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Effects of Dolutegravir and Protease Inhibitors based Regimen on Renal and Liver Function Markers of HIV Patients Attending Daughters of Charity Hospital Abuja

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Abstract

Human immunodeficiency virus (HIV) is a serious public health that has been managed by different HAART regimen for people infected to live a healthier live and also reduce the risk of HIV transmission. But this has come without its undesirable effects which may last only a few days or weeks or that may not appear for months or years after starting the drugs. The aim of this study was to determine the effects of dolutegravir and protease inhibitors (ATV/r, LPV/r) based regimen on liver and renal function markers of HIV patients attending Daughters of Charity Hospital Kubwa Abuja. The objectives were to determine the serum levels of renal (creatinine and Microalbumin) and liver (ALB, ALT, TB & DB) function markers of HIV positive patients on dolutegravir and protease inhibitors based regimen and HIV negative (control) subject, to determine the concentration of renal (creatinine and Microalbumin) and liver (ALB, ALT, TB & DB) function markers of HIV positive patients on dolutegravir and protease inhibitors based regimen and HIV negative (control) subject, based on age and gender (sex), determine the correlations between dolutegravir and protease inhibitors based regimens on renal and liver function markers of HIV patients, to determine the effects of these drugs on renal and liver function markers of HIV patients attending Daughters of charity hospital Abuja. A structured questionnaire was used to collect patient's information on age, sex and duration on drugs while information such as drug regimen and last viral load value of patients were extracted from individual patient's folder of those that consented to participate, while ethical approval was sought and obtained from the Hospital Coordinator Daughters of Charity Hospital. Ninety (90) consented participants comprising of sixty (60) HIV positive patients and thirty (30) negative controls were enrolled for this study. Five milliliters (5ml) of venous blood samples were aseptically collected by venipuncture from the median cubital vein of each participant. Five to ten milliliter of random urine samples were also collected from participants. Serum samples were analyzed for Albumin, Alanine aminotransferases, Bilirubin (total & direct) and Creatinine, which were quantified photometrically using Chemwell chemistry auto analyzer (Chemwell 2910) while urine samples were assayed for urinary microalbumin using turbidimetric immunoassay method. Data collected were processed and analyzed using statistical product and service solutions (SPSS) statistical software version 27, categorical variables were analyzed using chi-square and presented in frequency table, quantitative data on liver and kidney markers were analyzed using independent t-test and presented as mean and standard deviation. Pearson's correlations were used to find out the correlation between protease inhibitors and dolutegravir based regimen, renal &liver function markers. Multiple logistic regressions were used to evaluate the effects of these drugs on renal and liver function markers of HIV patients on HAART. A p-value of less than 0.05 was statistically significant. Results showed that dolutegravir based regimen group shows a relatively lower mean values for DB (2.6 ± 1.2), TB (9.8 ± 3.4) and MA (16.3±20.0) compared to protease inhibitors based regimen while having a higher Cr (58.6±12.4), ALB (40±2.0) values and ALT activity (28.1±11.3). But when compared with controls there were significant

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difference (P ≤0.05) that exists in DB, TB, Cr, and MA whereas there were no significant difference (P ≥0.05) in ALB value and ALT activity. The mean concentrations of various parameters based on age and gender indicates that there were lower significant differences observed for females when compared with males in the protease inhibitors based regimen for ALB (p= 0.012), and TB (p= 0.013), while among age groups significant differences were seen for TB (p= 0.020). In the dolutegravir based regimen, lower significant differences were observed for females when compared with males for TB (p = 0.003), while for age groups significant differences were observed for ALT activity (p = 0.036) and Cr levels (p = 0.008). For the correlation between protease inhibitors and dolutegravir, renal and liver function markers, a significant negative correlation was observed between protease inhibitors and dolutegravir with regards to Direct Bilirubin (DB) (p = 0.001), Total Bilirubin (TB) (p = 0.001), and Microalbumin level (p = 0.034). For multiple logistic regression tests, both dolutegravir and protease inhibitors based regimen have an effects on the variables Albumin (ALB), Alanine Transaminases (ALT), Direct Bilirubin (DB), Total Bilirubin (TB), Creatinine (Cr), and Microalbumin (P value=0.0001) when considered jointly while protease inhibitors based regimen exerts a higher effects on DB, TB and Microalbumin (p value= 0.001, 0.001, 0.034 respectively). This highlights the importance of monitoring these parameters in individuals receiving protease inhibitors and dolutegravir based regimen as part of their HAART regimen. Nigeria HIV treatment guidelines should introduce urinary microalbumin testing as part kidney markers to be used in monitoring of HIV patients on the drugs studied and also includes testing of serum bilirubin among the liver markers especially for patients on second line receiving protease inhibitors based regimen. Also, more studies should be done in other HAART centers across Nigeria on the safety or otherwise of dolutegravir and protease inhibitors based regimen.

***Keywords**: dolutegravir, protease inhibitors, HIV, renal function, liver function, markers, patients, DOC Abuja.*

Introduction

HIV, the virus that causes AIDS is one of the world's most serious public health and developmental challenges worldwide. Since the beginning of the epidemic, 85.6 million people have been infected with the virus and about 40.4 million people have died of HIV (UNAIDS/WHO, 2023).

Although, the burden of the epidemic continues to vary considerably between countries and regions, African regions remains the most severely affected with nearly 1 in every 25 adults (3.2%) living with HIV and accounting for more than two-thirds of the people living with HIV worldwide (UNAIDS/WHO, 2023).

The backbone for HIV treatment has been HAART(highly active antiretroviral therapy) which is a combination of at least three drugs from different classes such as nucleoside (and nucleotide) reverse transcriptase inhibitors (NRTIs)-tenofovir disoproxil fumarate (TDF), lamivudine (3TC), and emtricitabine (FTC),

Non-nucleoside reverse transcriptase inhibitors (NNRTIs)-nevirapine (NVR) and efavirenz (EFV), Protease inhibitors (PIs)-lopinavir/ritonavir (LPV/r), atazanavir/ritonavir (ATV/r) and Integrase strand transfer inhibitors (INSTIs)-dolutegravir (DTG), raltegravir (RAL) (WHO, 2018).

But due to increased resistance, complications and mortality, the World Health Organization (WHO) in 2018 has recommended that all countries using TLE as a first-line regimen should transit all eligible clients to a different combination, which contains dolutegravir (DTG) in place of efavirenz-that is, TLD, with the "D" standing for dolutegravir.

According to WHO, the transit to TLD is because; TLD is more potent, suppressing viral load more quickly compared to EFV-based regimens. Eighty-one percent of individuals who started with a DTG-based regimen presented a viral load below 50copies/ml after 3 months of treatment, compared to 61% for those on an EFV-based regimen (WHO, 2018).

WHO also recommends that individuals to transit to the new regimen should include; Patients currently failing on an NNRTI-based first-line regimen or who have failed on an NNRTI-containing regimen in the past and are currently on a PI-based second-line regimen in programs that can confirm virologic suppression 3-6 months after transition to TLD (WHO, 2018).

Dolutegravir (DTG) is relatively new antiretroviral drug that targets the HIV integrase, one of the three viral enzymes involved in HIV replication. Dolutegravir blocks the binding site of the HIV integrase and prevents the strand transfer activity and integration of the provirus into the host genome (Dolutegravir Sodium Monograph for Professionals, 2019).

Studies by Surgers & Lacombe (2013) on Dolutegravir identified a few instances of acute liver injury with jaundice, with the latency to onset varied from 1 to 8 months and the pattern of serum enzyme elevations was hepatocellular. Also, studies by Milburn et al (2017) identified that dolutegravir can cause significant increase in serum creatinine concentrations.

Protease inhibitor based regimen is recommended as the preferred ARV drug for second-line ART among adults, adolescents and children. However, DTG may be used as an alternative second-line regimen if an individual is intolerant of Lopinavir/ritonavir boosted (LPV/r) or has a contraindication to Atazanavir/ritonavir boosted (ATV/r) or if the first-line regimen does not contain DTG (National guideline for HIV prevention, care and treatment, 2020).

Studies by Bethesda (2017) found out that protease inhibitors have been associated with transient and usually asymptomatic elevations in serum aminotransferase levels, mild to moderate elevations in indirect and total bilirubin concentration and hepatotoxicity.

Tenofovir disoproxil fumarate (TDF) is a medication used to treat hepatitis B and to prevent and treat HIV/AIDS. It is generally recommended for use in combination with other anti-retroviral drugs (The American Society of Health-System Pharmacists, 2016).

Lamivudine commonly called 3TC is an antiretroviral drug used to prevent and treat HIV/AIDS. It is also used to treat chronic hepatitis B when other options are not possible. It is effective against both HIV 1 and HIV 2 (The American Society of Health-System Pharmacists, 2016).

Approximately 1.9 million Nigerians lived with HIV/AIDS in 2021 and the country noted 74,000 new infections in the same year alongside 51,000 AIDS-related mortalities. Nigeria has suffered the most significant HIV epidemic in West and Central Africa and Abuja is ranking behind Benue and Plateau State in North Central with a prevalence rate of 1.4% (NAIIS, 2018). People living with HIV/AIDS have been on different highly active antiretroviral therapy (HAART), but this has been with its undesirable effects. The effects of dolutegravir and protease inhibitors used in this study had not been evaluated in HIV patients attending Daughters of charity hospital Abuja hence the need for this study.

Materials and Method

Research Design

This was a comparative cross-sectional case control study conducted among two groups of HIV patients, one group on dolutegravir based regimen and the other on protease inhibitors based regimen attending Daughters of charity of St. Vincent de Paul hospital Abuja and a HIV negative control group.

Study Area

This study was carried out in Daughters of Charity of St.Vincent de Paul Hospital, Plot 505, Cadastral zone, F01-Kubwa, Abuja-Federal Capital Territory. The Coordinates of the hospitals location within Abuja is 9.1619^{o}N, 7.3575^{o}E. The hospital is one of the centers in Abuja were HIV treatment is carried out and serves over three thousand (3,000) patients.

Study Population

The study population comprised HIV positive individuals who are on HAART receiving first line dolutegravir based regimen and a comparative group who are on second line therapy receiving protease inhibitors based regimen and HIV negative (control) group.

Inclusion Criteria

HIV positive patients who attended Daughters of charity hospital and consented to participating in the study and must fulfill these criteria to be included:

1. Patients must be virally suppressed <1000copies/ml within the last six months of drug therapy.
2. Patients who are on tenofovir/lamivudine/dolutegravir based regimen for at least one year.
3. Patients who are on tenofovir/lamivudine/ATV/r or LPV/r based regimen for at least one year.

Exclusion Criteria

HIV positive patients who attended Daughters of charity hospital and declined to participate in the study

1. Patients who had received either of the dolutegravir or protease inhibitors (ATV/r, LPV/r) based regimen for not up to one year.
2. Patients who are currently on enhanced dose of dolutegravir or protease inhibitors based regimen due to other co-morbidity.
3. Patients on any other based regimen other than dolutegravir (TLD)/tenofovir (TDF)/lamivudine (3TC) or tenofovir/lamivudine/lopinavir (LPVr), or atazanavir (ATVr).
4. Patient's who developed renal and/or liver problem before the commencement of their drug therapy.

Ethical Consideration

Ethical clearance was obtained from the Hospital Coordinator, Daughters of Charity of St. Vincent de Paul Hospital, Plot 505 Cadastral Zone F01 Kubwa Abuja before carrying out this study.

Also consent forms, assent forms and questionnaires were issued to participants who consented to be included and fulfill the inclusion requirement criteria.

Sample Size Calculation

The sample size was calculated from NAIIS, 2018 report using prevalence formulae stated below:

$$n = \frac{Z^2P(1-P)}{d^2}$$

Where:
n = sample size
Z = level of confidence at 95% (1.96)
p = local prevalence= 1.4% (0.014)
d = precision at (5%) = (0.05)

$$n = \frac{1.96^2*0.014(1-0.014)}{0.05^2}$$

$$n = \frac{3.8416*0.014(0.986)}{0.0025}$$

$$n = \frac{3.8416*0.013804}$$

0.0025
n= 0.0530294464
0.0025
n= 21.21177856
n= 21

Plus 10% attrition value which is 2.1, therefore minimum sample size was equal to 23 individuals on drug therapy.

Sampling Technique

All HIV patients attending Daughters of charity of St. Vincent de Paul hospital was pooled and recruited consecutively as many as those that met the inclusion criteria and consented to participate.

Methodology

Data Collection Technique

A structured interviewer administered questionnaire was used to collect patient's information on age, sex and duration on drugs while information such as drug regimen and last viral load value of patients was extracted from individual patient's folder of those that consented to participate. 5ml whole blood sample was aseptically collected from both HIV positive and negative individuals attending Daughters of charity hospital Kubwa Abuja, allowed to clot, retract and centrifuged at 3000rpm for 5 minutes. Serum was collected into plain tubes using Pasteur pipette and stored at -20^{o}C until analysis was carried out. Random urine samples were also collected and stored at -56^{o}C until analysis was carried out.

Specimen Analysis

Serum samples were analyzed for Albumin, Alanine aminotransferase, Bilirubin (total & direct) and Creatinine, using Chemwell chemistry auto analyzer (Chemwell 2910) which uses the principle of enzyme immunoassay and photometric assays while urine samples were assayed for urinary microalbumin.

Albumin was determined using Bromocresol green methodology adapted from Bartholomew and Delaney 1966,

Alanine amino transferase was determined using modified IFCC method,

Bilirubin (T&D) was determined using modified diazo method,

Creatinine was determined using modified Jaffe's method,

Urine samples were assayed for urinary microalbumin using turbidimetric immunoassay method.

Data Analysis

Data collected was processed and analyzed using statistical product and service solutions (SPSS) statistical software version 27.

Categorical variables of participants such as age, gender and HAART type were analyzed using chi-square and presented in frequency tables.

Quantitative data on liver and kidney markers were analyzed using independent t-test and presented as mean and standard deviation.

Pearson's correlation was used to find out the correlation between protease inhibitors and dolutegravir based regimen and renal and liver function markers.

Multiple logistic regressions were used to evaluate the effects of these drugs on liver and kidney function markers.

A 95% confidence interval was used in this study and a p-value of ≤ 0.05 was considered statistically significant.

ESULTS

From the study, there were sixty (60) HIV patients and thirty (30) HIV negative subjects. Thirty (30) patients were on dolutegravir based regimen while (30) patients were on protease inhibitors based regimen. There were equal number of HIV patients on dolutegravir and protease inhibitors based regimen aged 8-17 years, 18-59 years, 60 years and above age categorization as 10 (38.5%), 10 (29.4%), & 10 (33.3%) respectively while the healthy non-HIV (control) subjects were 6 (23.1%), 14 (41.2%), 10 (33.3%).

Twenty (20) (36.4%), and 18 (32.7%) patients respectively were females on dolutegravir and protease inhibitors based regimen while 17 (30.9%) were healthy non-HIV controls. Ten (10) (28.6%) and 12 (34.3%) patients were males on dolutegravir and protease inhibitors based regimen while 13 (37.1%) were healthy non-HIV control subjects.

Table 1 Age ranges and gender of participants

		Dolutegravir based regimen	Protease inhibitors based regimen	Control group
		N (%)	N (%)	N (%)
Age ranges (years)	Children/Adolescents (8-17)	10 (38.5)	10 (38.5)	6 (23.1)
	Adults (18-59)	10 (29.4)	10 (29.4)	14 (41.2)
	Elderly (60 & above)	10 (33.3)	10 (33.3)	10 (33.3)
Gender	Females	20 (36.4)	18 (32.7)	17 (30.9)
	Males	10 (28.6)	12 (34.3)	13 (37.1)

Key: N= number of participants in each category

Table 2 presents the mean concentrations of renal and liver function biomarkers of HIV patients based on HAART type and healthy non-HIV (control) subjects. Serum direct bilirubin (DB) (2.6±1.2 µmol/L), total bilirubin (TB) (9.8±3.4 µmol/L) and microalbumin (MA) (16.3±2.0 mg/L) were significantly lower ($p<0.05$) in patients on dolutegravir based regimen compared to those on protease inhibitors and the control group while serum creatinine (Cr) (58.6±12.4 µmol/L) was significantly higher ($p<0.05$) compared with patients on protease inhibitors (56.4±16.6

µmol/L) but significantly lower compared with control (73.4±19.3 µmol/L). Serum albumin was non-significantly higher (p>0.05) in patients on dolutegravir based regimen (40±2.0 g/L) compared with those on protease inhibitors based regimen (39±3.0 g/L) and the controls (39±2.0 g/L) while serum alanine transaminases (ALT) was non-significantly higher (p>0.05) in patients on dolutegravir based regimen (28.1±11.3 U/L) compared with those on protease inhibitors (25.7±8.2 U/L) and non-significantly lower (P>0.05) when compared with the control (29.8±8.7 U/L).

Table 3A presents the mean concentrations of renal and liver functions biomarkers of HIV patients based on HAART type and non-HIV (control) subjects by age.

For patients on dolutegravir based regimen, serum ALB, DB, TB were non-significantly (p>0.05) lower in subject aged 18-59years when compared with other age groups while serum MA was non-significantly (p>0.05) lower in subjects aged 18-59years when compared with 8-17years but higher when compared with ≥ 60years. Serum ALT activity was significantly (p<0.05) lower in subjects aged 18-59years when compared with other age groups but serum creatinine was significantly (p<0.05) lower in subjects aged 18-59years when compared with ≥ 60years but significantly higher when compared with 8-17years.

For patients on protease inhibitors based regimen, serum ALB and Cr were non-significantly (p>0.05) lower in subjects aged 18-59years when compared with other age groups while serum ALT, DB and MA were non-significantly (p>0.05) lower in subjects aged 18-59years when compared with ≥ 60years but non-significantly higher when compared with 8-17years. Serum TB was significantly (p<0.05) lower in subjects aged 18-59years when compared with other age groups.

For the control group, serum ALT and TB were non-significantly (p>0.05) higher in subjects aged 18-59years when compared with other age groups. Serum ALB was non-significantly (p>0.05) lower in subjects aged 18-59years when compared with ≥ 60years but had same value with subject's 8-17years. Serum DB and Cr were significantly (p<0.05) higher in subjects aged 18-59years when compared with other age groups while serum MA was significantly (p<0.05) higher when compared with 8-17years but lower with ≥ 60years.

Table 2 Mean concentration of renal and liver function biomarkers of HIV patients based on HAART type and healthy non-HIV (control) subjects

	ALB	ALT	DB	TB	Cr	MA
	(g/L)	(U/L)	(µmol/L)	(µmol/L)	(µmol/L)	(mg/L)
HAART type	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Dolutegravir based regimen	40.0±2.0	28.1±11.3	2.6±1.2	9.8±3.4	58.6±12.4	16.3±20.0
Protease inhibitors based regimen	39.0±3.0	25.7±8.2	6.3±2.9	40.2±18.8	56.4±16.6	31.2±31.9
Control group	39.0±2.0	29.8±8.7	3.1±1.3	12.0±4.1	73.4±19.3	1.0±0.2
P value	0.325	0.242	**0.001**	**0.000**	**0.001**	***0.001**

Key: HAART= highly active antiretroviral therapy, ALB= Albumin, ALT= Alanine transaminases, DB= Direct bilirubin, TB= Total bilirubin, Cr= Creatinine, MA= Microalbumin, *= Kruskal Wallis test

Table 3A Mean concentrations of renal and liver functions biomarkers of HIV patients based on HAART type and non-HIV (control) subjects by age

	DTG based regimen				PI based regimen				Control group			
	8-17years	18-59years	≥60years	P value	8-17years	18-59years	≥60years	P value	8-17years	18-59years	≥60years	P value
ALB (g/L)	40.0±2.0	39.0±2.0	40.0±3.0	0.437	39.0±4.0	38.0±2.0	40.0±3.0	0.626	39.0±2.0	39.0±2.0	40.0±3.0	0.637
ALT (U/L)	25.7±12.1	23.4±7.4	35.3±10.9	**0.036**	21.9±8.0	25.7±9.0	29.4±6.5	0.123	24.6±7.3	31.6±9.4	30.4±8.0	0.247
DB (µmol/L)	2.7±1.4	2.0±0.7	3.1±1.3	0.176	5.3±1.0	6.3±2.2	7.3±4.4	0.291	2.3±0.9	3.8±1.4	2.7±1.2	**0.032**
TB (µmol/L)	9.7±2.5	8.2±2.2	11.5±4.5	0.100	36.8±15.1	31.0±19.7	52.8±15.3	**0.020**	9.4±4.8	12.7±3.7	12.5±4.0	0.240
Cr (µmol/L)	53.9±8.8	53.9±10.6	68.1±12.5	**0.008**	54.8±12.4	52.5±21.8	61.9±14.4	0.437	51.6±11.7	80.8±17.6	76.0±16.3	**0.004**
MA (mg/L)	20.6±27.7	15.1±16.1	13.2±15.1	0.682	20.2±25.3	36.4±34.6	37.0±35.2	0.409	0.9±0.1	1.0±0.2	1.1±0.2	**0.040**

Table 3B presents the mean concentrations of renal and liver functions biomarkers of HIV patients based on HAART type and non-HIV (control) subjects by gender.

For patients on dolutegravir based regimen, serum ALB and ALT were non-significantly (p>0.05) higher in females when compared with the male subjects while serum DB, Cr and MA were non-significantly (p>0.05) lower in females

when compared with the male subjects. Meanwhile serum TB was significantly (p<0.05) lower in females compared with the male subjects.

For patients on protease inhibitors based regimen, serum ALT and Cr were non-significantly (p>0.05) lower in females when compared with the male subjects while serum DB and MA were non-significantly (p>0.05) higher in females when compared with the male subjects. Meanwhile serum ALB and TB were significantly (p<0.05) lower in females when compared with the male subjects.

For control groups, serum ALB and ALT were non-significantly (p>0.05) higher in females when compared with the male subjects while serum DB, TB and Cr were non-significantly (p>0.05) lower in females when compared with the male subjects. Meanwhile serum MA was non-significantly (p>0.05) higher or lower (same value) in females and the male subjects.

Table 4 reveals the correlation between HAART type, renal and liver function markers. There were a significant negative correlation between the serum levels of Direct bilirubin (r= -0.645, p= 0.001), Total bilirubin (r= -0.753, p= 0.001), and urinary microalbumin levels (r= -0.274, p= 0.034) in patients on dolutegravir based regimen and those on protease inhibitors based regimen. There were no significant correlations between the serum levels of Albumin (r = 0.184, p = 0.158), Alanine Transaminases (r = 0.125, p = 0.342), and Creatinine (r = 0.077, p = 0.558) in patients on dolutegravir and those on protease inhibitors based regimens. Table 5 presents the multivariate regression analysis for protease inhibitors and dolutegravir based regimen on renal and liver function markers. The table presents the multivariate tests, tests within subjects (ANOVA) and estimated means. There were significant differences between protease inhibitors and dolutegravir based regimens when considered jointly on the variables Albumin, Alanine Transaminases, Direct Bilirubin, Total Bilirubin, Creatinine, and Microalbumin with Wilk's ∧= 0.302, P value=0.0001. A separate ANOVA was conducted for each independent variable, with each ANOVA evaluated at an alpha level of 0.05. There were significant differences observed between protease inhibitor and dolutegravir based regimen on DB, TB and Microalbumin (p value= 0.001, 0.001, 0.034 respectively).

Table 3B Mean concentrations of renal and liver functions biomarkers of HIV patients based on HAART type and non-HIV (control) subjects by gender.

	DTG based regimen			PI based regimen			Control group		
	Females	Males	P value	Females	Males	P value	Females	Males	P value
ALB (g/L)	40.0±2.0	39.0±3.0	0.566	38.0±2.0	40.0±3.0	**0.012**	39.0±3.0	39.0±2.0	0.652
ALT (U/L)	28.7±10.7	26.9±12.8	0.676	24.4±7.1	27.5±9.7	0.327	31.3±8.0	27.9±9.5	0.310
DB (µmol/L)	2.4±1.2	3.1±1.3	0.161	6.5±3.5	6.0±1.7	0.608	2.9±1.2	3.4±1.6	0.322
TB (µmol/L)	8.5±2.3	12.3±4.0	**0.003**	33.4±16.6	50.3±17.9	**0.013**	10.8±2.9	13.5±4.9	0.065
Cr (µmol/L)	55.7±11.1	64.5±13.2	0.064	51.8±18.2	63.4±11.2	0.060	71.8±19.0	75.5±20.3	0.610
MA (mg/L)	15.1±21.7	18.7±16.8	0.593	34.2±33.9	26.7±29.5	0.552	1.0±0.2	1.0±0.2	0.440

Table 4 Correlation between HAART type, renal and liver function biomarkers

	DTG	ALB(g/L)	ALT(U/L)	DB(µmol/L)	TB(µmol/L)	Cr(µmol/L)	MA(mg/L)
PIs							
Pearson's correlation		0.184	0.125	-0.645*	-0.753*	0.077	-0.274*
P value		0.158	0.342	**0.001**	**0.001**	0.558	**0.034**
Total no.		60	60	60	60	60	60

*= significant correlation, -=negative correlation

Key: DTG= dolutegravir, PIs= protease inhibitors, ALB= Albumin, ALT= Alanine transaminases, DB= Direct bilirubin, TB= Total bilirubin, Cr= Creatinine, MA= Microalbumin

Table 5 Multiple logistic regression analysis of Protease inhibitor and Dolutegravir based regimen on renal and liver function biomarkers

Multivariate Tests

Section A

Effect		Value	F	Hypothesis df	Error df	P value	PartialEta Squared
DTG/ PIs based regimen	Pillai's Trace	0.698	20.464[b]	6.000	53.000	**0.0001**	0.698
	Wilks' Lambda	0.302	20.464[b]	6.000	53.000	**0.0001**	0.698

Section B

Source	Dependent Variable	Type III Sum of Squares	Df	Mean Square	F	P value	PartialEta Squared(R^2)
(PIs/DTG based regimen)	ALB (g/L)	14.017	1	14.017	2.042	0.158	.034
	ALT (U/L)	89.304	1	89.304	.919	0.342	.016
	DB (µmol/L)	205.350	1	205.350	41.425	**0.001**	.417
	TB (µmol/L)	13856.321	1	13856.32	76.157	**0.001**	.568
	Cr (µmol/L)	74.371	1	74.371	.346	0.558	.006
	Microalbumin(mg/L)	3330.150	1	3330.150	4.702	**0.034**	.075

Section C: Estimated means

Dependent Variable	HAART type	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
ALB (g/L)	Protease inhibitor	38.800	0.478	37.842	39.758
	Dolutegravir	39.767	0.478	38.809	40.724
ALT (U/L)	Protease inhibitor	25.673	1.800	22.071	29.276
	Dolutegravir	28.113	1.800	24.511	31.716
DB (µmol/L)	Protease inhibitor	6.307	0.406	5.493	7.120
	Dolutegravir	2.607	0.406	1.793	3.420
TB (µmol/L)	Protease inhibitor	40.183	2.463	35.254	45.113
	Dolutegravir	9.790	2.463	4.860	14.720
Cr (µmol/L)	Protease inhibitor	56.410	2.675	51.055	61.765
	Dolutegravir	58.637	2.675	53.282	63.991
Microalbumin (mg/L)	Protease inhibitor	31.200	4.859	21.474	40.926
	Dolutegravir	16.300	4.859	6.574	26.026

Key: PIs= Protease inhibitors; DTG= Dolutegravir; ALB= Albumin; ALT= Alanine Transaminases; DB= Direct Bilirubin; TB= Total Bilirubin; Cr= Creatinine

Discussion

This study was aimed at investigating the effects of dolutegravir and protease inhibitors based regimen on renal and liver function markers of HIV patients on highly active antiretroviral therapy (HAART).

This study observed that there were significant differences ($p \leq 0.05$) in the serum levels of direct bilirubin (DB), total bilirubin (TB), Creatinine (Cr), and Microalbumin (MA) in patients on dolutegravir (DTG) and protease inhibitors (PIs) based regimen when compared with the control group. The dolutegravir based regimen showed a relatively lower mean values for DB, TB, and MA when compared with protease inhibitors based regimen and the control but have higher mean values for Creatinine, albumin levels and ALT activities. The higher values of DB, TB, and MA exhibited by protease inhibitors group shows the capacity of protease inhibitors to induce jaundice as well as kidney injury to its recipients if not closely monitored. The high bilirubin levels observed among the PIs based regimen group shows that protease inhibitors interferes with the livers ability to process circulating serum bilirubin and/or that protease inhibitors can induce hemolytic anemia among its users (Roche & Kobos, 2004). High serum levels of bilirubin is known as hyperbilirubinemia which can deposits on the sclera of the eyes, under the skin and can equally cross the blood brain barrier to cause damage to the brain cells.

This finding on the increase in direct and total bilirubin levels compares with Bethesda (2017) who documented that protease inhibitors induces hyperbilirubinemia without other evidence of liver injury. He stated that indinavir and atazanavir is associated with elevations in unconjugated and total serum bilirubin and can cause clinically apparent jaundice in up to 10% of patients. Also, increase in Microalbumin which is a renal marker equally compares with the report of Alfano et al (2019) who stated that protease inhibitors indinavir (IDN), lopinavir (LPV), and atazanavir (ATZ) have all been associated with renal stone formation. They stated that atazanavir is generally well tolerated and widely used with boosting dose of ritonavir but atazanavir has been well associated with cases of crystalluria and nephrolithiasis, and withdrawal avoided new renal stone formation whereas continuum exposed patients to recurrence of the disease.

The higher value of Cr, ALB levels, and ALT activity in the dolutegravir group indicates that dolutegravir interferes with the kidneys ability to excrete creatinine out of the body. Elevation of creatinine with dolutegravir use has been attributed to the inhibition of the renal organic cation transporter (OCT2) and multidrug and toxin extrusion protein (MATE-2K), which causes a decrease in creatinine secretion (Lee et al., 2016).

Alfano et al (2019) equally noted that dolutegravir increases serum creatinine concentration of its users. This is because DTG augments plasma creatinine concentration through inhibition of organic cation transporter-2 (OCT2), a co-transporter of the basolateral side of proximal tubule cells responsible for urinary excretion of creatinine and drugs such as metformin and ranitidine. In this way, the unsecreted creatinine tends to increase in blood without an effective reduction of glomerular filtration rate (GFR) due to drug induced nephrotoxicity.

Also, the increase in urinary microalbumin level, a renal marker compares with what was reported by Milburn et al (2017) who stated that dolutegravir induces a noticeable increase in serum creatinine following initiation of therapy due to non pathologic tubular blockade of creatinine secretion through the inhibition of OCTs. Milburn et al (2017) also noted that protease inhibitors indinavir, lopinavir and atazanavir have all been associated with renal stone formation while atazanavir have been shown to cause acute tubular injury and tubule-interstitial nephritis.

Also, the higher value observed for alanine transaminases in the dolutegravir based regimen group shows that dolutegravir have the capacity to alter liver functions or cause slight alteration of the hepatocytes which was reflected in the elevation of ALT though the elevation was not significant when compared with controls, while high albumin value observed shows that dolutegravir based regimen may be causing a lower harm to the liver when compared to protease inhibitors based regimen. This is because the loss in the synthetic ability of the liver is manifested by lower than normal value of albumin level in the blood.

The finding of this work that both protease inhibitors and dolutegravir based regimen can initiate renal or liver injury is similar to the report of Rwegerera et al (2019) who observed that dolutegravir induced sub-acute hepatic failure in HIV positive treatment naïve man in Botswana. This highlights the need for patients' education in order to report any observed side effect of their HAART and as well monitoring of renal and liver function markers of these patients.

This study also observed that there is a correlation between protease inhibitors and dolutegravir based regimen by age and gender on the biomarkers investigated. Significant differences was observed between age groups for patients on dolutegravir for serum ALT activity with subjects aged 18-59years having a lower value when compared with children/adolescent and the elderly with the elderly having a higher ALT activity (p =0.036). Also, serum creatinine levels was significantly lower in subjects aged 18-59years age group when compared with the elderly but higher when compared with 8-17years age group (p =0.008). These findings could be attributed to a drop in liver and renal functions among the elderly due to a gradual loss of some kidney and liver cells among the aged. The kidneys are affected by the aging process, which may results in numerous effects on the renal system. Renal mass decreases between the ages of 30 and 80 years, with the steepest decline observed after age 50. Fat and fibrosis scarring may replace some of the remaining functional parenchyma tissue. This scarring and loss of kidney parenchyma tissue occurs primarily in the renal cortex; therefore, scarring affects the nephrons that are important for maximal urine concentration. Even in normal aging kidneys, 30% of the glomeruli are destroyed and display diffuse glomerular sclerosis by age 75, and the remaining glomeruli exhibit impaired filtering ability.

In the elderly, the production of creatinine also decreases with age whereas the secretion of creatinine from the tubules increases although the glomerular filtration rate declines in aging kidneys (Kanasaki et al., 2012). This finding also supports the phenomenon that renal functions decreases with age.

For patients on protease inhibitors, serum TB was significantly lower in subjects aged 18-59years age group when compared with other age groups with the elderly having the highest value (p =0.020). This may be attributed to aging with gradual alteration of hepatic functions and other changes associated with liver cells such as tissue scarring and gradual reduction of hepatocytes as one ages.

For patients on dolutegravir based regimen with respect to gender, significant differences was observed between males and females for total bilirubin (p =0.003), with males having a higher value. For patients on protease inhibitors based regimen, significant differences were also observed between males and females, for albumin (ALB) and total bilirubin (TB) (p =0.012 & p =0.013) with males again having a higher value when compared with the females. This observation may suggest that male gender is more predisposed to HAART induced jaundice but with better biosynthetic capacity of the liver. This findings contradicts the report of a study carried out by Samje et al (2020) in Cameroon where their study showed that female in HAART are more predispose to HAART induced liver toxicity though they used Aspartate transaminases (AST) and alanine transaminases (ALT) activities as their liver markers.

It was also observed from this study that a significant negative correlation exist between protease inhibitors (PIs) and dolutegravir (DTG) based regimen, renal and liver function biomarkers with regards to direct (conjugated) bilirubin (DB) (p = 0.001, r= -0.645), total bilirubin (TB) (p = 0.001, r= -0.753), and Microalbumin (MA) (p = 0.034, r= -0.274) suggesting that protease inhibitors and dolutegravir based regimen leads to an increase in DB, TB, and MA levels, though protease inhibitors based regimen has a stronger effect and caused a higher DB, TB and MA levels than dolutegravir based regimen. This shows that the two drugs studied has the ability to alter bilirubin metabolism by the liver by interfering with the enzyme uridine-5'-diphosphate (UDP) glucuronyl transferase found in the endoplasmic reticulum of the liver to and diglucuronide (Oliver et al., 2010).

Finally, the two drugs showed statistically significant effects on the parameters studied. This shows that these drugs have both hepatotoxic and nephrotoxic effects in HIV patients studied with protease inhibitors based regimen having a higher effect on direct (conjugated) bilirubin, total bilirubin, and microalbumin. This highlights while these parameters should be routinely monitored in individuals receiving protease inhibitors and dolutegravir based regimen as part of their HAART regimen. Patients on protease inhibitors should be closely monitored for presence of jaundice so that they can be quickly switched to a safer regimen that has lesser propensity to induce jaundice. Also, lopinavir which is a protease inhibitor can exacerbate an underlying chronic hepatitis B or C in HIV co-infected individuals (Bethesda, 2017). This equally underscores the importance of screening all eligible HAART recipients for hepatitis B or C co-infection before starting them on protease inhibitors based regimen.

The increase in urinary Microalbumin in the two groups studied highlights the importance of regular monitoring of kidney functions among patients on HAART.

Conclusion

There are statistically significant elevations in serum direct bilirubin, total bilirubin and urinary Microalbumin levels in HIV patients managed with this HAART group when compared with the negative control population. This highlights the importance of monitoring these parameters in individuals receiving these drugs as part of their HAART regimen. There is also the need to monitor serum creatinine level in patients on dolutegravir based regimen since it was observed that dolutegravir based regimen group have higher serum creatinine compared to protease inhibitors based groups. As observed from this study there is a need to incorporate a more sensitive marker for

monitoring of renal functions such as urinary microalbumin or serum Cystatin C since urinary microalbumin level was elevated in both patients receiving either of protease inhibitors or dolutegravir based regimen which was not observed with serum creatinine. Also patients on protease inhibitors based regimen whose mean serum total bilirubin is twice the upper limit of normal should be monitored for signs of jaundice.

Author contributions

All authors made a significant contribution to this work, from the conception through the study design, execution, and acquisition of data, analysis and interpretation of results. They equally took part in drafting, revising and critically reviewing the manuscript, gave final approval of the version to be published.

Conflict of interest

There is no conflict of interest between and among authors in any aspect of this work either financially or otherwise.

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