Stages	
Stage 1 (Asymptomatic)	83 (39.5)
Stage 2 (Mildly symptomatic)	50 (23.8)
Stage 3 (Moderately symptomatic)	48 (22.8)
Stage 4 (Severely symptomatic/AIDS)	29 (13.8)
Total	**210 (100)**

Chi-square analysis was used to show the relationship between CD4 count and Viral load in adult patients with HIV/AIDS. Chi-square analysis was used to show the relationship between Abnormal transabdominal ultrasound findings and CD4+ count, Viral load and clinical fiindings in adult patients with HIV/AIDS. Spectrum of CD4+ classification, viral load . clinical findings and Transabdominal Ultrasound (U/S) findings in adult patients with HIV/AIDS are shown in Tables 3,4,5

Table 3: Spectrum of CD4+ classification and Transabdominal Ultrasound (U/s) findings in adult patients with HIV/AIDS

Ultrasound Findings	Freq (%) (n=210)	CD4+ Classification				χ^2value	p-value
		Not significant(%)	Mild (%)	Advanced (%)	Severe (%)		
Normal	64 (30.5)	17 (8.1)	19 (9.0)	15 (7.1)	13(6.2)	10.578	0.014*
Ascites	25 (11.9)	3 (1.4)	0	6 (2.9)	16(7.6)	14.125	0.003*
Hepatomegaly	122(58.1)	18 (8.6)	25(11.9)	31 (14.8)	48(22.9)	7.370	0.288
Coarse Liver	6 (2.8)	0	1 (0.5)	1 (0.5)	4 (1.9)	14.015	0.029*
Hyperechoic Liver	43 (20.5)	5 (2.4)	5 (2.4)	10 (4.8)	23(11.0)	14.015	0.029*
Dilated bile duct	3 (1.4)	1 (0.5)	0	0	2 (0.9)	2.668	0.532
Gall bladder Sludge	7 (3.3)	0	0	3 (1.4)	4 (1.9)	9.499	0.416
Gallstones	12 (5.7)	1 (0.5)	2 (0.9)	4 (1.9)	5 (2.4)	9.499	0.416
Thickened gall bladder wall	5 (2.4)	2 (0.9)	0	1 (2.0)	2 (0.9)	9.499	0.416
Splenomegaly	47 (22.4)	10 (4.8)	9 (4.3)	11 (5.2)	17 (8.1)	0.641	0.887
Enlarged Kidney	29 (13.8)	3 (1.4)	4 (1.9)	9 (4.3)	13 (6.2)	6.951	0.330
Shrunken Kidney	7 (3.3)	0	2 (0.9)	2 (0.9)	3 (1.4)	6.951	0.330
Hyperechoic Kidney							
(Bil grade 1)	18 (8.5)	1 (0.5)	5 (2.4)	3 (1.4)	9 (4.3)	38.759	<0.001*
(Bil grade 2)	28 (13.3)	2 (0.9)	1 (0.5)	7 (3.3)	18 (8.6)	38.759	<0.001*
(Bil grade 3)	27 (12.8)	0	6 (2.9)	5 (2.4)	16 (7.6)	38.759	<0.001*
Hydronephrosis	8 (3.8)	0	1 (0.5)	0	7 (3.3)	10.569	0.018*
Hydroureter	6 (2.8)	0	0	0	6 (2.9)	11.591	0.013*
Renal mass/cyst	9 (4.3)	1 (0.5)	2 (0.9)	2 (0.9)	4 (1.9)	0.547	0.965
Thickened urinary Bladder wall	5 (2.4)	1 (0.5)	1 (0.5)	0	3 (1.4)	2.182	0.568
Lymphadenopathy	18 (8.6)	0	2 (0.9)	3 (1.4)	13 (6.2)	13.214	0.004*

**= significant p-value <0.05 Bil =Bilateral. Renal calculi were also found in 3(1.4%) patients. Enlarged and echogenic pancreas were observed in 2(0.9%) patients while shrunken liver, liver mass, liver abscess, coarse spleen and cervical mass were seen in 1(0.5%) patients each. These findings were not statistically significant. Many of the ultrasound findings above wer statistically significant.*

Table 4: Spectrum of Viral Load Classification and Transabdominal Ultrasound findings in adult patients with HIV/AIDS

Ultrasound Findings	Freq(%) (n=210)	Viral Load Classification			χ^2 value	p-value
		<10copies/ml (%)	10-100,000 copies/ml (%)	>100,000 copies/ml (%)		
Normal	64 (30.5)	30 (14.3)	21 (10.0)	13 (6.2)	8.159	0.017*
Ascites	25 (11.9)	4 (1.9)	6 (2.9)	15 (7.1)	10.596	0.005*
Hepatomegaly	122(58.1)	35 (16.7)	43 (20.5)	44 (21.0)	6.971	0.087
Coarse Liver	6 (2.8)	1 (0.5)	2 (1.9)	3 (1.4)	6.193	0.151
Hyperechoic Liver	43(20.5)	9 (4.3)	19 (9.0)	15 (7.1)	6.193	0.151
Dilated bile duct	3 (1.4)	1 (0.5)	0	2 (0.9)	2.182	0.336
Gall bladder Sludge	7 (3.3)	1 (0.5)	2 (0.9)	4 (1.9)	6.593	0.397
Gallstones	12 (5.7)	2 (0.9)	6 (2.9)	4 (1.9)	6.593	0.397
Thickened gall bladder wall	5 (2.4)	1 (0.5)	1 (0.5)	3 (1.4)	6.593	0.397
Splenomegaly	47(22.4)	11 (5.2)	19 (9.0)	17 (8.1)	3.220	0.200
Enlarged Kidney	29 (13.8)	4 (1.9)	9 (4.3)	16 (7.6)	10.640	0.021*
Shrunken Kidney	7 (3.3)	2 (0.9)	2 (0.9)	3 (1.4)	10.640	0.021*
Hyperechoic Kidney						
(Bil grade1)	18 (8.5)	5 (2.4)	6 (2.9)	7 (3.3)	42.459	<0.001*
(Bil grade2)	28 (13.3)	2 (0.9)	9 (4.3)	17 (8.1)	42.459	<0.001*
(Bil grade3)	27 (12.8)	3 (1.4)	6 (2.9)	18 (8.6)	42.459	<0.001*
Hydronephrosis	8 (3.8)	1 (0.5)	1 (0.5)	6 (2.9)	7.109	0.036*
Hydroureter	6 (2.9)	1 (0.5)	1 (0.5)	4 (1.9)	3.435	0.287
Renal mass/cyst	9 (4.3)	2 (0.9)	4 (1.9)	3 (1.4)	0.719	0.704
Thickened urinary Bladder wall	5 (2.4)	0	4 (1.9)	1 (0.5)	5.215	0.065
Lymphadenopathy	18 (8.6)	1 (0.5)	6 (2.9)	11 (5.2)	10.004	0.007*

**= significant p-value <0.05 Bil = Bilateral. Ascites, enlarged kidney, shrunken kidneys, Hydronephrosis, Lymphadenopathy and Grades of renal parenchymal disease were statistically significant.*

Table 5: Spectrum of clinical and Transabdominal Ultrasound (U/s) findings in adult patients with HIV/AIDS

Ultrasound Findings	Freq(%) (n=210)	Clinical Findings				χ^2 value	p-value
		Stage1(%)	Stage2(%)	Stage3(%)	Stage4(%)		
Normal	64 (30.5)	3 (15.7)	14(6.7)	15(7.1)	2(0.9)	11.143	0.011*
Ascites	25 (11.9)	1 (0.5)	5 (2.4)	12(5.7)	7(3.3)	21.220	<0.001*
Hepatomegaly	122(58.1)	46 (21.9)	28(13.3)	26(12.4)	22(10.5)	7.651	0.212
Coarse Liver	6 (2.8)	1 (0.5)	0	5 (2.4)	0	28.702	<0.001*
HyperechoicLiver	43 (20.5)	7 (3.3)	11 (5.2)	15 (7.1)	10 (4.8)	28.702	<0.001*
Dilated bile duct	3 (1.4)	0	0	1 (0.5)	2 (0.9)	8.231	0.027*
Gall bladder Sludge	7 (3.3)	0	3 (1.4)	1 (0.5)	3 (1.4)	16.967	0.014*
Gallstones	12 (5.7)	4 (1.9)	4 (1.9)	4 (1.9)	0	16.967	0.014*
Thickened gall bladder wall	5 (2.4)	0	1 (0.5)	2 (0.9)	2 (0.9)	16.967	0.014*
Splenomegaly	47 (22.4)	13 (6.2)	14 (6.7)	10 (4.8)	10 (4.8)	5.5763	0.134
Enlarged Kidney	29 (13.8)	6 (2.9)	11 (5.2)	5 (2.4)	7 (3.3)	13.751	0.034*
Shrunken Kidney	7 (3.3)	3 (1.4)	1 (0.5)	3 (1.4)	0	13.751	0.034*
Hyperechoic Kidney							
(Bil grade1)	18 (8.5)	4 (1.9)	6 (2.9)	7 (3.3)	1 (0.5)	43.3213	<0.001*
(Bil grade2)	28 (13.3)	7 (3.3)	5 (2.4)	11 (5.2)	5 (2.4)	43.3213	<0.001*
(Bil grade3)	27 (12.8)	3 (1.4)	7 (3.3)	5 (2.4)	12 (5.7)	43.3213	<0.001*
Hydronephrosis	8 (3.8)	1 (0.5)	2 (0.9)	1 (0.5)	4 (1.9)	9.820	0.034*
Hydroureter	6 (2.8)	0	2 (0.9)	1 (0.5)	3 (1.4)	8.638	0.016*
Renal mass/cyst	9 (4.3)	5 (2.4)	0	2 (0.9)	2 ()	3.333	0.262
Thickened urinary bladder	5 (2.4)	1 (0.5)	1 (0.5)	0	3 (1.4)	9.609	0.036*
Lymphadenopathy	18 (8.6)	0	2 (0.9)	8 (3.8)	8 (3.8)	26.508	<0.001*

**= significant p-value <0.05 Bil = Bilateral. Renal mass/cyst, Hepatomegaly and Splenomegaly were not statistically significant.*

DISCUSSION

The female preponderance demonstrated in this study was similar to that of some other authors such as Igbinedion et al,[21] Obajimi et al,[16] and Atsukwei et al.[22] This was however at variance with a study in central Africa by Tshibwabwa *et al*[23] which showed a higher male preponderance. The finding of female preponderance in this study may not be unrelated to the fact that females have better health seeking behaviour than males and are counselled during their antenatal visits for HIV testing, thus leading to the discovery of their status.[22] The female predominance in this study, could also be due to the consecutive recruitment method adopted. Over 76% of the patients in this study were within the age range 18 to 47 years, similar to what was documented by Atsukwei *et al*.[22] This age group was noted by Adeoye [24] as the economically productive segment of the Nigerian society as well as the age group at the greatest risk of HIV/AIDS.[24] Only about 6% of the patients were above 57 years of age, possibly because most of the older population are less sexually active or promiscuous as described by Igbinedionet al.[21] However, many more elderly HIV infected patients may be seen in the future from the HIV-infected youths that survived due to the availability and accessibility of free anti-retroviral therapy, which showed that AIDS- related deaths have declined by 33% since 2010.[25] Using the WHO classification of CD4+ immunological profile in adult patients with HIV/AIDS,[7] a higher proportion of patients (34.8%) were in the severe CD4+ class with the least number of patients in the "Not significant" class (18.5%), which was similar to what was found by Igbinedionet al[21] who recorded majority of the patients (46.3%) in the severe CD4+ category and the least number of patients in the "Not significant" class (11.7%). This was however at variance with the findings of Atsukwei et al[22] who found the least proportion of patients (8.2%) to be in the severe CD4+ class with the majority of the patients in that study (47.2%) documented to be in the "Not significant" class. The high proportion of patients in the severe CD4+ class, in this study, may be due to late presentation in hospital by most of the patients, with a large number of patients presenting only when symptoms are unamenable to other medications other than ART. The changes in intra-abdominal organs of HIV/AIDS could be specific, non-specific, single or multiple.[16,21,23,26] This was consistent with the findings in this study, (p = 0.012,

0.017 and 0.011 respectively). This was however at variance with the findings of Atsukwei et al[22] who noted that the "Not significant" CD4+ class had the highest number of patients with abnormal ultrasound findings. Sixty-four (30.5%) patients had normal abdominal findings while 69.5% of the 210 patients had various abnormal abdominal sonographic findings, which is consistent with the observation made by Atsukwei et al,[22] that out of 500 patients studied in the correlation between abdominal ultrasonogaphic findings and CD4+ count, 60% of the patients had abnormal ultrasound findings while 40% had normal findings. Igbinedion et al[21] and Smith et al[26] documented similar but higher abnormal trans-abdominal ultrasound findings of 85% and 81% respectively. The pattern of abnormal transabdominal ultrasound findings in this study were comparable to those documented by some local and foreign authors.[6,21,22,23] Hepatomegaly was the most common abnormal transabdominal ultrasound finding in this study as seen in 58.1% of the patients. Previous studies documented figures such as 41%, 39%, 13.3%, 35% and 23.4% respectively.[16,21,22,23,26] This showed that the frequency of hepatomegaly in this study, to the best of the author's knowledge, was the highest, when compared with previous studies. The hepatomegaly in these patients could be due to infections, non-specific response to infective hepatitis, fatty change or neoplastic infiltration from kaposi sarcoma or lymphoma.[13,21] Hepatomegaly did not correlate with the patient's CD4+ count, viral load and clinical findings (p = 0.288, 0.087 and 0.212 respectively). This was consistent with the findings by Atsukwei et al [22] who documented that hepatomegaly did not correlate with CD4+ count. Increased liver echogenicity was the second most common abnormal hepatic finding in 20.5% of patients. This was consistent with the findings of 18.0% and 25.0% by Igbinedion.[21]

Even though the highest number of patients had undetectable viral load (34.3%) and the highest number of patients were in the clinical stage 1 (Asymptomatic stage) (26.1%), the majority of the patients still had low CD4+ count and were in the severe CD4+ class (34.8%) and in addition, the highest number of patients with abnormal transabdominal ultrasound findings were in the severe CD4+ class (28.6%), with high viral load (25.7%) and in the worse clinical stages (stages 3 and 4) (28.6%). This was partly similar to the findings of Igbinedion et al[21] who recorded even a higher proportion of patients with abnormal sonographic abdominal findings in the severe CD4+ class(41.7%). Thus, there was statistically significant correlation between CD4+ class, viral load, clinical findings and transabdominal ultrasound findings in Igbinedionet al[21] and Atsukwei et al [22] respectively. Increased liver echogenicity showed statistically significant correlation with CD4+ count and clinical findings (p = 0.029 and< 0.001 respectively). This was however at variance with the findings in a study by Atsukwei et al [22] in which increased liver echogenicity had no correlation with patient's CD4+ count (p = 0.526).

This study demonstrates a high proportion of patients(34.8%) with increased renal parenchymal echogenicity which is a statistically significant finding as there was correlation between increased renal parenchymal echogenicity and CD4+ count, viral load and clinical findings (p< 0.001). Increased renal parenchymal echogenicity, particularly grade 3 renal parenchymal echogenicity, was seen more in patients with < 200 cells/microlitre (severe CD4+ class), high viral load (< 100,000 copies/ml) and clinical stages 3 and 4 (moderately and severely symptomatic/AIDS stage). The pattern of increased renal cortical echogenicity seen in this study is comparable to that described by Igbinedion et al,[21] Obajimi et al[16] and Smith *et al*[26] as significant findings. The pattern of increased renal cortical echogenicity seen in this study was described by some authors as HIV associated nephropathy (HIVAN)which has been shown to occur primarily in black patients.[26] This was however at variance with the findings by Atsukwei *et al*[22] who demonstrated no correlation between enlarged kidney, increased renal parenchymal echogenicity and CD4+ count. This high proportion of abnormal renal transabdominal ultrasound findings in patients with HIV/AIDS suggest that even if a patient has no laboratory evidence of background renal disease, a baseline renal ultrasound scan could be useful for comparison if the patient ultimately presents with proteinuria or uraemia. In addition, there is evidence to suggest that HIVAN will respond remarkably well to HAART.[21] Therefore, prompt diagnosis of

HIVAN and follow-up abdominal ultrasound scan may be invaluable to this group of patients.

Splenomegaly was seen in 22.4% of the patients but did not correlate with CD4+ count and viral load in this study (p = 0.391, r = -0.059 andp = 0.319, r = 0.069 respectively). This finding was consistent with those of Atsukwei et al[22]. This was however at variance with Igbinedion *et al*[21] who demonstrated splenomegaly in 44.3% of the patients with significant correlation with their CD4+ count. The high proportion of splenomegaly seen in this study was consistent with those reported in some previous studies, [6,16,21,23]

Ascites showed statistically significant correlation with CD4+ count, viral load and clinical findings in this study (p = 0.003, 0.005 and < 0.001 respectively). These findings were similar to those of Tshibwabwa et al[23] and Atsukwei *et al* [22] in which ascites showed statistically significant correlation with CD4+ count.

In this study, gall stones, gall bladder sludge, thickened gall bladder wall and dilated bile duct, showed statistically significant correlation with clinical findings (p = 0.014 each, for the gall bladder anomalies and p = 0.027 for dilated bile duct), most of these findings were noted in symptomatic clinical stages 2 to 4. They however did not show statistically significant correlation with CD4+ count and viral load. This finding was comparable to other authors who had fewer gall stones and biliary abnormalities,[6,16,21,23] but at variance with Pawar et al[27] who showed statistically significant correlation between AIDS Cholangitis and CD4+count. In this study, enlarged lymph nodes were seen in 8.6% of the 210 patients studied and it showed statistically significant correlation with the patient's CD4+ count, viral load and clinical findings (p = 0.004, 0.007 and < 0.001 respectively). This was similar to other studies,[6,16,21-23,28] in which lymphadenopathy was noted as a statistically significant finding.

To the best of the authors' knowledge, no previous study has correlated CD4+ count, viral load and clinical findings with transabdominal ultrasound findings in adult patients with HIV/AIDS in our environment, thus this study was carried out. It has shown the usefulness of ultrasound in the evaluation of HIV/AIDS patients with the positve correlation between the aforementioned parameters and transabsominal ultrasound findings.

The limitations of the study include its cross-sectional design. In addition, ultrasonography is operator dependent and there could be intra and inter-observer bias. This was also partly addressed by more than one consultant radiologist scaning each patient. However, the absence of histopathological data (obtained from biopsies of the diseased organs), owing to scarcity of resources in developing countries, made the sensitivity and specificity of ultrasound difficult to assess. It is of the opinion that this work will provide the platform for future studies to address these issues.

CONCLUSION

Many of the transabdominal ultrasound findings showed statistically significant correlation with CD4+ count, viral load and clinical findings. Thus, the values of these surrogate markers (CD4+ count and viral load), could be used as an indication for ultrasonography. It is therefore recommended that ultrasound be used to investigate HIV-infected patients with low CD4 count and high viral load as well as a baseline screening tool.

Acknowledgement: We sincerely appreciate the assistance of the patients who participated in the research. We are grateful to the Head, Department of Radiology as at the time of this study, Associate Prof M.E. Aronu as well as the Chief Medical Director of NAUTH, Prof. J. Ugboaja, for allowing us access to the study site and materials needed for the study.

Author contributions: KGO conceptualized the study. KGO, URE, KCE, SAN designed the study. URE, KCE and SAN contributed to implementation of the project. KGO, URE, KCE, SAN were involved in the writing and revision of the manuscript. The authors read, approved the final manuscript and agreed to be accountable for all aspects of the work.

Data availability: The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Funding: No funding sources.

Conflict of Interest: None declared.

Ethical Approval:
Prior to the commencement of the study, ethical clearance was obtained from the Research and Ethics Committee of NAUTH, Nnewi, bearing NAUTH /CS/66/VOL.9 /145/2016/95. Informed consent was obtained from the subjects before they were enrolled into the study. The subjects were informed of the safety of the procedure and could withdraw from the study at any stage without consequences. The overall benefit to the subjects was that those with abnormal abdominal findings were referred appropriately for treatment. Also, the subjects may gain psychological reward from knowing that he or she contributed to studies advancing the use of ultrasound in monitoring disease progression in HIV/AIDS. The subject's confidentiality was maintained all through the study period and beyond.

REFERENCES

1. Ford N, Meintges G, Victoria M, Greene G, Chller T. The evolving role of CD4 cell count in HIV care. Review: Current Opinion. HIV and Diagnosis. 2017;12:123-128.
2. Kaplan JE. HIV Myths and Infacts: How CD4 counts help treat HIV and AIDS. Available from; https: // www.webmd.com. Accessed on 23rd July, 2019.
3. Debalke S, Cheneke W, Tassew H, Awol M. Urinary tract infection among retroviral therapy users and non-users in Jimma University Specialized Hospital, Jimma, Ethiopia. Int. J. Microbial. 10.1155/2014/968716.
4. Adhanom G, Gebreegziabiher D, Weldu Y, Gebreyesus WA, Araya T, Legese H, et al. Species, risk factors and antimicrobial susceptibility profiles of bacterial isolates from HIV-infected patients suspected to have pneumonia in Mekelle zone, Tigrgy, Northern Ethiopia. Bio Med Res. Int. 10.1155/2019/8768439
5. Kisembo HN, Kawooya MG, Keyon C, Worodria W, Colebunders R. Abdominal sonographic findings in severely immunosuppressed human immunodeficiency virus-infected patients treated for tuberculosis. J TubercRes 2014; 2: 65-74.
6. Anjana T, Aarti T, Manicha P, Maulik J, Manish Y. Abdominal ultrasonography findings in HIV-infected patients. Sch J App Med Sci 2015; 3: 504-507.
7. WHO clinical staging of HIV/AIDS for adults and adolescents with confirmed HIV infection. Geneva, World Health Organization. Last edited 25 Mar 2018.
8. Santos ASeAdC, Silveira EA, Falco MdO. Gastrointestinal Symptoms in HIV-infected pati ents: Female Sex and Smoking as risk factors in an outpatient cohort in Brazil. 2016. PLoS ONE 11(10):e0164774. Doi:10.1371/journal.pone.0164774
9. Panel on Antiretroviral guidelines for adults and adolescents. Guidelines for the use of antiretroviral agents in HIV-1-infeced adults and adolescents. Department of health and human services. Available at: https://clinicalinfo.hiv.gov/en/guidelines/adult-and-adolescent-arv. Accessed February 27 , 2024.
10. Todd WTA, Lockwood DNJ, Nye FJ, Wilkins EGL, Carey PB. Infection and immune failure, Human Immunodeficiency Virus infection and the human Acquired Immune Deficiency Syndrome In: Davidson's principles and practice of medicine. 19th ed. Churchill Livingstone, Philadelphia. 2002: 3-146.
11. AIDS.gov : Access to U.S. Government HIV/AIDS information. Last revised: 14/7/2016.
12. Deepika P, Pallavi A. Initiation of antiretroviral therapy. Indian J Sex Transm Dis AIDS. 2014;35:1-11.
13. Medical monitoring: viral load testing available online at www.aidsmap.com/hatip. Accessed on 26/10/16.
14. Subbaraman R, Chaguturu SK, Mayer KH, Flanigan TP, Kumarasamy N. Adverse effects of highly active antiretroviral therapy in developing countries. Clin Infect Dis 2007; 45: 1093 – 1101. doi: 10. 1086/521150.
15. Sutton D. The Pancreas. In: Textbook of Radiology and imaging. 7th ed. Churchill Livingstone, India. 2009: 787 – 823.

16. Obajimi MO, Atalabi MO, Ogbole GI, Adeniji-Sofoluwe AT, Agunloye AM, Adekanmi AJ *et al*. Abdominal ultrasonography in HIV/AIDS patients in South Western Nigeria. BMC Med Imaging 2008; 8: 5 - 10.
17. Parsons L, Winter T. Right upper Quadrant mass In: Clinical Sonography. A practical guide. 4th ed. Lippincott Williams and Wilkins, Philadelphia. 2007: 68 - 79.
18. Matheson JR, Cooperberg PL. The spleen In: Diagnostic ultrasound. Mosby, St. Louis, year book. 1991: 87 - 105.
19. Ledwidge M, Winter T. Right upper Quadrant pain in: Clinical Sonography. A practical guide. 4th ed. Lippincott Williams and Wilkins, Philadelphia. 2007: 80 - 93. Drose JA. Epigastric pain (upper abdominal pain), Pancreatitis? In: Clinical Sonography. A practical guide. 4th ed. Lippincott Williams and Wilkins, Philadelphia. 2007: 54 - 67.
20. Igbinedion BOE, Marchie T, Ogbeide E. Trans-abdominal ultrasonic findings correlated with CD4 counts in adult HIV-infected patients in Benin, Nigeria. S Afr J Radiol 2009; 13: 34-40.
21. Atsukwei D, Eze ED, Chom ND, Igoh EO, Oweye SC, Angbalaga A *et al*. Correlation between abdominal Ultrasonographic findings and CD4+ cell count in adult patients with HIV/AIDS in Jos, Nigeria. Adv J Mol Imaging 2017; 7: 49 – 66.
22. Tshibwabwa ET, Nwaba P, Bogle-Taylor J, Zumla A. Four-year study of abdominal ultrasound in 900 central African adults with AIDS referred for diagnostic imaging. Abdom Imaging 2000; 25: 290 - 296.
23. Adeoye S. Sexual behaviour, perception of HIV/AIDS and condom use among commercial motorcyclists in Benin-city. Nig Postgrad Med J 2005; 12: 262 - 265.
24. UNAIDS, Global HIV & AIDS statistics – 2019 fact sheet.
25. Smith FJ, Matheson JR, Cooperberg PL. Abdominal abnormalities in AIDS: Detection at ultrasound in a large population. Radiology 1994; 192: 691-695.
26. Pawar SS, Biradar SP, Meenakshi IG, Khadse GJ. Correlation of hepatobiliary ultrasound findings with CD4+ count and Liver function tests in HIV positive patients. Med J of Western India 2013; 41: 86-88.