ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

SCHOOL OF MEDICINE, DEPARTMENT OF BIOCHEMISTRY

ASSESSMENT OF RENAL FUNCTION AMONG HIV POSITIVE PATIENTS TAKING TENOFOVIR AND NON-TENOFOVIR CONTAINING HIGHLY ACTIVE ANTIRETROVIRAL THERAPY (HAART) AT AYDER COMPREHENSIVE SPECIALIZED HOSPITAL, MEKELLE, ETHIOPIA

BY:

ZEMENFES GEBREMEDHIN

A THESIS SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES OF ADDIS ABABA UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTERS OF SCIENCE IN MEDICAL BIOCHEMISTRY

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Table of Contents

Acronyms

ACSH	Ayder comprehensive specialized hospital
AIDS	Acquired immunodeficiency syndrome
ARF	Acute renal failure
ART	Antiretroviral therapy
ESRD	End stage renal disease
CD4	Cluster of Differentiation 4
CKD	Chronic kidney disease
CrCl	Creatinine clearance
DDI	Didanosine
eGFR	Estimated glomerular filtration rate
GLDH	glutamate-Dehydrogenase
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
hOAT1	Human organic anion transporter 1
IL1	Interleukin1
IL6	Interleukin 6
mDNA	Mitochondrial DNA
MDRD	Modification of Diet in Renal Disease

MRP2	Multidrug-resistance protein 2
NSAID	Non-steroidal anti-inflammatory drug
NNRTIs	Non-nucleoside reverse transcriptase inhibitors
NRTIs	Nucleoside reverse transcriptase inhibitors
PLHIV	People living with HIV
Pol γ	DNA polymerase γ
STAT	Short Turnaround Time
TASH	Tikur Anbesa Specialized Hospital
TDF	Tenofovir disoproxil fumarate
TFV	Tenofovir
TNFα	Tumor necrosis factor α

List of tables

List of figures

Abstract

Background: *kidney disease is becoming a global burden on public health. The use of HAART worsens the progression of renal failure in HIV patients. Previous studies have incoherent TDF outcomes on renal wellbeing.*

General objective*: To evaluate renal function among HIV positive adult patients taking tenofovir compared with non-tenofovir containing highly active antiretroviral therapy (HAART)*

Methods*: Institutional based cross-sectional comparative study was conducted using a convenience sampling method from May 20/ 2019 to December /20/ 2019 at ACSH, Mekelle, Ethiopia. Socio-demographic and clinical characteristics of the study participants were assessed using medical records, structured questionnaire, anthropometric measuring tools (electronic weighing scale, adult height board), BDFACA Presto for CD4 determination, Pentra machine for analyzing renal function was used and the obtained data was analyzed using SPSS version 25.0.*

Result: *During the enrolment to HAART, a total of(9.1%,n=21) respondents had a lowered GFR level but after the intake of HAART for at least six months, the number of patients with lowered GFR level raised to 16.8%, n=39(9.5% for the TDF group and 7.3% for the non-TDF group). Among the total 39(16.8%) declined GFR cases, thirty (12.9%) of study participants had mild renal dysfunction, 18(81.1%) vs. 12 (70.6%) for TDF and non-TDF groups respectively. But there was no statistically significant correlation between the severity of renal dysfunction as described by GFR and the form of HAART regimen (P=0.31). After HAART initiation, there was no significant mean difference in almost all of the test parameters for renal function. In those who received TDF-group, renal dysfunction is significantly predicted by older age ≥50 years, being male, advanced immune-suppression (CD4<200cell/ml) and ≥ 10 years of HAART intake; whereas those on the non-TDF group, renal dysfunction was significantly predicted by older age ≥50 years and being male.*

Conclusion: *We found no statistically significant differences in renal dysfunction of HIV positive patients on TDF and non-TDF containing HAART regimen, but the overall prevalence of renal dysfunction after six months of HAART follow-up was 16.8%. Therefore, to enhance the early detection of patients at high risk of kidney failure after initiation of HAART, renal function of all patients on HAART must be checked regularly by the health care providers.*

Keywords*- HAART, TDF, duration of ART, non TDF, renal function test parameters, renal dysfunction, renal safety*

INTRODUCTION

1.1. Background

According to the global statistics agency, there were around 37.9 million people living with Human immune virus (HIV) or acquired immune deficiency syndrome(AIDS) worldwide, and an additional 1.7 million people were newly infected worldwide which means 5,000 new infections every day and 7.7 million people have died of HIV in 2018 (UNAIDS, 2019). Although the burden of the epidemic varies across countries and continents, Sub-Saharan Africa, which has just 12% of the global population, accounts for 71% (nearly two-thirds) of the global burden of HIV infection (Kharsany and Karim, 2016). In Ethiopia, nearly 690,000 people were living with HIV, 23,000 newly diagnosed with HIV, and 11,000 died in 2018 from an AIDS-related disease (UNAIDSEthiopia, 2018).

The initiation of highly active antiretroviral therapy (HAART) has been a key to reduce overall morbidity and mortality associated with human immunodeficiency virus-1 (HIV-1) infection and acquired immune deficiency syndrome (AIDS) (Kharsany and Karim, 2016). However, several complications of long-standing infection and long-term treatment have been recognized with increasing frequency. Renal disease is one of the highly prevalent co-morbidity in patients living with HIV (Calza, 2012). According to the National Kidney Foundation Kidney Disease Outcomes Quality Initiative classification; 18.9% patients were in stage one of kidney disease, 10.7% in stage two of kidney disease, 5% in stage three of kidney disease, 0.3% in stage four of kidney disease and no patient was in stage five of kidney disease (Dauchy *et al*., 2011).

HIV-positive patients may have abnormal kidney functions that may be either due to HIV itself known as HIV-associated renal disease, a condition characterized by nephritic range of proteinuria that rapidly progresses to end-stage renal disease (ESRD) (Maggi *et al*., 2012), or Drug-induced kidney injury which often leads to acute renal failure (ARF) indicated by an increase in serum creatinine level more than 30%(>1.3mg/dl (in females) and >1.5 mg/dl (in males), or blood urea nitrogen >20 mg/dl of baseline (Ibrahim *et al*., 2010, Izzedine *et al*., 2005). However, the exact frequency of ART medication-induced renal insufficiency becomes difficult to determine (Izzedine *et al*., 2005). HIV infected patients who are at risk of developing ARF are older and more likely to be men of black ethnicity, had lower CD4 T-cell counts, high viral load and hepatitis C co-infection, hypertension, diabetes, advanced WHO clinical stage of AIDS and abnormal BMI)(Ibrahim et al., 2010, Kumarasamy et al., 2018a,

Labarga et al., 2009, Mekuria et al., 2016a). The occurrence of acute renal failure in peoples living with HIV is also associated with delayed HIV diagnosis (Post and Holt, 2009). A review of over 200 patients in New York with HIV-associated nephropathy also indicated that 90% were black and 70% were male despite the prevalence of HIV is three times more among the white people (D'Agati and Appel, 1997).

Usually, first-line therapy offered to ART-naïve patients included one Non-Nucleoside Reverse Transcriptase (NNRTI) generally called efavirenz (EFV) or nevirapine (NVP), plus two Nucleoside Reverse Transcriptase NRTIs, either lamivudine (3TC) or emtricitabine (FTC), in addition to TDF (Kumarasamy *et al*., 2018a). Studies have shown, however, that some antiviral medications such as indinavir, tenofovir, and atazanavir induce defects in renal function including a reduction in glomerular filtration rate, proximal tubular damage, and acute kidney injury (Calza, 2012, Ibrahim *et al*., 2010, Izzedine *et al*., 2005)

Tenofovir disoproxil fumarate (TDF) belongs to the nucleoside reverse- transcriptase inhibitors (NRTI) used for the treatment of HIV/AIDS since 2001 which was approved by the US Food and Drug Administration (Calza, 2012, Kumarasamy *et al*., 2018a, Tourret *et al*., 2013). It is needed to inhibit HIV replication by halting DNA synthesis from the RNA-dependent DNA polymerase of HIV and is a poor inhibitor of host cell α and β DNA polymerases and of mitochondrial γ DNA polymerase (Tourret *et al*., 2013). TDF is renaly excreted via a combination of glomerular filtration and active tubular secretion causing proximal renal tubular dysfunction (Gallant *et al*., 2005). Despite its proven efficacy, the frequent clinical use of TDF is associated with increased risk of kidney tubular dysfunction, which can manifest as reduced glomerular filtration rate, increased serum Creatinine level, Fanconi syndrome, proximal tubulopathy, nephrogenic diabetes insipidus, acute and chronic kidney injury (Maggi *et al.*, 2012, Ojeh *et al*., 2018, Scherzer *et al*., 2012). Chronic kidney disease (CKD), defined as an estimated glomerular filtration rate (eGFR) below 60 ml/min or the presence of proteinuria, is present in 15–20% of patients infected with HIV (Ibrahim *et al*., 2010). Some other studies have shown that although small decreases in the estimated glomerular filtration rate (eGFR), an increase in glucose and low molecular weight proteins in patients' urine after the use of TDF, they are not associated with increased nephrotoxicity compared to other regimens (Gallant *et al*., 2008, Yombi *et al*., 2014).

Some other studies have also indicated that TDF use is safe for the kidney in clinical practice with an only modest decline in renal function which generally has been reported in patients with advanced HIV disease, decreased renal function at baseline or co-morbidities such as diabetes, and hypertension (Eneyew *et al*., 2016, Gallant *et al*., 2005, Labarga *et al.*, 2009). Co-administration of TDF with other ART drugs may compete for the same pathway in order to be removed by active tubular secretion which may increase concentrations of either TDF itself or the other co-administered drug, resulting in renal tubular damage (Gallant *et al*., 2005).

Therefore, since there are limited data available on renal dysfunction associated with TDF and non-TDF containing HAART in Ethiopia, the aim of this study is to evaluate the harmful effects of TDF as compared to non-TDF alternative NRTIs containing combination ART on renal function in Ayder Comprehensive Specialized Hospital and to suggest early identification and effective management.

1.2. Statement of the problem

The renal complication of HIV is increasingly prevalent (e.g. ARF incidence rate was 2.8 (95% CI 2.41–3.24)(Ibrahim *et al*., 2010) and becoming a global health issue as the prevalence of new infection is increasing from time to time (D'Agati and Appel, 1997).

Whether or not long-term use of TDF is detrimental to the kidney is widely debated and the effect of renal insufficiency associated with TDF is variable in various settings (Tourret *et al.*, 2013). Studies on the assessment of renal function in TDF uses have suggested that the use of this drug induces defects in renal function (Brennan et al., 2011, Calza et al., 2013, Jung et al., 2018, Kumarasamy et al., 2018b, Mulenga et al., 2008), on the other hand, multiple studies indicate that there is no significant difference in renal function in TDF and non-TDF patients with HAART. (Eneyew et al., 2016, Gallant et al., 2008, Labarga et al., 2009, Salome et al., 2016b).

According to a study conducted in Nigeria, the incidence of renal dysfunction among TDF-exposed patients is twice as compared with patients taking non TDF drug regimen (Ojeh *et al.*, 2018). A prospective observational study conducted by Taklo Simeneh and his colleagues on the impact of TDF on renal function at TASH, Ethiopia, revealed that renal dysfunction (defined as a decline in eGFR greater than 25 percent) was found in 25 percent of the participants. However, because the medication (TDF) is administered in conjugation with other drug regimens, it creates difficulties in deciding whether TDF will cause renal dysfunction (Yazie *et al*., 2019). The two opposing ideas need therefore to be negotiated through further study. In addition, PLWH with moderately/ severely impaired renal function before their initiation to ART was found to improve their eGFR after the initiation of ART(Deckert et al., 2017b). Therefore, further study is needed to improve the evidence pointing to its cause.

Co-administration of tenofovir with didanosine has resulted in a significant increase (28%) in maximum serum concentrations of didanosine because both of the drugs compete for the same transporter hOAT1 to enter tubular epithelial cells of the kidney in order to be excreted which leads to an increased risk of didanosine(ddi) toxicity resulting in an increased risk of mitochondrial damage and nephropathy (Poizot-Martin *et al*., 2013).

Mitochondrial DNA(mtDNA) of Kidney depletion is also associated with HIV infection and concurrent TDF/ddi therapy use, while kidney ultra-structural mitochondrial abnormality was seen with TDF use alone so that the findings of this study recommends to reduce doses of ddI

when used in conjunction with TDF (Côté *et al*., 2006). So this study will also assess the severity and magnitude of renal function abnormalities of the combination of these two drugs.

1.3. Justification of the study

This research aims at determining the extent and nature of renal function abnormalities in HIV positive patients who are taking TDF and non-TDF containing ARTs by evaluating renal test parameters comparatively. After the advent of antiretroviral therapy, the life expectancy of HIV patients has been increased. It has been documented that when HIV patients are exposed to HAART, a high rate of viral suppression and increment in CD4+ count is observed but followed up with kidney disease and renal toxicity problems. Furthermore, the safety of TDF and predisposed patients to renal diseases and the risk of developing CKD in adults have been observed and predicted in African populations and drawn many different conclusions. Therefore this study aims to provide useful information that will negotiate the two reported opposing ideas on TDF's to the kidney. So far, no published research was conducted regarding the effect of TDF on renal function disorders in Ethiopia compared to other alternative ART medicines. Since fewer studies are conducted, this will have a great role in providing useful information on renal function tests in association with ART drugs. There is also a scarcity of documenting on renal changes due to 2^{nd} line ART in sub-Saharan Africa. Therefore, the finding of this study is expected to help both the health care providers and HIV positive patients taking HAART on dose adjustment and proper drug switch to minimize renal impairment caused by the medications. In addition, this may serve as baseline data for the researchers in order to initiate further studies on the risk of development of end-stage renal diseases.

2. LITERATURE REVIEW

2.1. Incidence and risk factors for TDF induced nephropathy

According to the retrospective cohort study conducted at Lusaka, Zambia, the point prevalence of renal dysfunction was 18.6% (95% CI; 0.2-28.3) (Wantakisha *et al*., 2017). Another comparative cross-sectional study done on ART naïve and ART experienced patients in southern Ethiopia found that the overall prevalence of renal function impairment was 18.2% [95%CI: 14.6–21.7] (Mekuria et al., 2016a). Renal disease is more common and an increasingly prevalent cause of morbidity and mortality in HIV Positive individuals than in the general population (Calza, 2012), with ARF incidence rate of 2.8 (95% CI 2.41–3.24) episodes per 100 person-years (Labarga *et al*., 2009). The development of renal disease among HIV positive patients can be due to the HIV itself known as HIV-associated renal disease or drug-induced kidney injury that is a major side effect in clinical practice, frequently leading to acute renal failure (ARF)(Ibrahim *et al*., 2010, Maggi *et al*., 2012).

Patients who developed ARF are older and more likely to be men of black ethnicity and hepatitis C confection (Ibrahim *et al.*, 2010). The overall prevalence of CKD was 18.6% for the age of 50–59, 28.5% for the age of 60–69, and 47% for over 70 years old. CKD has been associated with older age, heavier body weight, diabetes mellitus, hypertension, and longer duration of ART but not specifically due to TDF exposure (Nishijima et al., 2017a). Another drug Indinavir is associated with increased risk of CKD, whereas efavirenz and zidovudine are associated with decreased risk (Scherzer *et al*., 2012). Another study also indicated that advanced immune-suppression (defined as a baseline CD4 cell count of <50 cells/mm3 was associated with a change of 14% (vs. a change of 8% for patients with a baseline count of >50 cells/mm3 ($P < .001$) and patients who are diabetic and taking TDF was associated with a 13% change in CrCl (vs. a change of 8% in patients taking TDF but without diabetes [$P < .04$) (Gallant *et al*., 2005). Patients who are 40 years of age or more (the higher risk being 50–59-years age group) (Kumarasamy *et al*., 2018a), male patients, those with low hemoglobin, hypertensive patients, those with low BMI and taking TDF are also at increased risk of nephrotoxicity (Brennan et al., 2011, Kumarasamy et al., 2018a, Nishijima et al., 2017a, Ojeh et al., 2018). A review of over 200 patients done in New York with HIV-associated nephropathy indicated also that 90% were black and 70% were male despite the prevalence of HIV is three times more among the white people (D'Agati and Appel, 1997).

A medical review done on Antiviral Drug-Induced Nephrotoxicity indicated that renal injury was associated with the use of ART (Izzedine *et al*., 2005). Another retrospective cohort study done on patients taking ART in Lusaka, Zambia also found that among 1118 PLWH (63,3% female with a mean age of 41.8 years, 83% ever on TDF with a median duration of 1461 [range 98 to 4342] days on ART, 28.3% had an eGFR <90 ml/min, and 5.5% <60 ml/min(Deckert et al., 2017a). According to a study done in Nigeria, the incidence of renal impairment among the TDF-exposed and TDF-unexposed groups was 4.6% and 2.3% respectively at 48 weeks (Ojeh *et al*., 2018). Another prospective observational study conducted at Tikur Anbessa Specialized Hospital(TASH), Ethiopia by Taklo Simeneh and his colleagues indicated that renal dysfunction (defined as a decline in eGFR greater than 25%) found in a 25% of the study participants (Yazie *et al*., 2019). A prospective study conducted in Zambia also found a high prevalence of renal dysfunction among HIV positive adults on TDF containing HAART. The risk of renal dysfunction in these populations was more common among older patients with low CD4 counts, suggesting that they need close monitoring of their renal function when they want to initiate HAART-containing TDF(Wantakisha *et al*., 2017).

According to a study conducted on 344 patients who received TDF and 314 Patients who received an alternative NRTI, The TDF group had significantly greater increases in serum Creatinine levels and decreases in absolute and percentage ClCr, compared with the NRTI group (Mekuria et al., 2016a). TDF use was also associated with an 11% increased risk of rapid decline in creatinine clearance per year of exposure (P<0.0033) (Scherzer *et al*., 2012) and 4% (Gallant *et al*., 2005). Another cohort study in Johannesburg, South Africa, conducted in 890 patients initiated on tenofovir claimed that 573 (64.4%) had normal renal function (90 ml/min), 271 (30.4%) had mild renal dysfunction (60–89 ml/min) and 46 (5.2%) had moderate renal dysfunction (30–59 ml/min)(Brennan *et al*., 2011). Despite that, PLWH who had moderately/ severely impaired renal function before their initiation to ART are found to improve their eGFR during ART (Maggi *et al*., 2012).

A prospective cohort study conducted during the period 2012 through 2016 evidenced that the cumulative mean change in creatinine level was significantly related to the TDF exposure period(P<.001)(Jung *et al*., 2018). According to a study conducted in Bologna, Italy, on HIV infected patients taking TDF containing second-line ART, revealed TDF use is the only statistically significant predictor (p <0.05) of a greater than 20% decline in eGFR(Calza *et al*., 2013). Another study done on 3209 individuals starting tenofovir in Lusaka, Zambia, found

that when GFR was calculated by the MDRD equation, (12.4%; 95%, CI: 12.0%, 12.9%) had renal insufficiency: 2397 (74.7%) of them were mild, 642 (20.0%) were moderate, and 170 (5.3%) were severe (Mulenga *et al.*, 2008). In a cohort study of 10841 HIV-infected patients exposed and non-exposed to TDF found that Proportion with baseline eGFR less than 60 ml/min per 1.73m2 was slightly higher among those unexposed to TDF. However, the incidence was low for both eGFR less than 45 ml/min per 1.73m2 (237 events) and eGFR less than 30 ml/min per 1.73m2 (124 events) respectively (Scherzer *et al*., 2012). The median change from baseline to week 144 in estimated GFR by Cockcroft–Gault was 2 and 0 ml/min in the TDF and control groups, respectively (P=0.81) (Gallant *et al*., 2008). Following exposure to TDF, the median glomerular filtration rate (GFR) was 100.82 ml/min per 1.73m2 (IQR: 84.63–115.89), and Proteinuria was present in 46 (11.6%) patients and albuminuria in 128 (31.8%) (Dauchy *et al.*, 2011). According to a prospective cohort study done in Tikur Anbesa Hospital of 6 months follow up, the prevalence of proteinuria was higher than its baseline (27% and 20.6%, respectively). Among the 27% of patients with proteinuria; 14.3% of them were found to have renal dysfunction. However, the prevalence of glycosuria was the same as the prevalence of baseline glycosuria (4.8%) *(*Yazie *et al.*, 2019).

In a study of 284 consecutive HIV patients, 154 on TDF, 49 on other HAART regimens and 81 drug-naïve found that no significant difference in Creatinine clearance was observed among the comparing distinct groups but the proportion of patients with tubular damage in groups on TDF, on another HAART and treatment naïve patients was 22, 6 and 12%, respectively suggesting that, the only independent predictors of tubular dysfunction is TDF use (P<0.001) (Labarga *et al*., 2009). A year exposure to TDF increases in 33% prevalence of CKD and 30% of proteinuria (P<0.0001) but the risk of proteinuria and incidence of rapid decline in Creatinine clearance appeared after 3 years exposure to TDF (Scherzer *et al*., 2012). An Indian cohort study conducted during the period 2002 through 2017 of 7171 patients on TDF containing ART evidenced that the proportion of patients who developed grade 3 or 4 renal dysfunction was 5.6%, with an incidence rate of two cases per 100 person-years (Kumarasamy *et al*., 2018a). According to a retrospective cross-sectional comparative study conducted in 2016 in Tikur Anbesa Hospital disclosed that Serum Creatinine level was higher in patients on HAART groups than in control groups but there is no significant difference in serum Creatinine clearance and glomerular filtration rate between control groups and patients on HAART (Eneyew *et al*., 2016). Another prospective cohort study in France, also claimed that out of 22

603 subjects initially with normal eGFR of (≥90 mL/min) followed until their eGFR <60 ml/min and found that 131 (0.6%) experienced CKD during a median follow-up duration of 4.5 years (interquartile range [IQR of 2.7–6.1 years]) (Ryom *et al*., 2013).

TDF treatment was discontinued for patients taking TDF containing HAART that have abnormal laboratory findings and glomerular filtration rate shows a slight increment after a mean duration of follow-up of 7.5 months (range, 3–20 months) (Zimmermann *et al*., 2006). This is supported by another study concluding that, among those who discontinued TDF use, the period following cessation was not significantly associated with either higher or lower risks of proteinuria, Creatinine clearance and CKD which suggests that the effects of TDF on kidney disease risk is not reversible following discontinuation when compared with those never exposed (Scherzer *et al*., 2012).

According to study done on the renal safety of TDF concluded that the rate of discontinuation of therapy at the time of maximum decline in renal function is slightly higher among other NRTI-treated patients 21 (6.7%) compared to TDF-treated patients 19 (5.5%) (Gallant *et al*., 2005). Another three years follow up study conducted in 1111 patients on renal safety of TDF compared to the other drugs either stavudine or zidovudine in combination with efavirenz or lamivudine found that a similar proportion of patients experienced urine proteinuria at least 100 mg/dl (TDF, 5%; control, 6%) and The median change of glomerular filtration rate from baseline to week 144 was 2 and 3 ml/min in the TDF and control groups respectively ($P<0.05$)(Gallant *et al*., 2008). Considering discontinuation of TDF due to renal adverse events, no patient in the TDF group discontinued, whereas one patient in the control group discontinued due to acute renal failure (Gallant *et al*., 2008). A study conducted in Johannesburg, South Africa, claimed also that despite TDF use was associated with a statistically significant loss of renal function, the clinical magnitude of this effect is modest, so that the findings do not support the need to restrict TDF use (Cooper *et al.*, 2010). To another prospective cohort study conducted in Ugandan adults on long-term antiretroviral therapy, there is no difference in renal function abnormalities among patients on Tenofovir and non-Tenofovir containing ART for almost a decade follow up. Therefore Tenofovir based first-line ART can safely be initiated even in settings without routine renal function monitoring (Salome *et al.*, 2016b)(Table 1).

Table 1: Pharmacologic classification of antiretroviral drugs

NRTIs	NNRTIs	PIs
Zidovudine (AZT)	Efavirenze (EFV)	ritonavir-boosted lopinavir= LPV/r
Lamivudine (3TC)	Nevirapine (NVP)	Ritonavir boosted atazanavir =ATV/r
Abacavir (ABC)		
Didanosine (ddi)		
Stavudine (d4T)		
Tenofovir Disoproxil Fumarate (TDF)		

Note: NRTIs =nucleoside reverse transcriptase inhibitors, NNRTIs =non- nucleoside reverse transcriptase inhibitors; PI =protease inhibitors (Gilks et al., 2006)

Concerning first line and second line TDF containing HAART, an Indian cohort study conducted during the period 2002 through 2017 disclosed that there is no difference in the incidence of tenofovir toxicity between the first- and second-line drug regimens (Kumarasamy *et al*., 2018a)(Table 2).

Table 2: First line and second line drug regimens recommended to adults

Fist line drug regimens	**Second line drug regimens**
1a=d4T+3TC+NVP	2a= ABC+ddi +LPV/r
1b=d4T+3TC+EFV	2b= TDF+ ddi+ LPV/r
1c=AZT+3TC+NVP	2c=TDF + 3TC+ LPV/r
1d=AZT+3TC+EFV	2d = AZT + 3TC + LPV/r
1e=TDF+3TC+EFV	
1f=TDF+3TC+NVP	

Note: d4T= Stavudine ; AZT= Zidovudine ; LPv/r = ritonavir, enhanced Lopinavir;

ABC = Abacavir ; ddI = Didanosine ; 3TC = Lamivudine; EFV= Efavirenze; TDF= Tenofovir Disoproxil Fumarate; NVP= Nevirapine (Gilks et al., 2006)

2.2. Pathogenesis of TDF induced nephropathy

Following oral administration, TDF is converted in the gut to tenofovir (TFV). TFV, the renal active moiety, enters the bloodstream and has a half-life elimination of almost 30 hours in people with normal kidney function (Yombi *et al*., 2014). TDF can be toxic to mitochondria by creating structural abnormality and depleting Kidney mitochondrial DNA (mtDNA) count (Herlitz *et al*., 2010, Rodriguez-Novoa *et al.,* 2009). The successful uptake of nucleotides from the blood into the proximal tubule of nephrons occurs through hOAT1, which is located in the basal-lateral membrane of the proximal tubules. Once the nucleotides had accumulated, they secreted into the urine on the apical side of the proximal tubular cell via the multidrug-resistance protein (MRP2) and multidrug-resistance protein (MRP4).

The intracellular concentrations of TDF can be modified by drugs such as lopinavir and/or ritonavir; that specifically inhibit these receptors resulting in an increment of TDF concentrations that could increase the development of tenofovir-associated ARF (Zimmermann *et al*., 2006). Once inside a mitochondrion, TDF inhibits DNA polymerase γ, which results in a progressive depletion of mitochondrial DNA, a decreased synthesis of respiratory chain proteins, and morphologic abnormalities of mitochondria (enlargement, loss of cristae). Due to damage in the cristae, certain proteins in the respiratory chain are released into the cytoplasm, and they can be detected by the caspase pathway and then triggered cell apoptosis (Tourret *et al*., 2013).

Co-administration of TDF with didanosine has resulted in a significant increase (28%) in serum concentrations of didanosine, Since didanosine competes with TDF in the proximal tubules for the same hOAT1 transporter, this results in an increase in didanosine concentration which ultimately aggravates mitochondrial damage and nephropathy (Poizot-Martin *et al*., 2013). TDF's renal clearance is significantly higher than the glomerular filtration rate, which shows that tenofovir has a renal tubular secretion (Zimmermann *et al*., 2006)(figure 1).

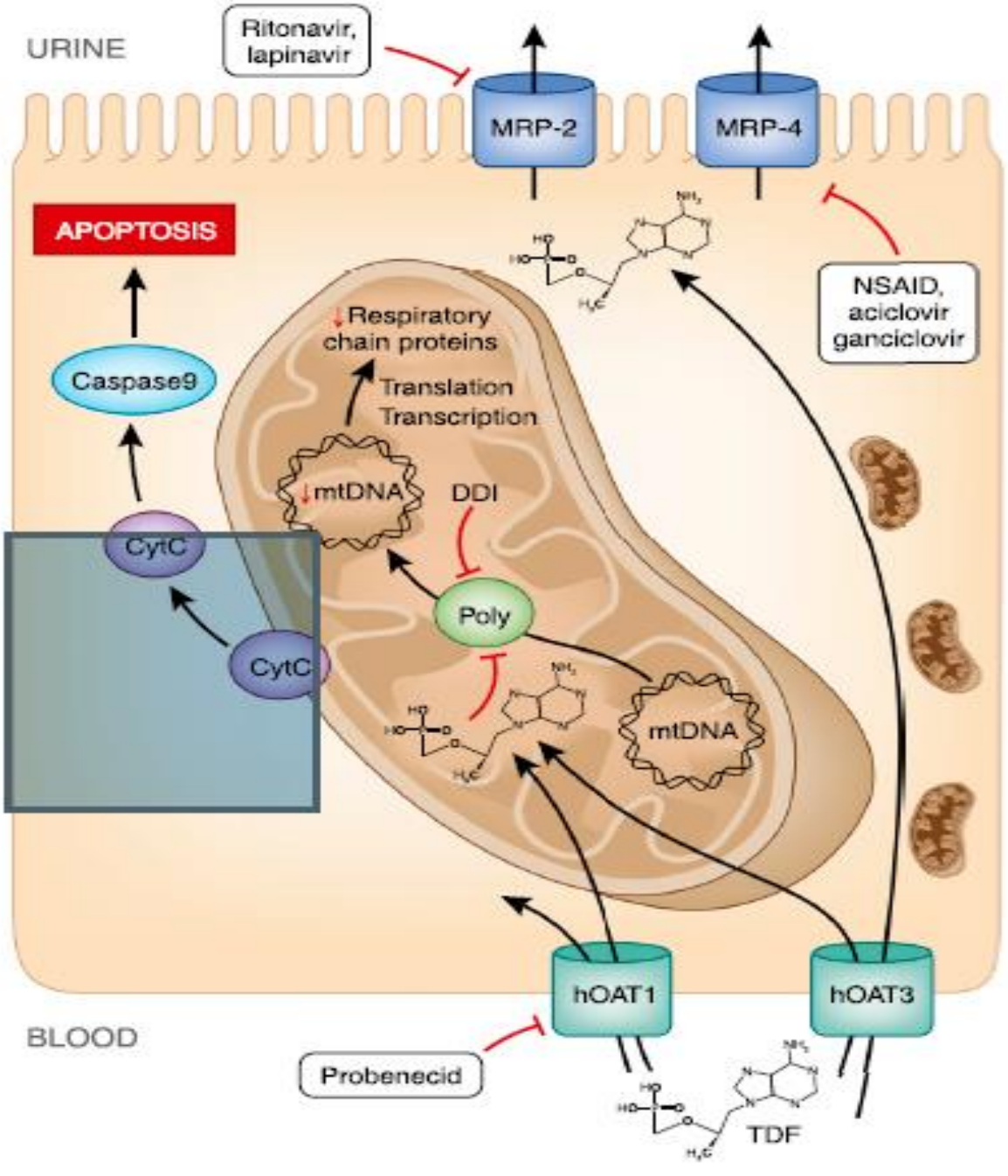


Figure 1: *Pathogenesis of TDF induced nephropathy*

Cytoplasmic accumulation of TDF is responsible for the degradation of mDNA and dysfunction of the respiratory chain; resulting in apoptosis of the renal epithelial cells. (Adopted from (Tourret *et al*., 2013)). Polγ= DNA polymerase γ; CytC= cytochrome C; NSAID=non-steroidal anti-inflammatory Drug; HOAT= human organic anion transporter 1; MRP2 =multidrug-resistance protein 2; DDI=didanosine

2.3. Pathogenesis of HIV associated nephropathy

HIV associated nephropathy is a unique pattern of sclerosing-glomerulopathy that ranges in prevalence from 1-10% of HIV infected patients. This complication of HIV will likely become a global health challenge as the prevalence of new infection is increasing from time to time (D'Agati and Appel, 1997). In HIV related nephropathy, the nature of the host response to viral infection is likely to be critical to the development of nephropathy. HIV infection can deregulate a variety of host cytokines such as IL6, IL1, and TNFα which could be important in the development of nephropathy (Buonaguro *et al*., 1992). HLA- linked responses particularly to a subset of blacks and biological heterogeneity in the strains of HIV-1 that could account for a particular nephritogenic strain (D'Agati and Appel, 1997). Mechanism of HIV leading to nephropathy could follow any of the following schemes:

1. Direct injury to renal epithelial (visceral and tubular cells) by cytopathic effects of viral infection of renal parenchymal cells, 2. Indirect injury to the kidney by renal cellular uptake of circulating virally encoded molecules; or 3. Indirect injury to the kidney through the release of cytokines by infected lymphocytes or monocytes in the circulation or infiltrating the kidney. The final common pathway in the development of HIV-associated nephropathy is the involvement of altered patterns of gene expression of renal parenchymal cells by cytokines and growth factors leading to interstitial fibrosis (figure 2).

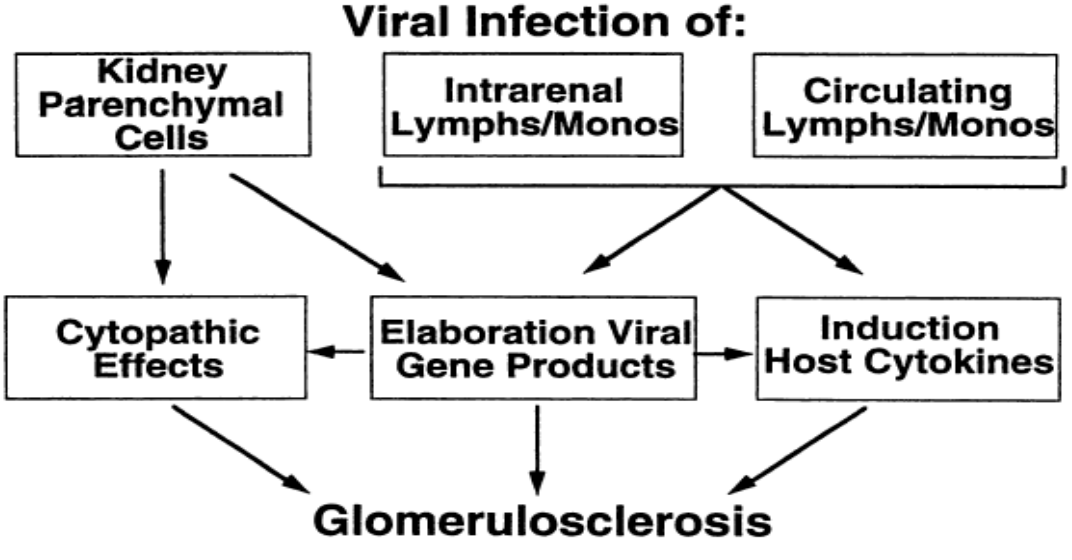


Figure 2: *Hypothetical pathogenesis of HIV-induced nephropathy(adapted from*(D'Agati and Appel, 1997). Lymphs= lymphocytes; Monos= monocytes

3. OBJECTIVES

3.1 General objective

➢ To assess renal function among HIV positive adult patients taking tenofovir and non-tenofovir containing highly active antiretroviral therapy (HAART) at ACSH, Mekelle, Ethiopia, 2020.

3.2 Specific objective

- To measure serum creatinine level of the study participants
- To estimate the glomerular filtration rate of the study participants
- To test blood urea nitrogen of the study participants
- To analyze the urine of the study participants
- To compare the severity of renal function abnormalities among TDF and non-TDF containing ART drug users based on the operational definition

Hypothesis

Null hypothesis (Ho):

- There is no difference in renal function abnormalities in patients taking TDF and non-TDF containing HAART

Alternative hypothesis (Ha):

- There is a difference in renal function abnormalities in patients taking TDF and non-TDF containing HAART

4. METHODOLOGY

4.1. Study design and study period

A hospital-based comparative cross-sectional study was performed from May 20, 2019-December 20, 2019. Because the prevalence of exposure to TDF and non-TDF containing ART drugs in the comparing group was assessed at a single point in time in association with renal function abnormalities.

4.2. Study Area and Study Population

Ayder comprehensive specialized hospital (ACSH) is located in Tigray, Ethiopia. It is one of the specialized teaching hospitals in Tigray regional state. ACSH began its referral and non-referral services in 2008 to more than 8 million people in its catchment areas of Tigray, Northeastern parts of the Amhara Regional States, and Afar. It gives a broad range of medical services to both in- and out-patients of all age groups. It has a total capacity of about 500 inpatient beds in four of the major departments and other specialty units such as ART clinic. In 2018/19, there were about 1360 adult patients in the ART Clinic. Of these, 1265 were in first-line and 95 were in second-line therapy (HMIS, april 2019).

4.3. Inclusion and exclusion criteria

4.3.1. Inclusion criteria

The research included HIV positive patients over 18 years of age (adults) who had been taking ART at ACSH for at least 6 months and who were willing to participate in the study.

4.3.2. Exclusion criteria

- The study excluded patients who had acute or chronic kidney disease, diabetes mellitus, and hypertension.
- Patients who were taking nephrotoxic drugs; or pregnant; or patients who were HBsAg, HAV and HCV positive patients were excluded from the study.
- Patients with CD4+ count <50mm/dl were excluded from the study
- NB: Since patients with the above-mentioned medical conditions are at high risk of developing renal dysfunction, this contributes to the study's false-positive test results. Those patients, therefore, need to be excluded from the study.

4.4. Study variables

4.4.1. Dependent variable

- Creatinine, eGFR, Blood Urea Nitrogen(BUN), serum total protein, uric acid, Glucosuria, and proteinuria in all the two groups

4.4.2. Independent variable

- Socio-demographic characteristics (age, educational status, residence, marital status, and occupation)
- Alcohol usage and smoking
- Duration of HAART intake, BMI, viral load and CD4+ level
- Type of ART drug regimen

4.5. Sample size determination

Sample size was determined using a formula $n = z^2 p (1-p)/E^2$

Where n=required sample size

z=reliability coefficient at 95% confidence interval (standard value 1.96)

p= proportion of the target population that have renal impairment among TDF exposed. This was estimated from the previous study done on Lusaka, Zambia, the population proportion of renal impairment among TDF exposed was found to be 18.6% (Wantakisha et al., 2017).

E =marginal error at 5 %(standard value 0.05)

$n= (1.96)^2(0.186) (0.814)/ (0.05)^2$

n=232

These number of patients who full fill the inclusion criteria were split in to two and participants were given equal chance to recruited in the study

4.6. Sampling technique & procedures

Convenience sampling method was employed. Participants in the study was given equal opportunities to be enrolled, but since all consecutive patients who fulfilled the inclusion criteria were involved until the desired sample were reached in each of the study groups, it was found that 158 individuals were on TDF containing HAART group and the remaining 74 were on HAART group containing non-TDF.

4.7. Data collection materials and Data processing methods

Before the data was collected, the data collectors were trained by the principal investigator. Trained clinical nurses gathered socio-demographic and related clinical data by interviewing the study participants using a structured, local language based questionnaire (Tigrigna). Laboratory professionals obtained 5 ml of Blood specimens from each of the study participants for laboratory testing.

4.8. Data quality control issue

The whole data collection process was controlled by the principal investigator and the collected data was checked each day for its completeness, consistency, and clarity. After the sample was collected, known two standard controls (normal and abnormal) sample were run every 24 hours in order to ensure the functionality of the machine. Following the established quality control procedures that meet the acceptance criteria; the patients sample were run by the machine.

4.9. Medical conditions and other associated risk factors

A prior medical condition such as kidney disease, diabetes mellitus, hypertension, and hepatitis A, B, and C was either asked orally or their medical card was checked to exclude patients with this disease from the sample. Study participants' weight was measured using electronic weighing scale and height measured using meter reading, and body mass index was obtained by the formula; body mass index = weight (kg)/(height(m))^2. The research participants' blood pressure was assessed using a sphygmomanometer (Hgmm) and individuals with the reading unit beyond the WHO recommendations were excluded from the study. Time of HAART initiation, viral load, and CD4+ counts were recorded using a standard questionnaire from the SMART CARE and medical record card.

4.10. Blood sample collection

After tying up the patient's upper arm using a tourniquet, 5 ml of blood was drawn from the ante-cubital vein of each study participant using 5 cc syringes by qualified professionals. The blood samples from each of the study participants were collected on serum separator tubes and sent to the central laboratory for about 4 minutes to be centrifuged at 6000 RPM to extract serum. The serum was used for the determination of biochemical analysis like Serum total protein, blood urea nitrogen, uric acid, and Creatinine. The eGFR was calculated from the serum Creatinine level, age, gender, and weight of the subjects studied.

4.11. Determination of Biochemical Analysis

4.11.1. Determination of Creatinine from Jaffe's assay

The first suggesting evidence of renal dysfunction can be an elevated serum Cr (SCr) level but it may not be an accurate measure of renal function since it is low in the elderly and persons with low muscle mass and can be high in African Americans and persons with high muscle mass. At an alkaline pH, Creatinine reacts with a picrate to form an orange-red Creatinine-picrate complex. As a result of Creatinine-picrate complex formation, the rate of absorbance increase at 510 nm is directly proportional to the Creatinine concentration present in the sample (JOURILABS, 2019).

Creatinine + alkaline picrate $\longrightarrow$ Creatinine-picrate complex

The detail of the procedure is included in annex part.

4.11.2. Measurement of eGFR

GFR indicates the rate of fluid filtering through the kidney. Serum Creatinine (SrCr) is a useful measure for approximating GFR; however, SrCr is slightly higher than the actual GFR level because, in addition to being filtered by the glomerulus, Creatinine is secreted through proximal tubule. Thus, it is difficult to diagnose renal insufficiency by looking at the serum Creatinine level alone. Therefore, accurate detection of chronic renal disease is achieved by estimating the glomerular filtration rate (GFR). In clinical practice, GFR estimated from serum Creatinine using either the Modification of Diet in Renal Disease (MDRD) study equation or the Cockcroft-Gault (CG) equation but the best method being the MDRD equation.

Calculation: GFR = 175 × (SCr)−1.154 × (Age)−0.203 × (0.742 if female) × (1.212 if African American) (Imai *et al*., 2007)

4.11.3. Determination of BUN

BUN is a waste product that is excreted by the kidney from dietary products. It is often used to measure the extent of kidney function but is less accurate than GFR as how much fluid someone drinks, how much dietary protein eats and some other variables can influence it. The concentration of BUN in serum shall be calculated using the enzymatic method of conductivity scale. In a reaction cup containing an electrode that responds to changes in the conductivity of the solution, a serum volume of pieces was inserted into the urease reagent and water. In the presence of water and urease, then, Urea is hydrolyzed to produce ammonia and carbon

dioxide. The ammonia from this reaction, in the presence of glutamate-dehydrogenase (GLDH), combines with 2-oxoglutarate and NADH to produce glutamate and NAD+. The test was designed in such a way that the GLDH is the enzyme limiting factor. The decrease in absorbance during the given time intervals is proportional to the concentration of the urea. This test is preferably designed for analyzer application because the kinetics is very fast. Electronic circuits determine the rate of conductivity increase in the sample which is direct proportional to the urea(JOURILABS, 2019).

$$\text{Urea} + 2H_2O \xrightarrow[\text{Urease}]{} 2NH_4^+ + CO_3^{2-}$$

$$\text{2-Oxoglutarate} + 2NH_4 + \text{NADH} \xrightarrow[+\text{GLDH}]{} \text{L-glutamate} + H_2O + NAD^+$$

4.11.4. Determination of total serum protein

Serum total protein is a valuable measure for testing the activity of the kidney and identifying various disorders involving renal problems. After 2drops of serum are poured into the refractometer, the serum protein will be calculated by digital refractometry and switch on the battery, then press the red button. The color absorbance is immediately proportional to concentration. The typical range of blood for protein levels is 6.0-8.3 g / dl. In an alkaline solution, the cupric ion interacts with protein to form a purple complex. This complex is absorbed proportionally to the concentration of proteins in the sample

Reaction principle

$$\text{Protein} + Cu^{++} \xrightarrow{\text{Alkali}} \text{Protein-Copper Complex(JOURILABS, 2019)}$$

4.11.5. Determination of uric acid

Uric acid reagent is used by a timed-endpoint method to measure the concentration of uric acid. Uric acid is oxidized to form allantoin and hydrogen peroxide by uricase. The hydrogen peroxide reacts in a reaction catalyzed by peroxidase to create a colored substance with 4-amino-antipyrine (4-AAP) and 3, 5-dichloro-2-hydroxybenzene sulfonate (DCHBS). The machine automatically proportions the corresponding sample and reagent volumes into a cuvette. The ratio used is between one part sample and 25 part reagent. The machine tracks 520 nanometers of change in absorbance. Such an increase in absorbance is directly proportional to the sample concentration of Uric Acid, which is used by the system to measure which express the concentration of Uric (JOURILABS, 2019).

Reaction Principle

$$\text{Uric acid} + 2H_2O + O_2 + \text{Uricase} \longrightarrow \text{Allantoin} + CO_2 + H_2O_2$$

$$H_2O_2 + 4AAP + EHSPT \xrightarrow[Mg^{++}]{\text{Peroxidase}} \text{Quinoneimine}$$

4.12. Determination of Urine analysis

After the collection of urine by a 30 ml urine cup, the dipstick was placed into the urine sample ensuring all zone of the dipstick is immersed. Then remove the dipstick from the sample, lay the strip horizontally on a paper towel in order to avoid cross-contamination. Then each of the tests was interpreted at the appropriate time interval using the dipstick analyzer chart**.** From this test proteinuria and glycosuria were determined.

4.13. Statistical analysis

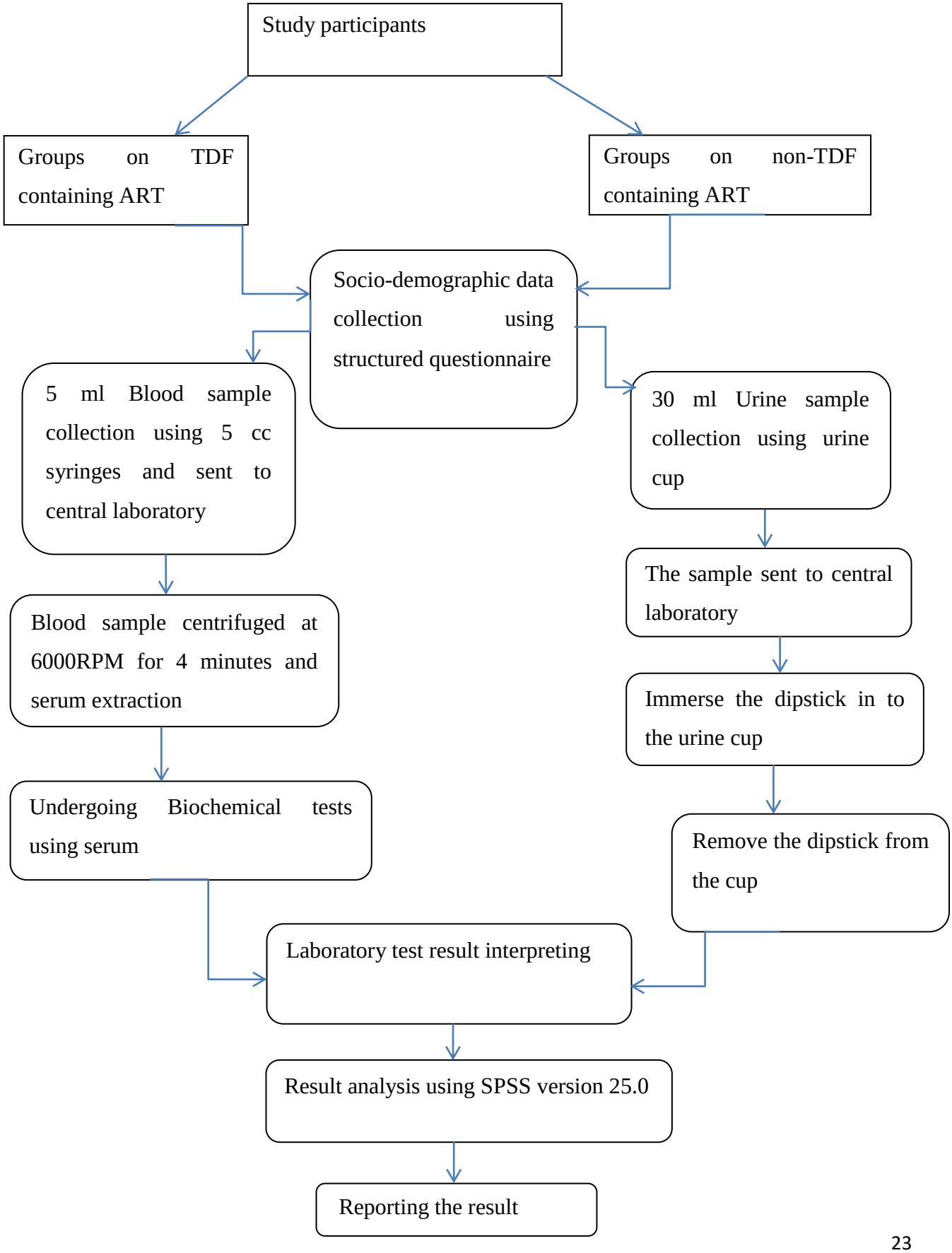
Before analyzing the data, it was cleaned manually and entered into SPSS window version 25.0 software. Descriptive statistics of numeric variables presented in medians with interquartile range (IQR) and categorical variables presented using frequency and percentages. Association among categorical variables was assessed using a Chi-square test (χ^2) and the strength of association was assessed using Pearson's correlation. For this analysis, significance level was set at 0.05. Frequencies, percentage, mean, and median were used to describe the data. Finally, data was presented in the form of text, tables, and figures.

4.14. Operational definition

- Patients on TDF containing HAART: If the patient was taking TDF containing ART drug regimen at least for the last six months
- Patients on non TDF containing HAART: if the patient was taking non TDF containing ART drug regimen at least for the last six months
- Normal renal function: eGFR>90 ml/min
- Stage 1 renal dysfunction: eGFR >90 ml/min but with abnormal laboratory renal test results
- Mild renal dysfunction (stage2): eGFR =60-89 ml/min

- Moderate renal dysfunction (stage 3): eGFR =30-59 ml/min
- Severe renal dysfunction (stage 4): eGFR =15-29 ml/min
- Extremely severe renal dysfunction (stage 5): eGFR < 15ml/min
- Alcoholic: an individual who drinks at least one bottle of beer, one glass of liquor or wine per day
- Cigarette smoker: an individual who smokes ≥ one piece of cigarette per day
- Chat chewer: an individual who chews ≥ 70 g of chat almost daily
- Addiction: an individual who is alcoholic and/or cigarette smoker and/or chat chewer
- Early-stage of AIDS: an individual who is on the WHO clinical stage of one or two
- advanced AIDS stage: an individual who is on WHO clinical stage three or four
- normal BMI: a client who has a BMI measure of 18.5 kg/m^2 up to 24.9 kg/m^2
- abnormal BMI: a client who has a BMI measure of less than 18.5kg/m^2 and/or ≥25 kg/m^2

5. Flow chart


Study participants
Groups on TDF containing ART
Groups on non-TDF containing ART
Socio-demographic data collection using structured questionnaire
5 ml Blood sample collection using 5 cc syringes and sent to central laboratory
30 ml Urine sample collection using urine cup
Blood sample centrifuged at 6000RPM for 4 minutes and serum extraction
The sample sent to central laboratory
Immerse the dipstick in to the urine cup
Undergoing Biochemical tests using serum
Remove the dipstick from the cup
Laboratory test result interpreting
Result analysis using SPSS version 25.0
Reporting the result

6. Ethical consideration

The study was approved by the Research and Ethics Committee of the Department of Biochemistry, School of medicine, College of Health Science, Addis Ababa University with the reference number of SOM / BCHM/122/2011, meeting number of DRERC 01/19 and protocol number of M.Sc.13/19 as of 18/03/2019. Written Formal letter of support from the Department of Biochemistry, Addis Ababa University has been sent as of May/09/2011 E.C to Ayder Comprehensive Specialized Hospital with reference number SOM / BCHM/120/2019. The objective of the study was explained to the concerned bodies of Ayder Comprehensive Specialized Hospital to obtain permission. The head of the Dean College of Ayder Comprehensive Specialized Hospital showed his consent by sending the letter to the ART clinic. The research was also given priority to the study participants and the right they have to withdraw from the study. All of the participants in the study had written informed consent. Participants of the study were coded in order to ensure confidentiality, and their information was kept secret during data analysis.

7. Results

7.1. Socio-demographic characteristics of the study participants

The study included a total of 232 HIV-positive individuals (158 on TDF and 74 on non-TDF-containing regimen) who had regular ART clinic follow-up at ACSH for a minimum of six months.

The age of the respondents included in this study ranged from 18 to 67 years with a median age overall of 40.5 years and an IQR of (35, 48 years).

The majority of study participants 136 (58.6%) were female, 183 (78.9%) were currently married, 152 (65.5%) attended high school and/or above and 175 (75.4%) were urban dwellers.

With respect to the respondents' income, 65.5% had a regular income and there was no significant difference in almost all of their socio-demographic characteristics in the two study groups (table 3).

Table 3: Socio-demographic and clinical characteristics of patients who received TDF and no-TDF containing HAART regimen in ACSH, Mekelle, Ethiopia, 2020.

Baseline variables		Exposure category		Total n(%)	P-value(χ^2)
		TDF containing HAART, n (%)	Non-TDF containing HAART, n (%)		
Age(years)	18-29	14(6.0)	9(3.9)	23(9.9)	0.835
	30-39	54(23.3)	23(9.9)	77(33.2)	
	40-49	57(24.6)	25(10.8)	82(35.3)	
	=50+	33(14.2)	17(7.3)	50(21.6)	
sex	male	59(25.4)	37(15.9)	96(41.4)	0.086
	female	99(42.7)	37(15.9)	136(58.6)	
Marital status	married	124(53.4)	59(25.4)	183(78.9)	0.62
	single	19(8.2)	10(4.3)	29(12.5)	
	divorced	13(5.6)	5(2.2)	18(7.8)	
	widowed	2(0.9)	0	2(0.9)	
Educational status	Unable to read and write	17(7.3)	5(2.2)	22(9.5)	0.056
	Able to read and write	43(18.5)	15(6.5)	58(25)	
	Attended elementary school	36(15.5)	11(4.7)	47(20.2)	

	Attended high school(9-12)	44(19)	25(10.8)	69(29.7)	
	Attended university/college	18(7.8)	18(7.8)	36(15.5)	
residence	urban	112(48.3)	63(27.2)	175(75.5)	0.019 *
	rural	46(19.8)	11(4.7)	57(24.5)	
Occupational status	House wife	53(22.8)	19(8.2)	72(31.0)	0.055
	Government employee	25(10.8)	17(7.3)	42(18.1)	
	farmer	23(9.9)	5(2.2)	28(12.1)	
	trader	40(17.2)	17(7.3)	57(24.6)	
	others€	17(7.3)	16(6.9)	33(14.2)	
Regular income	yes	103(44.4)	49(21.1)	152(65.5)	0.878
	no	55(23.7)	25(10.8)	80(34.5)	
* Significant difference at 5% level; €=implies that Commercial sex workers (5), daily laborers (1), drivers (20) and students (7)					

7.2. Baseline and currently general clinical characteristics of the study participants

The study participants' baseline clinical characteristics were assessed from each of their medical records, whereas the current clinical characteristics were obtained from interviews, anthropometric measurements, and laboratory testing. Regarding the participants' BMI, the median BMI for those on TDF containing HAART was 20, IQR (18.5-22), and 20.2, IQR (18-22.4) for those on non-TDF.

Concerning to the respondents' ART exposure status, the median duration of ART exposure was 74 months for those on TDF containing HAART, IQR (47-103) months and 102.5 months for those on non-TDF containing HAART, IQR (86-124) months.

Concerning the median CD4 baseline of the study participants who were on TDF containing HAART was 235, IQR (120-419.5) and for those on non-TDF was 149.5, (72.5-233.3). Whereas the median current CD4 level of the study individuals for those on TDF containing group was 385.5, IQR (235.5-556.5) but for those who are on non-TDF containing HAART was 399, IQR (174.5-579).

About half of the study participants had a baseline CD4+ count of less-than 200cells/ml but after the initiation of HAART, the majority (83.6%, n=194) of the participants had a CD4+ count above 200 cells/ml. The majority (84.5%, n= 196) of the study subjects were on 1st line of ART drug regimen.

Almost all (94%, n=218) of the study participants were on the early stage of WHO clinical treatment (T1&T2) (table 4).

Table 4: Baseline and clinical characteristics of HIV positive patients taking TDF and Non-TDF containing HAART regimen in ACSH, Mekelle, Ethiopia, 2020.

variables		Exposure category		Total (%)	P-value(χ2)
		TDF containing HAART, n (%)	Non-TDF containing HAART, n (%)		
baseline CD4 (cells/ml)	≤200	68(29.3)	49(21.1)	117(50.4)	0.000*
	201-350	39(16.8)	12(5.2)	51(22)	
	351-499	22(9.5)	8(3.4)	30(12.9)	
	≥500	29(12.5)	5(2.2)	34(14.7)	
Baseline viral load(copies/ml)	<400	136(58.6)	50(21.6)	186(80.2)	0.001*
	≥400	22(9.5)	24(10.3)	46(19.8)	
Line of ART	1^{st} line	146(62.9)	50(21.6)	196(84.5)	0.000**
	2^{nd} line	12(5.2)	24(10.3)	36(15.5)	
Addiction	yes	17(7.3)	5(2.2)	22(9.5)	0.332
	no	141(60.8)	69(29.7)	210(90.5)	
Addiction to	Chat	2(1.2)	0(0)	2(1.2)	0.228
	cigarette	1(0.6)	0(0)	1(0.6)	
	alcohol	14(8.9)	5(6.7)	19(15.6)	
BMI (Kg/m^2)	<18.5	36(15.5)	25(10.8)	61(26.3)	0.175
	18.5-24.9	111(47.8)	41(17.7)	152(65.5)	
	25-29.9	9(3.9)	7(3)	16(6.9)	
	30-39.9	2(0.9)	1(0.4)	3(1.3)	

Current CD4 level (cells/ml)	≤200	18(7.8)	20(8.6)	38(16.8)	0.01**
	201-350	51(22)	14(6)	65(28)	
	351-499	37(15.9)	13(5.6)	50(21.6)	
	≥500	52(22.4)	27(11.6)	79(34.1)	
Current viral load(copies/ml)	<400	143(61.6)	61(26.3)	204(87.9)	0.079
	≥400	15(6.5)	13(5.6)	28(12.1)	
Medication use other than ART	CPT	67(64.4)	27(26)	94(90.4)	0.731
	INH	5(4.8)	3(2.9)	8(7.7)	
	Anti-TB	1(1)	1(1)	2(1.9)	
Status of the medications taken other than ART	finished	60(57.1)	28(26.7)	88(83.8)	0.497
	Currently taking	13(12.4)	4(3.8)	17(16.2)	
Adherence to ART	poor	18 (7.8)	7 (3)	25(10.8)	0.625
	good	140(60.3)	67(28.8)	207 (89.2)	
Current Stages of AIDS	Stage I/II	148(63.8)	70(30.2)	218(94.0)	0.754
	Stage III	7(3.0)	2(0.9)	9(3.9)	
	Stage IV	3(1.3)	2(0.9)	5(2.2)	

* indicates there was statistically significant difference (at 5% level

** indicates statistically significant difference at the 1 % level (P<0.01)

*** indicates statistically significant difference at the 0.1 % level (P<0.001)

More than half (57.8%, n=134) of the study participants were on their ART follow up for more than five years with the mean duration of about 77 months for TDF group and 100 months for Non-TDF group (figure 3).

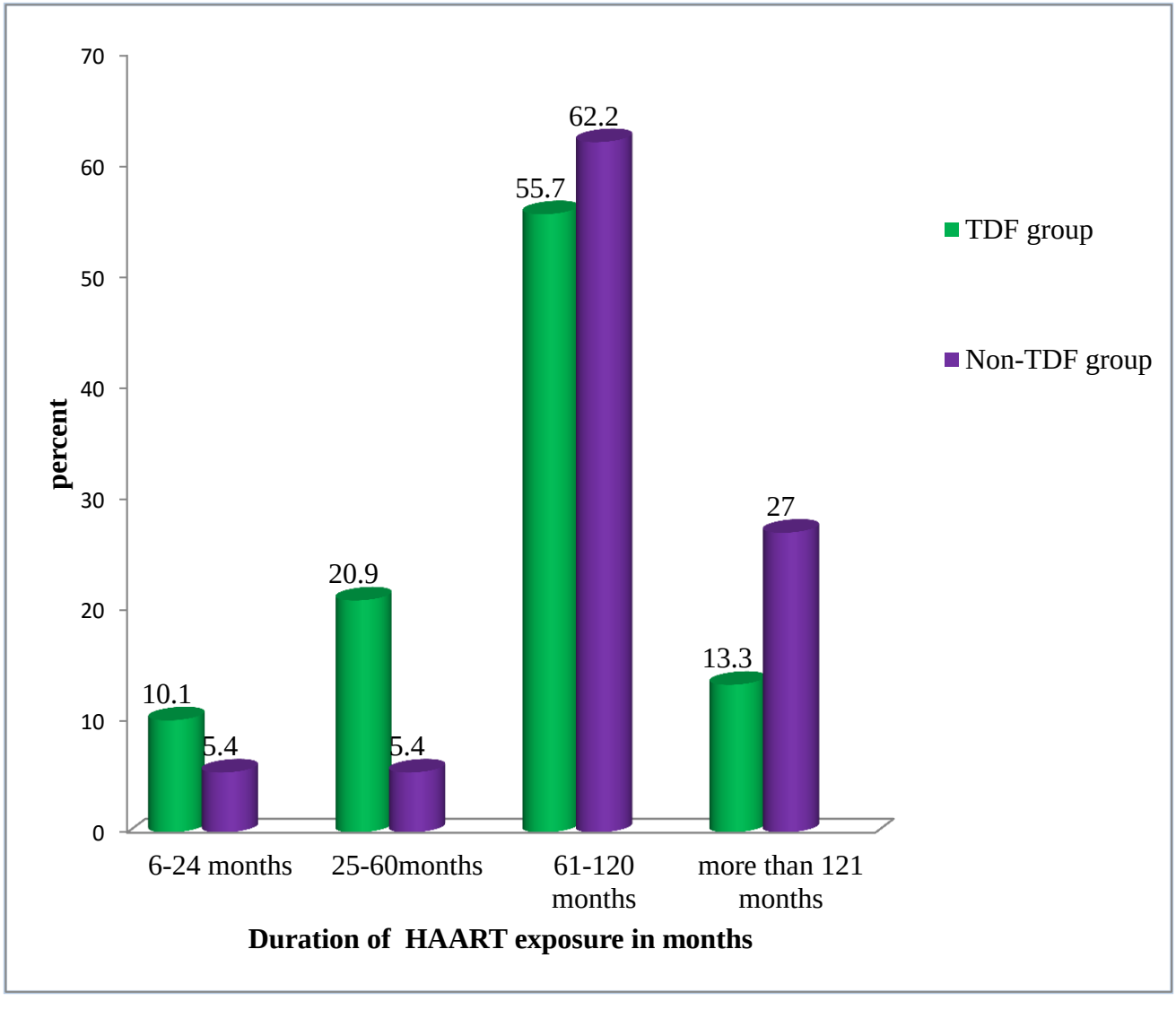
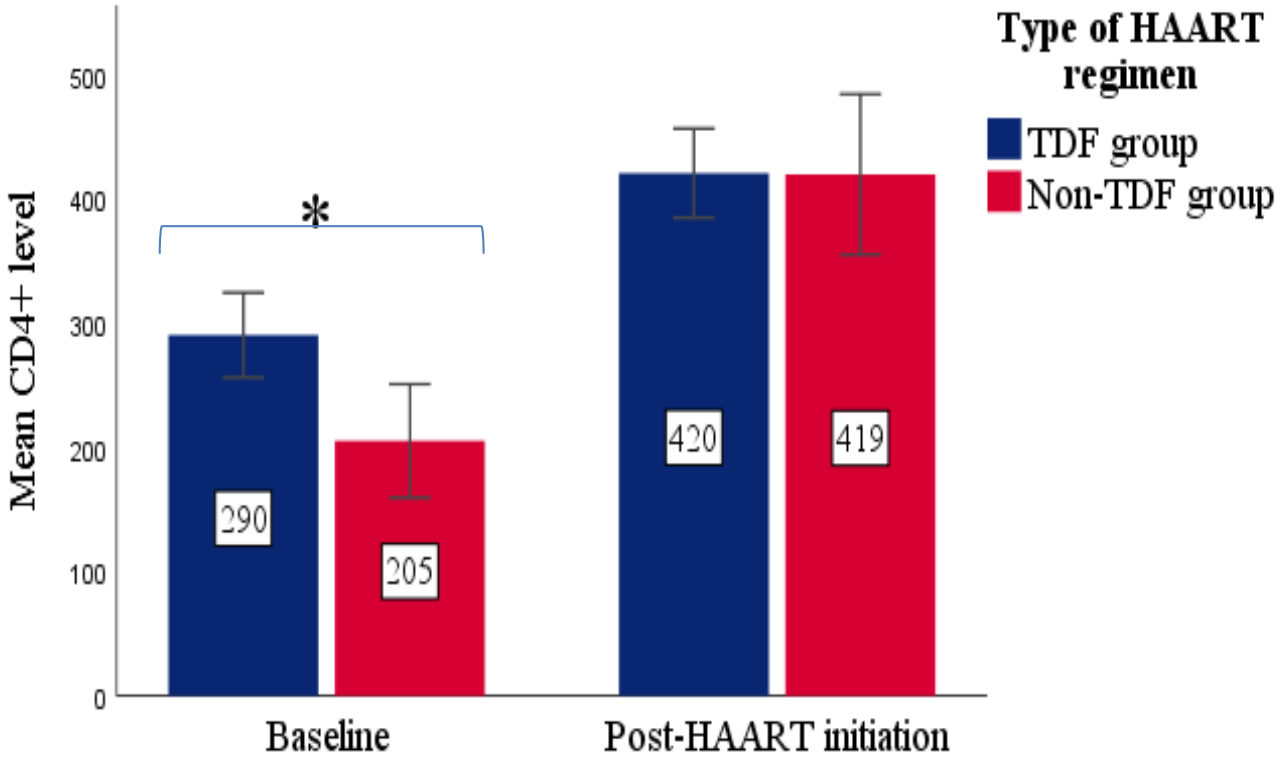


Figure 3: *duration of ART exposure in months among HIV positive individuals on HAART follow up in ACSH, Mekelle, Ethiopia, 2020.*

Before the initiation of HAART, the mean CD4 count was significantly higher (P=0.005) among those who started TDF containing HAART drug regimen, but there was no significant difference in mean CD4 count after six months of HAART in both of the study groups (figure 4).



*indicates significant mean CD4+ difference at 5% level between the study groups

Figure 4: *Mean and standard deviation values of CD4 count at baseline and after initiation of HAART among HIV infected patients in ACSH, Mekelle, Ethiopia, 2020.*

7.3. Prevalence and severity of renal dysfunction

Independent samples t-test revealed that there was no significant mean difference in baseline renal function test parameters between the two study groups (table 5).

Table 5: Independent samples t-test analysis of renal function test parameters at time of initiation of HAART (baseline) of HIV positive patients receiving TDF and Non-TDF containing HAART regimen in ACSH, Mekelle, Ethiopia, 2020.

variables	TDF group, N=158	Non-TDF group, N=74	95%CI of the mean difference		P-value
	Mean(SD)	Mean(SD)	lower	upper	
Creatinine(mg/dl)	0.73(0.21)	1.38(3.63)	-1.49	0.19	0.128
BUN(mg/dl)	21.20(11.00)	24.56(14.53)	-6.75	0.39	0.053
Serum total protein (g/dl)	7.20(0.66)	7.19(0.55)	-0.16	0.18	0.95
Uric acid(mg/dl)	3.85(3.73)	6.03(2.53)	-5.02	0.65	0.131
GFR(ml/min/1.73/m2)	147.78(49.26)	138.36(61.2)	-5.46	24.31	0.213
Proteinuria	0.31(0.20)	0.13(0.47)	-0.21	0.01	0.078
Glucosuria	0.00	0.00	-0.06	0.01	0.159

As can be seen from the table below there was no significant mean difference in almost all the renal function test parameters after the initiation of HAART except that mean of serum total protein was slightly higher for those on Non-TDF group(P=0.001) (table 6).

Table 6:Independent t-test analysis of renal function test parameters following initiation of HAART (current) among patients taking TDF and non-TDF containing HAART regimen at ACSH, Mekelle, Ethiopia, 2020.

variables	TDF group, N=158	Non-TDF group	95%CI of the mean difference		P-value
	mean	mean	lower	upper	
Creatinine(mg/dl)	0.94	1.27	-0.94	0.26	0.272
BUN(mg/dl)	21.21	27.55	-16.68	3.99	0.226
Serum total protein (g/dl)	7.66	8.05	-0.63	-0.15	0.001**
Uric acid(mg/dl)	4.54	5.47	-1.93	0.08	0.071
GFR(ml/min/1.73/m2)	120.06	116.06	-6.39	14.39	0.449
Proteinuria	0.11	0.13	-0.15	0.10	0.738
Glucosuria	0.00	0.00			NA
**implies statistically significant difference at p<0.001(0.1%level).					

Paired sample t-test statistics revealed that there was a statistically significant increase in mean of serum Creatinine level, total serum protein, uric acid level, proteinuria, and a significant decrease in mean GFR after the intake of TDF containing HAART. On the other hand except for a statistically significant increase in mean serum total protein and a significant decrease in mean GFR level, there was no significant difference in the mean baseline and current level of the other renal function test variables following the intake of non-TDF containing HAART. Concerning BUN, except it showed a slight increment after the intake of HAART in both the study groups there was no significant difference in baseline and current mean BUN values in both study groups (table 7).

Table 7: Paired samples t-test analysis of baseline and current renal functions among patients taking TDF and non-TDF containing HAART regimen at ACSH, Mekelle, Ethiopia, 2020.

Variables		TDF-group, N=158		Non-TDF group, N=74	
		Mean	P-value	Mean	P-value
Creatinine(mg/dl)	baseline	0.73	0.019*	1.38	0.817
	current	0.94		1.28	
BUN(mg/dl)	baseline	21.20	0.993	24.56	0.530
	current	21.21		27.55	
Total serum protein (g/dl)	baseline	7.20	0.000**	7.19	0.000**
	current	7.66		8.05	
Uric acid(mg/dl)	baseline	3.51	0.000**	4.28	0.496
	current	4.63		4.38	
GFR(ml/min/1.73/m2)	baseline	147.78	0.000**	138.36	0.001*
	current	120.06		116.06	
Proteinuria	baseline	0.03	0.023*	0.13	1.000
	current	0.11		0.13	
Glucosuria	baseline	0.00		0.02	0.159
	current	0.00		0.00	

*significant mean difference at 5% level, **significant mean difference at 1% level

The overall prevalence of renal dysfunction of study participants (eGFR < 90 ml/ min) was 16.8 %,(n =39). The prevalence of renal dysfunction among the study participant on TDF group was 9.5% whereas among the non-TDF group was 7.3%.

A total of (9.1%, n=21) respondents had a decreased GFR level during HAART enrolment, but after HAART intake for at least six months, the number of patients with a decreased GFR level increased to 16.8%, n=39. Of the overall reduced GFR level, 30 of respondents had moderate renal dysfunction [81.1% were among the TDF group and 70.6% among the non TDF group)]. However, Chi square test analysis showed that there was no statistically significant association between severity of renal dysfunction as described by GFR and type of HAART regimen (P=0.43) (figure 5).

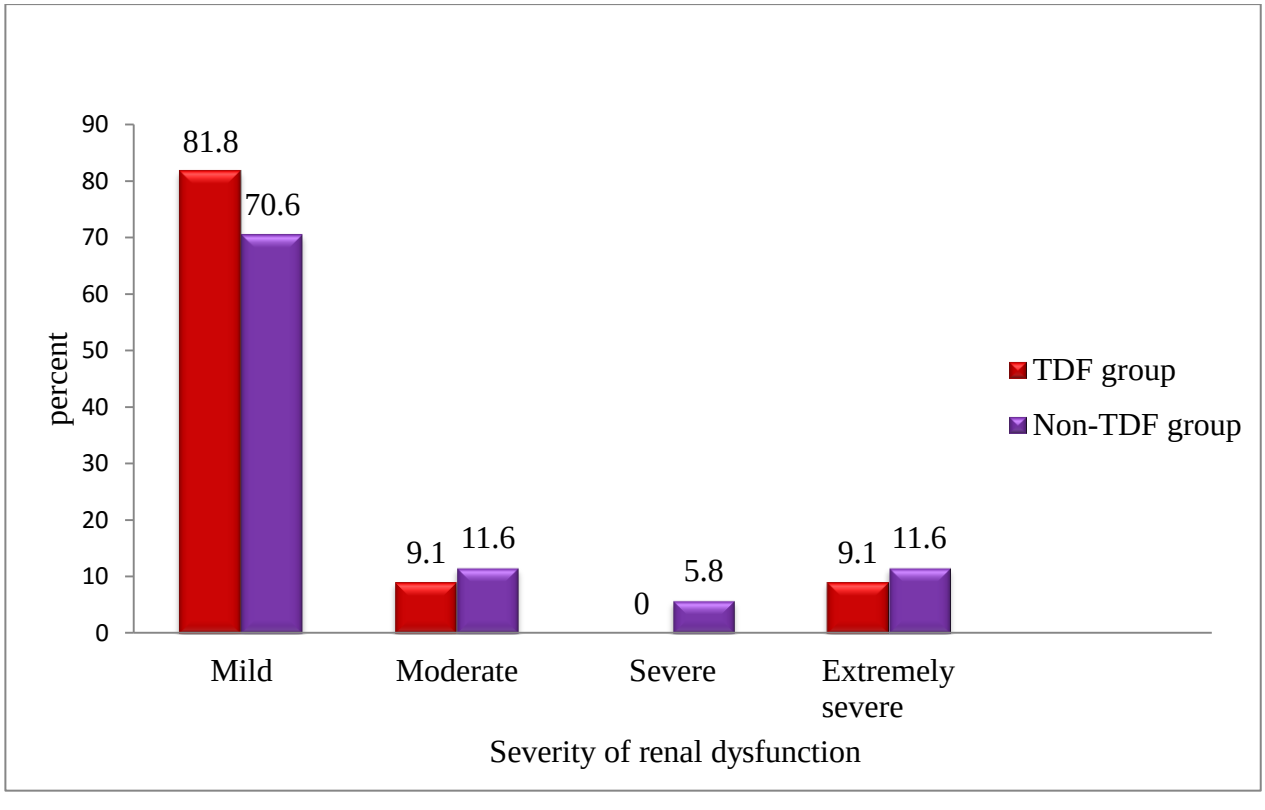


Figure 5*: Percentage of severity of renal dysfunction among HIV positive patients taking TDF and Non-TDF containing HAART regimen in ACSH, Mekelle, Ethiopia, 2020.*

7.4. Predictors of renal dysfunction

Binary logistic regression revealed that in those who received TDF-group, the odds of decrease in GFR was significantly associated with the age of ≥50, abnormal BMI, intake of cotrimoxazole prophylaxis therapy, intake of 2nd line ART, above ten years of HAART intake

and CD4 of <200cells/ml. On the other hand for those who received a non-TDF group of ART drug regimens the odds of decrease in GFR was significantly associated with age of ≥ 50, abnormal BMI, and above ten years of HAART intake. In addition being male decreases the GFR by 0.145 for each female increment. However, other variables did not maintain their statistical significance in multivariable logistic regression (table 8).

Table 8: Binary logistic regression analysis for the independent predictors of declined GFR among HIV positive patients taking TDF and Non-TDF containing HAART regimen in ACSH, Mekelle, Ethiopia, 2020.

Variables		TDF group, N=158			Non-TDF group, N=74		
		P-value	COR	95% CI	P-value	COR	95% CI
residence	rural urban	0.422 Ref(1)	1.474	0.572,3.795	0.260 Ref(1)	0.294	0.035,2.478
Age category(years)	≥ 50 <50	0.001** Ref(1)	5.18	2.00,13.42	0.010* Ref(1)	4.741	1.444,15.569
sex	Male female	0.134 Ref(1)	0.447	0.156,1.282	0.005* Ref(1)	0.145	0.037,0.562
BMI(kg/m^2)	abnormal normal	0.036* ref(1)	0.202	0.45,0.903	0.045* Ref(1)	3.300	1.026,10.614
Regular income	Yes no	0.519 Ref(1)	1.355	0.539,3.403	0.192 Ref(1)	2.092	0.690,6.338
CD4+(cells/ml)	<200 ≥200	0.036* Ref(1)	0.202	0.045,0.903	0.801 Ref(1)	1.167	0.352,3.867
Line of ART drug	2^{nd} line 1^{st} line	0.008* Ref(1)	5.420	1.547,18.996	0.762 Ref(1)	0.833	0.256,2.711
CPT intake	Yes no	0.011* Ref(1)	0.289	0.110,0.756	0.907 Ref(1)	1.069	0.345,3.314

Addiction to alcohol and/or chat	Yes no	0.786 Ref(1)	0.807	0.171,3.789	0.999 Ref(1)	0.000	0.000
Stage of HIV/AIDS	Early stage Advanced stage	0.569 Ref(1)	0.625	0.124,3.157	0.921	0.889	0.893,9.144
Duration of ART exposure	≥ 10 years <10 years	0.009* Ref(1)	0.246	0.086,0.705	0.003*	0.167	0.052,0.539
Baseline viral RNA load(copies/ml)	<400 ≥400	0.945 Ref(1)	1.057	0.222,5.039	0.465	0.6609	0.162,2.299
Current viral load (copies/ml)	<400 ≥400	0.485 Ref(1)	1.724	0.374,7.955	0.762	1.200	0.369,3.904

*indicates significant at 5% level, COR: crude odds ratio, BMI: body mass index, CPT: Cotrimoxazole prophylaxis therapy

Multivariate logistic regression analysis in TDF group revealed that older age ≥50 years, being male, advanced immune-suppression (CD4<200cell/ml)and ≥ 10 years of HAART intake were significant predictors of decline in renal function test as defined by GFR when other confounding variables are controlled for each predictor. On the other hand, multivariate analysis on the non-TDF group revealed; older age ≥50 years and being male were the significant predictors of decline in renal function test as defined by GFR (table 9).

Table 9: Multiple logistic regression analysis for the predictors of declined GRF among patients taking TDF and non-TDF containing regimen in ACSH, Mekelle, Ethiopia, 2020.

categories	TDF-group(N=158)			Non-TDF group(N=74)		
	P-value	AOR	95% CI	P-value	AOR	95% CI
Age ≥50 years	0.001*	0.127¶	0.036,0.446	0.006*	0.113	0.024,0.527
Abnormal BMI	0.052	4.810¶	0.985,23.493	0.452	0.622	0.180,2.147
Male sex	0.047*	3.779	1.019,14.015	0.008*	9.351¶	1.788,48.897
Intake of CPT	0.057	0.314¶	0.095,1.036	0.321	2.397	0.426,13.489
Intake of 2nd line ART drug regimen	0.329	0.430¶	0.079,2.341	0.987	0.987	0.216,4.515
≥ 10 years of HAART intake	0.045*	0.251¶	0.065,0.970	0.742	0.779	0.176,3.447
CD4<200 cells/ml	0.039*	1.432¶	1.240,11.552	0.745	0.736	0.116,4.673

*Implies significant association at 5% level for multivariate analysis, CI: Confidence Interval

¶ implies significant association at 5% level for bivariate analysis, AOR: Adjusted Odds Ratio

8. Discussion

8.1. Prevalence and severity of renal dysfunction

The objective of this cross-sectional comparative study was to evaluate the renal function abnormalities in HIV positive adult patients receiving tenofovir and non-tenofovir containing a combination of highly active antiretroviral therapy (HAART) at ACSH, Mekelle, Ethiopia. The study population was homogenous as in most socio-demographic characteristics the two study groups (TDF and non-TDF group) were comparable.

The study showed that more than half (50.4%) of participants in both study groups had advanced immune-suppression (CD4 count less than 200 cells/ml) at the time of HAART enrollment, and 19.8% of participants in both study groups had at the same time a viral load of over 400 copies/ml.

The possible explanation for the immune suppression at baseline could be, fear of being identified as having HIV may discourage patients from attending health facilities(Fido *et al*., 2016). In addition, previously there was a lack of in-depth knowledge about HIV/AIDS and clear policies and guidelines on combating stigma and discrimination of the patients that arrive to the hospital (Feyissa *et al*., 2012). So these factors may have contributed to the advanced immune-suppression of patients before they started HAART.

After at least six months treatment with HAART, nearly all (94%) of the patients in both study groups were on clinical WHO treatment stage one or two, while the majority (83.8%) of the study participants in both the study groups had a CD4 count of more than 200cells/ml and 12.1% of the participants had more than 400 copies/ml of the viral load simultaneously. This increment in immune status may be due to the recovery of the antigen-specific proliferation of CD4 T-cells that was observed in most patients after six months of HAART (Wendland *et al*., 1999).

Following at least six months of HAART treatment, the overall prevalence of kidney dysfunction in our study based on the glomerular filtration rate defined by eGFR < 90ml / min using the study equation Renal Disease Diet Modification (MDRD) was found to be 16.8 % (TDF group: 9.5%, non-TDF group: 7.3% and P=0.31) with an average 74-month follow-up. This result was almost similar to the studies carried out in southern Ethiopia (18.2%) (Mekuria et al., 2016b)and Lusaka, Zambia (18.6%) (E *et al*., 2017). However, this is lower than a report

from Zambia (28.3%) (Deckert et al., 2017b) and TASH, Addis Ababa, Ethiopia (25%) (Yazie *et al.*, 2019).

This difference could be due to differences in HAART follow up duration, study design, sample size, and method of determining eGFR. For instance, the method used to determine eGFR in Zambya by (Deckert et al., 2017b) was CKD-Epi formula and had sample size of 1118 participants who had been on ART for a minimum of three months; and that of the study done in TASH, Ethiopia by (Yazie et al., 2019), the study design was prospective cohort study with total of 63 study participants followed prospectively.

We found a non-significant difference in renal dysfunction between TDF and non-TDF group (TDF group: 9.5%, non-TDF group: 7.3% and P=0.31). In agreement with our study, a study carried out in Nigeria (Ojeh *et al.*, 2018), reported that the incidence of renal dysfunction among TDF group and non-TDF group was 4.6% and 2.3% respectively. This finding is also supported by a study carried out in Uganda (Salome et al., 2016a), who reported no difference in renal function abnormalities among patients on Tenofovir and non-Tenofovir containing ART for almost a decade follow up. Such findings contradicted the study done on renal safety TDF(Gallant *et al*., 2008), according to which the frequency of renal dysfunction among the non-TDF population was slightly higher than that of the TDF. These results also contradicted the research in Maryland, USA (Gallant *et al.*, 2005), where TDF use was significantly correlated with increased renal dysfunction by 4% compared to alternative NRTIs. Majority of the study participants had been on HAART follow up for more than five years and those patients with renal dysfunction in both the study groups had HAART exposure for more than five years. In agreement with this finding, a cohort study from France (Salome et al., 2016a), reported that during a median follow-up duration of 4.5 years number of patients with renal dysfunction raises. The probable explanation for the prevalence differences among these studies may be due to the difference in study design, sample size, socio-demographic characteristics and duration of HAART follow-up and method of determining eGFR.

There was no statistically significant association between the severity of renal dysfunction as described by GFR and type of HAART regimen (P=0.43, except that of the total 39 (16.8%) reduced cases of GFR, the majority of participants in both study groups 30 (76.9%) had mild renal dysfunction, 10.3% had moderate dysfunction, 10.3% had extremely severe renal

dysfunction and 7 (2.5%) in the non- TDF group had severe renal dysfunction and we found no severe renal dysfunction among the TDF group. In accordance with our study, a study conducted in Lusaka, Zambia (Mulenga *et al*., 2008), found that 74.7% of patients had mild, 20.0% moderate and 5.3% severe renal dysfunction. This finding was higher from a study conducted in Johannesburg, South Africa (Brennan *et al*., 2011), which reported that they had mild renal dysfunction in 30.4% and moderate renal dysfunction in 5.2 %.

We found no discontinuation of HAART due to abnormal laboratory findings that lead to chronic kidney disease. This finding was in line with a prospective cohort study conducted in Uganda(Salome *et al*., 2016c), who reported that there was no difference in renal function abnormalities in Tenofovir and non-Tenofovir patients on ART for nearly a decade, so Tenofovir-based first-line ART can be safely implemented even in settings without regular monitoring of renal function. This is also supported by another study who claimed that no patient in the TDF group was discontinued due to renal dysfunction (Gallant *et al*., 2008). This is in contradiction with a study carried out in Maryland (Gallant *et al.*, 2005)and who reported that the rate of discontinuation due to renal dysfunction was slightly higher among other NRTI-treated patients 21 (6.7%) compared to TDF-treated patients 19 (5.5%). A study conducted in Johannesburg, South Africa (Cooper *et al*., 2010) , showed that despite TDF use was associated with a statistically significant loss of renal function; the clinical magnitude of this effect is minimal. In addition, a study carried out in Tokyo (Nishijima et al., 2017b) concluded that the use of TDF is not associated with CKD, so that these results do not support the need to restrict the use of TDF due to its adverse renal effect.

The present research found that there was no significant mean difference in serum Creatinine level between the study groups after HAART was initiated. This result contradicts a report conducted in South West Ethiopia (Mekuria et al., 2016b) who reported that serum Creatinine levels of the TDF group were significantly higher relative to the NRTI group. Similar to a study done in South West Ethiopia, another study (Jung *et al*., 2018) also reported that the content Creatinine was significantly correlated with the exposure to TDF (P< 001). The variation may be attributed to the difference in the cut-off point used by various test devices to calculate the amount of serum Creatinine.

This study showed that there was a significant increase in mean serum total protein from baseline after the use of TDF and non-TDF containing HAART. This is supported by a study

done in Ghana (Afari SK, 2018) who concluded that serum total protein increases significantly after intake of HAART. This result is contradicted with the study in Texas, USA (Serpa *et al.*, 2010), who reported that after one year intake of HAART, serum total protein decreases significantly. This might be due to differences in sample size and type of HAART used in the USA.

We found a statistically significant increase in mean proteinuria after the start of TDF containing HAART. This is in line with the studies in France (Dauchy *et al*., 2011)and the USA (Gallant *et al*., 2008*)* ,which disclosed that the level of proteinuria increases after the intake of TDF containing combined ART. Similarly, a study in the USA (Gallant *et al*., 2008) strengthens this finding, who reported that the prevalence of proteinuria of patients taking ART was higher than its baseline (27% and 20.6%, respectively).

Our study found that uric acid level was statistically increased after the intake of TDF containing HAART as compared to baseline (P<0.001). This finding is supported by a study done in Nigeria (Obiebi and Nwannadi, 2018), who reported serum uric acid levels was elevated in patients on TDF based regimen. The possible reason for the elevation of serum uric acid after the use of TDF may be associated with endothelial damage and thus, can be used as a marker for early detection of asymptomatic patients before they develop renal dysfunction.

The present study revealed that there was no statistically significant difference in the mean of BUN level after the start of HAART from baseline in both the study groups. In agreement with our study, a study carried out in Ethiopia (Eneyew et al., 2016) reported that serum BUN level of both treatment-naive and HAART experienced patients was not significantly different, but this finding is in contradiction to the study in Cameron (Nforbugwe et al., 2020) who reported that those HAART exposed group had a significantly higher mean BUN value (p= 0.001) as compared to the HAART unexposed. The discrepancy may be attributed by the dietary protein eaten and amount of fluids taken.

8.2. Predictors of renal dysfunction

In our study on TDF group, the significant predictors of renal dysfunction as defined by GFR when other confounding variables are controlled were older age ≥50 years, being male, advanced immune-suppression (CD4<200cell/ml)and ≥ 10 years of HAART intake. Our finding is consistent with the study done at the University of North Carolina (Overton *et al*., 2009) who reported that loss of renal function was associated with low CD4 count(<200

cells/mm3) and being female as compared to male. The possible reason could be the genetic difference and inclusion/exclusion criteria used.

On the other hand, for the non-TDF group, the predictors of renal dysfunction were older age ≥50 years and being male sex. In agreement with our finding, studies conducted in Maryland, USA (Gallant *et al*., 2005)and India (Kumarasamy *et al*., 2018b), found that advanced immune-suppression, older age (≥50 years), being male and low BMI were among the risks associated with the development of renal dysfunction in those receiving TDF containing HAART. Similarly, this finding is supported by the study done in southwest Ethiopia (Mekuria et al., 2016c), which stated that renal dysfunction in HIV positive individuals over 50 years of age was found to be 4.4 times more than under 50 years.

9. Conclusion

The findings of this study showed that renal dysfunction in HIV positive patients taking specific HAART regimens in the study area remain a public health issue.

The findings of the present study revealed that there is no statistically significant difference in renal dysfunction among patients on TDF and non-TDF containing HAART regimen but the overall prevalence of renal dysfunction following six months of HAART follow up was 16.8%. In the group who received TDF, renal dysfunction was significantly predicted by older age ≥50 years, being male, advanced immune-suppression (CD4<200cell/ml), and ≥ 10 years of HAART use. In the group who received non-TDF of ART drugs, renal dysfunction was significantly predicted by older age ≥50 years and being male. So this finding does not support the need to restrict TDF use. Our finding does not support the need for restriction of TDF based ART regimen use due to its renal adverse effect. Therefore, TDF can safely be initiated even in resource-poor settings such as in Ethiopia.

10. Strength and limitations of the study

Strengths:

- The baseline clinical characteristic of the study participants was obtained from each of the patient's medical record documents so as to avoid recall bias.
- The study reviewed baseline renal function test parameters to be used as a reference and comparative purposes with data after intake of HAART.
- The long duration of exposure in patients to ART drugs, in the present work permitted us to document the effect of TDF in the renal functioning. Most previous reports in Ethiopia were of shorter duration.
- The effect of 2nd line ART drugs on renal function was assessed.
- Availability of comparison group of patients on non-Tenofovir containing ART which enabled prediction of renal dysfunction after Tenofovir treatment.

Limitations: We acknowledge some limitations that might have affected our study findings.

First, as the study was a single-period study we cannot completely rule out potential selection bias.

Secondly, since a Convenience sampling approach has been used, we do not have the same number of participants in each of the study groups (most participants in the research were those who received TDF containing HAART).

Third, micro-albuminuria and hepatitis E were not evaluated because the reagents were not affordable.

Fourthly, the laboratory testing machine used to determine the renal function test parameters during enrollment (baseline) to HAART might not be the same as the machine used after six months of HAART intake, so the test results may not be equally comparable since the normal because the usual value cut-off point differs between the testing machines.

Fifth, some of the patients had changed their initial type of ART drug containing Tenofovir, but we did not consider the period as significant. Moreover, dietary habits of the patients under observation were not taken into consideration.

11. Recommendation

Health care providers: We suggest that health care providers enhance early detection of patients at high risk of kidney failure before and after initiation of HAART through routine renal function monitoring and prompt drug switching and/or dose adjustment. Nephrologists should be mindful of the potential for the development of kidney associated diseases after the use of HAART regimens. Awareness should also be created on patients at their every visit to the ART clinic by the health care providers on the possible side effect and adherence of the ART drugs of patients.

Researchers: We recommend researchers to conduct a prospective cohort study with a large sample size to draw the exact conclusion on the long-term safety of TDF to renal function of HIV patients who are on ART drugs. Because, early stage pharmacogenomics screening of patients who are potentially at risk of developing renal diseases may also prevent risk of developing CKD or ESRDs. We also suggest researchers to consider nutritional status and dietary habits of patients under study as a cofounder as well as the laboratory testing machine used to calculate the renal function test parameters should be the same type throughout their prospective study.

Policy makers: we recommend policy makers to revise the national ART treatment guidelines that TDF has a minimal renal side effect.

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12. Annex

12.1. Questionnaire

12.1.1. English version

Informed Consent Form

My name is _____. I am currently collecting data on the assessment of renal function among HIV positive patients taking ART drug regimens for the partial fulfillment of Msc program in medical biochemistry at AAU. The objective of the study is to examine the effect of ART drugs on kidney functions and the outcome of the result will help to generate information necessary for planning to improve, redesign and timely dose adjustment. You are randomly selected to participate in this study, because you are using the ART drugs.

No personal identifiers will be attached/ recorded to the interview. The data you provided will be kept strictly confidential by using only code numbers and will aggregated with the responses of others to establish common voices.

Your participation in the study is upon purely voluntary basis and no payment will be given for your participation. The interview will be conducted in private and will take 10-15 minutes. During the interview period, if you feel inconvenient, you can interrupt and clarify inconvenience, appoint to other time or even withdraw any time after you get involved in the study. Dear respondent, below are some questions which are designed to assess renal function among the ART drug users and 5ml of blood sample will be taken from your upper arm. Your honest and genuine participation in this study is very important & highly appreciated.

Would you be willing to participate?

Thank you for your cooperation!

Signature of Participant _____Date _____

Code no. _____

Questionnaire to assess renal function among HIV positive patients taking tenofovir and non tenofovir containing highly active antiretroviral therapy (HAART)

Section I: Socio Demographic Characteristics

s/no.	Category	Response	remark
101	Age of the respondent(yrs)		
102	Sex of the respondent	1. Male 2. Female	
103	Marital status	1. Currently married 2. Single – Never married 4. Divorced 5. Widowed	
104	Educational Status of the respondent	1. Unable to read and write 2. able to read and write 3. Attended elementary school (Grades 1– 8) 4. Attended high school (Grades 9 – 12) 5. attended University/college	
105	Residence of the respondent	1. rural 2. urban	
106	Occupation of the respondent	A. House wife B. Government employee C. Farmer D. Commercial sex worker E. House maid F. Daily laborer G. Other (specify)_____	
107	Income of the respondent(in Birr)		
108	Any addiction to ?(make circle if any)	1. Chat 2. Cigarette 3. Iv drugs 4. Alcohol 5. Specify others_____	

Section II. Anthropometric measures and Background Information

s/no.	Category	response	remark
201	Height (m)		
202	body weight(kg)		
203	body mass index (BMI)in kg/ m^2		
204	BP measure(mmHg)		
205	date of initiation of HAART		
206	CD4+ counts at time of initiation of HAART		
207	viral load at initiation of HAART		
208	duration of ART exposure(months)		
209	Current CD4+ count		
210	Current viral load		
211	Adherence to HAART	1. Poor 2. Good	
212	Current ART regimen	1. 1a=d4T+3TC+NVP 2. 1b=d4T+3TC+EFV 3. 1c=AZT+3TC+NVP 4. 1d=AZT+3TC+EFV 5. 1e=TDF+3TC+EFV 6. 1f=TDF+3TC+NVP 7. 2a=ABC+ ddI+ LPv/r 8. 2b=TDF+ ddI+ LPR/r 9. 2c=TDF+ 3TC+ LPR/r 10. 2d= AZT+ 3Tc+ LPv/r	
2113	Did you have ever changed your previous drug regimen? Hint : (look card to confirm)	1. Yes 2. No	
214	If (Q .213 is **yes**, What was the previous drug regimen you were using?		
215	What was the reason for changing the drug regimen		

216	Did you take medications other than ART	1. Yes 2. No	
217	If Q. 216, is **yes** , what type	**1.** cotrimoxazole prophylaxis therapy **2.** INH **3.** Others specify_____	
218	if Q. 216, is **yes** , the status of the medication ?	1. Finished 2. Currently taking 3. Discontinued	
219	WHO Stage of HIV/AIDS	1. I/II 2. III 3. IV	

Section III. **Renal function test of the respondents**

s/no.	**catagory**	**Baseline**	**current**	**remark**
301	serum creatinine level			
302	BUN			
303	Serum total protein			
304	eGFR			
305	proteinuria			
306	Glucosuria			
307	albuminuria			

12.1.2. Tigrigna version

<u>ኣዲስ ኣበባ ዩኒቨርሲቲ ጥዕና ሳይንስ ኮሌጅ ትምህርቲ ክፍሊ ሜዲካል ባዮኬሚስትሪ</u>
<u>መብርሂ ቅጥዒ</u> :- ኣብ ክልል ትግራይ ዓይደር ሓፈሻዊ እስፔሻላይድ ሆስፒታል ናይ ፀረ ኤች ኣይ ቪ/ኤድስ መድሓኒት ኣብ ኮላሊቶም ዘለዎ ሳዕቤን ንምፍላጥ ዝካየድ ፅንዓት ጥዕና ይሃበለይ

ሽመይ _____ ይበሃል:: ኣብ ኣዲስ ኣበባ ዩኒቨርሲቲ ንናይ 2ይ ድግሪ መመረቂ መፅናዕቲ ሓበሬታ እንዳኣከብኩ እንትኸውን ዕላማ እዚ ምፅናዕቲ ድማ ናይ ኤች ኣይቪ መድሓኒት ኣብ ናይ ኩላሊት ተጠቀምቲ እዚ መድሓኒት ዘስዕቦ ለውጢን መጠን እቲ ለዉጥን እንታይ ከምዝመስል ንምፍላጥ እዩ:: ክቡር ተሳታፊ ኣብዚ መፅናዕቲ እዚ ተሳታፊ ንክኾኑ/ና ዝተሓሰበሉ ዋና ምክንያት ንሶም/ን ናይዚ መድሓኒት ተጠቃሚ ሰለዝኾኑ/ና እንትኸውን ኣብዚ መፅናዕቲ ንምስታፍ ፍቃደኛ እንተኮይኖም/ነን ጥራሕ እዩ :: ቅኑዕን ትኽክለኛን መልሶም ደረጃ ሳዕቤን እቲ መድሓኒትን ንቀፃሊ ክግበሩ ዘለዎም ምንገድታትን ንምሕባር ይጠቅም:: እዚ ቃለመሕተት ካብ 10 ክ15 ደቂቃ ክውድእ ዝኽእል ኾይኑ 5ሚሊ ደም ንላብራቶሪ ምርመራ ዘድሊ ኮይኑ ኣብዚ ቃለ-መሕተት ሽሞም/ሽመን ምፅሓፍ ኣየድልን:: ብምኻኑ ንዝገብርዎ/ኦ ቅኑዕ ምትሕብባር ኣቀዲመ ከመስግኖም/ነን እፎቱ::

ናይ ተሳታፊ ፈርማ _____
ዕለት_____

ኮድ_____

ኣድራሻ ፅንዓት መካየዲ ስቁ 0914046790
<u>1ይ ክፋል</u> :- ማሕበራዊ ኩነታት ተሳተፍቲ ዝምልከት

ተ ቁ	ዝርዝር ምሕትት	መልሲ	መብርሂ
101	ዕድመ		
102	ፆታ	1 ተባ 2 ኣን	
103	ኩነታት ሓዳር	1 በዓል/ቲ ሓዳር 2 ኣይተመርዓኹን 3 ተፋቲሐ 4 በዓል /ቲ ገዛይ ሞይቱኒ /ታትኒ	
104	ደረጃ ትምህርቲ	1 ምንባብን ምፅሓን ኣይኽእልን 2 ምንባብን ምፅሓን እኽእል 3 ክስካዕ 8ይ ክፍሊ ተኸታቲለ 4 ክስካዕ ሃይስኩል (9-12) ክፍሊ ተኸታቲለ 5 ኮሌጅ ዩኒቨርስቲ ተኸታቲለ	
105	ዝነብርሉ/ዝነብራሉ ቦታ	1 ገጠር 2 ከተማ	
106	ኩነታት ስራሕ	1 በዓልቲ ቤት 2 ናይ መንግስቲ ስራሕ 3 ሓረስታይ	

		4 ፋይቶት 5 ናይ ገዛ ሰራሕተኛ 6 ካሊእይገለፅ_____	
107	ወርሓዊ ኣታዊኦም/አን ብብር		
108	ዝጥቀምሉ/ማሉ ወልፊ እንተሃልዩ ምልክት ይግበሩ	1 ጫት 2 ሽጋራ 3 መዐወኒ ሓሽሽ 4 ኣልኮል 5 ካሊእ ይገለፅ_____	

2ይ ክፋል :- **ናይ HIV መድሓኒት እንትዉሰድ ዘሎ ኩነታት ኣመላካቲ ሓበሬታ**

ተ ቁ	ዝርዝር ምሕትት	መልሲ	መብርሂ
201	ቁመት (ሜ)		
	ክብደት(ኪግ)		
202	BMI(ኪግ/ሜ)		
203	መጠን ፀቕጢ ደም		
204	ናይ ኤች ኣይ ቪ መድሓኒት ዝጀመሩሉ/ ራሉ ዕለት		
205	መጠን CD4+ መድሓኒት እንትጅምሩ/ ራ		
206	መጠን viral RNA መድሓኒት እንትጅምሩ/ ራ		
207	ናይ ኤች ኣይ ቪ መድሓኒት ንክንደይ ዝኣክል ወሲዶም /ደን?		
208	መጠን CD4+ ኣብዚ ሐዚ እዋን		
209	መጠን viral RNA ኣብዚ ሐዚ እዋን		
210	ክትትሌ ሕክምና (ካርድ ረአ)	1. ድኹም 2. ጽቡቕ	
210	ኣብዚ ሐዚ እዋን ዝወስድዎ/ ኦ ሀለዉ ዋ ዓይነት መድሓኒት	1. 1a=d4T+3TC+NVP 2. 1b=d4T+3TC+EFV 3. 1c=AZT+3TC+NVP 4. 1d=AZT+3TC+EFV 5. 1e=TDF+3TC+EFV 6. 1f=TDF+3TC+NVP 7. 2a=ABC+ ddI+ LPv/r	

		8. 2b=TDF+ ddI+ LPR/r 9. 2c=TDF+ 3TC+ LPR/r 10. 10.2d=AZT+3Tc+ LPv/r	
211	ቅድሚ ሐዚ ዝወስድዎ/ኦ ዝነበረ መድሓኒት ተቐይሩ ዶ?	1 እወ 2 ኣይፋሉን	
212	ሕ 211 እወ እንተኾይኑ ቅድሚ ሐዚ ዝወስድዎ ዝነበረ መድሓኒት እንታይ ዓይነት ነይሩ/ ካርዲ ረኢ/		
213	ምክንያት መቐየሪ መድሓኒት እንታይ ነይሩ /ካርዲ ረኢ/		
214	ካብ ናይ ኤች ኣይ ቪ መድሓኒት ወፃኢ ዝወሰድዎ /ኦ መደሓኒት ኣሎ ዶ?	1 እወ 2 ኣይፋሉን	
215	ሕ 214 እወ እንተኾይኑ ዝወሰድዎ መደሓኒት እንታይ ዓይነት እዩ /ካረዲ ረኢ/	1 cotrimoxazole prophylaxis therapy 1 INH 3 ካልእ ይገለፅ_____	
216	ሕ 214 እወ እንተኾይኑ ኩነታት እቲ መድሓኒት	1 ወሲደ ወዲእዮ 2 ሐዚ እወስድ ኣለኹ 3 ኣቃሪፀዮ	
217	WHO Stage of HIV/AIDS	1. I/II 2. III 3. IV	

የቀንየለይ

12.2. Standard operational procedures of the renal function parameters

12.2.1. Determination of Creatinine testing

Specimen

Serum on heparinsed bottle

Safety Considerations

- Be careful with the handling of the Probe
- Avoid ingestion of reagents and buffers and contact with the eyes
- Observe universal safety precautions.

Equipment Supplies and Reagents

Equipment	Supplies	Reagents
ISE Electrolyte machine or equivalent Centrifuge	5000ul-100ul pipette and tips Eppendorf tubes or equivalent	ISE Reagent. Calibrator

Procedures

- Switch on power from the mains and then the UPS
- Switch on the ISE electrolyte machine
- Allow the machine to boot and auto-calibrate Confirm that the slopes are satisfactory
- Press TEST on the ISE machine
- Place serum or heparinsed plasma at the base of the probe (for aspiration) .
- Press RUN on the ISE machine
- Remove sample from the probe as the ISE machine commands
- Allow for auto-analysis
- Wait for the ISE machine to automatically display and print out the results.

Quality control/Quality Assurance

- Check for correctness of the slope
- Always run the control sample along with the test

Reporting and recording of of Results

The calculated result should be copied into the chemistry result register and laboratory result form. Reference range and unit of reporting should be quoted. Sign, date and stamp the result. File the original print out from the machine for reference.

12.2.2. Determination of uric acid

Principle: Enzymatic colorimetric test. Uricase cleaves uric acid to form allantoin and hydrogen peroxide. In the presence of peroxidase, 4-aminophenazone is oxidized by hydrogen peroxide to a quinone-diimine dye. The red color intensity of the quinone-diimine formed is directly proportional to the uric acid concentration and is determined by measuring the increase in absorbance.

Clinical significance: Uric acid is the major end product of purine metabolism and is one of the components of the nonprotein nitrogen fraction in plasma. Most uric acid formation occurs in the liver and is derived either from ingested or endogenous nucleoproteins. Approximately half of the total uric acid in the body is eliminated daily by urinary excretion and destruction in the intestinal tract. Numerous disease states and physiological conditions are associated with alterations in serum uric acid concentrations. Increased levels are more frequent. Serum uric acid levels are characteristically elevated in gout, a disorder involving either uric acid synthesis or excretion. Other common etiologies of hyperuricemia include renal dysfunction, ketoacidosis, glucose-6-phosphate deficiency, and Lesch-Nyhan syndrome. Decreased uric acid levels have been described in renal tubular absorption defects, Hodgkin's disease, bronchogenic carcinoma, severe hepatocellular disease, and xanthinuria.

Specimen: Serum or heparinized plasma collected using standard sampling tubes or tubes containing separating gel.

Stability: Serum/plasma: 5 days at 2-8°C, 6 months at -20°C.

Reagents /materials: Uric Acid ver.2, 400 Tests – the reagent cassette is labeled as UA2. R1 is in position B and R3 is in position C. R1 - Phosphate buffer: 0.05 mol/L, pH 7.8; TOOS: 7 mmol/L; fatty alcohol polyglycol ether: 4.8%; ascorbate oxidase (EC 1.10.3.3; zucchini) ≥83.5 µkat/L (25°C); stabilizers R3 - Phosphate buffer: 0.1 mol/L; pH 7.8; potassium hexacyanoferrate (II): 0.3 mmol/L; 4- aminophenazone ≥3 mmol/L; uricase (EC 1.7.3.3; Arthrobacter protophormiae) ≥83.4 µkat/L (25°C); peroxidase (POD) (EC 1.11.1.7; horseradish) ≥50 µkat/L (25°C); stabilizers Diluent NaCl 9%, 50 mL – the diluent cassette is labeled as NACL.

12.2.3. Procedure for Urinalysis Tests

Procedures:

1. Specimens should be sent to the laboratory as soon as possible. Transportation shall be accomplished in such a way as to minimize any damage to the specimens or containers. Urine samples must not be left at a receiving area or in an office or laboratory unless a responsible individual takes custody of the material. Persons processing and/or shipping specimens will maintain training documentation as required per Institutional Biosafety Committee policy. Always use your personal protective equipment (PPE) and observe universal precautions. If the sample cannot be processed immediately it can be refrigerated; verify sample's stability in the Sponsors Reference Laboratory Manual.

2. Prepare your working surface with absorbent paper.

3. The study personnel will identify the urine cup with the subject number and/or initials; the date, the name of the protocol and the collection time (once it is collected). Also, the laboratory requisition will be completed by the study personnel.

4. The urine cup will be handled in a transport bag with the biohazard symbol.

5. After the participant fills up the urine cup with enough sample, the study personnel will deliver it to the laboratory.

6. The laboratory personnel will verify that the information in the label and the requisition is complete.

7. Transfer the urine from the cup to the urine transport tube.

8. Secure the tube cap tightly.

9. Verify the information in the transport tube before packing it.

10. Discard the urine cup, the absorbent paper and any other contaminated material(s) in the biohazard waste container.

11. Pack and ship the sample as stablished by the laboratory reference manual.

12. Decontaminate the working surface with germicidal wipes.

13. Discard the wipes in the biohazard waste container.

14. Sign the maintenance records for "Laboratory Daily Maintenance".

12.2.4. Serum total protein

Principles of procedure: Polypeptides containing at least two peptide bonds react with biuret reagent. In alkaline solution, cupric ion forms a coordination complex with protein nitrogen with very little difference between albumin and globulin on a protein-nitrogen basis

Reagent handling and storage: Reagent Handling Remove air bubbles, if present in the reagent cartridge, with a new applicator stick. Alternatively, allow the reagent to sit at the appropriate storage temperature to allow the bubbles to dissipate. To minimize volume depletion, do not use a transfer pipette to remove the bubbles. CAUTION: Reagent bubbles may interfere with proper detection of reagent level in the cartridge, causing insufficient reagent aspiration which could impact results. Reagent Storage Unopened reagents are stable until the expiration date when stored at 15 to 30°C. Reagent stability is 23 days if the reagent is uncapped and onboard.

Specimen collection and handling: Suitable Specimens Serum and plasma are acceptable specimens.

Serum: Use serum collected by standard venipuncture techniques into glass or plastic tubes with or without gel barriers. Ensure complete clot formation has taken place prior to centrifugation. Separate serum from red blood cells or gel as soon after collection as possible. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may take longer to complete their clotting processes. Fibrin clots may subsequently form in these sera and the clots could cause erroneous test results.

Plasma: Use plasma collected by standard venipuncture techniques into glass or plastic tubes. Acceptable anticoagulants are lithium heparin (with or without gel barrier) and sodium heparin. Ensure centrifugation is adequate to remove platelets. Separate plasma from red blood cells or gel as soon after collection as possible. Refer to the specimen collection tube manufacturer's instructions for processing and handling requirements.

Specimen Storage Serum and plasma: Total protein is stable in serum and plasma for 1 week at room temperature, for at least 1 month when refrigerated, and for up to 2 months at -20°C.5 An in-house study confirmed total protein is stable in serum for 34 days at 2 to 8°C.

Procedure

Automated Dilution Protocol: If using the Automated Dilution Protocol, the system performs a dilution of the specimen and automatically corrects the concentration by multiplying the result by the appropriate dilution factor

Calibration: Calibration is stable for approximately 23 days (552 hours) and is required with each change in reagent lot number. Verify calibration with at least two levels of controls according to the established quality control requirements for your laboratory. If control results fall outside acceptable ranges, recalibration may be necessary. For a detailed description of how to calibrate an assay, refer to Section 6 of the instrument-specific operations manual. For information on calibrator standardization, refer to the Multi-constituent Calibrator package insert.

Quality control: The following is the recommendation of Abbott Laboratories for quality control. As appropriate, refer to your laboratory standard operating procedure(s) and/or quality assurance plan for additional quality control requirements and potential corrective actions.

• Two levels of controls (normal and abnormal) are to be run every 24 hours.

• If more frequent control monitoring is required, follow the established quality control procedures for your laboratory.

• If quality control results do not meet the acceptance criteria defined by your laboratory, patient values may be suspect. Follow the established quality control procedures for your laboratory. Recalibration may be necessary.

• Review quality control results and acceptance criteria following a change of reagent or calibrator lot.

12.2.5. Determination of BUN

Purpose

This procedure provides instruction on how to measure the concentration of Urea in serum using Pentra C 400 Chemistry Analyzer

Materials

Reagent 1:

TRIS pH 7.8 150 mmol/L

2-Oxoglutarate 9 mmol/L

ADP 0.75 mmol/L

Urease ≥ 7 kU/L

GLDH (Glutamate dehydrogenase) ≥ 1 kU/L

Sodium azide < 1 g/L

Reagent 2:

NADH 1.3 mmol/L

Sodium azide < 1 g/L

Reagents preparation:

: 1. Remove both caps of the cassette.

2. If present, remove foam by using a plastic pipette.

3. Place the cassette into the refrigerated Pentra C400 reagent compartment

Reagents stability and storage:- Stock reagent, stored at 2 – 8 °C, until expiry date even after opening.

- Automated clinical chemistry analyzer: Pentra C400
- Calibrator: **ABX Pentra Multical**, Ref. A11A01652
- Controls:

  ABX Pentra N Control, Ref. A11A01653, and

  ABX Pentra P Control, Ref. A11A01654

  ABX Pentra Urine Control L/H, Ref. A11A01674

- Standard laboratory equipment.

Sample

Sample type	Amount required	Transport and Storage	Stability
Serum, heparinised plasma or EDTA plasma	0.3mL	•Transport whole blood at RT •Separate serum within 1 Hr.	-2 days at room temperature 1 week at 4-8°C

Retention: Specimens are discarded in accordance with ARHL Specimen retention policy.

Safety Precautions

- Use Universal safety precaution(wearing gloves lab coat and washing hands) when handling infectious materials
- Refer to the National Health and Safety Guidelines for standard safety procedures

Calibration

For calibration, use: ABX Pentra Multical,

Calibrator preparation: Reconstituted one vial of Multical with 5ml of distilled water.

Note: Mix gently to avoid formation of bubble. Verify calibration with two levels of control (controls should be within the range).

Quality Control

Control	Level	Stability	Frequency	Preparation (y/n)
ABX Pentra N Control,	1	Lyophilized*:* Until Expiry Date Reconstituted: 24hr at 20-30°C 7days at 2-8°C 1month at -20°C	Once per a testing date	Yes
ABX Pentra P Control,	1			

Control preparation: Reconstituted one vial of ABX Pentra control N/P with 5ml of distilled water. **Note:**

- Controls must be kept at room temperature before use
- Controls must be within range. If out of range, repeat the run. If still out of the range. Investigate for root cause. (Reagent, Calibration and QC Preparation….etc).

Procedure

Refer chemistry analyzer manual and reagent leaflet insert kit.

Result Interpretation

The results will be interpreted as concentration of TG in serum as mg/dl.

Expected Values

Analyte	**Reference Range**	**Analytical Range/linearity**	**Units**
urea	10 - 50	300	mg/dl

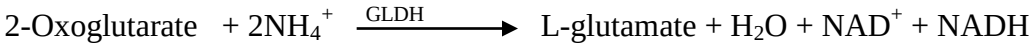
Limitations**: -** Gross hemolysis and icterus specimen.

Linearity: The test is linear up to 300 mg/dl. For urea concentrations exceeding 4500 mg/dl dilute the sample 1+1 with distilled water and repeat the determination. Multiply the result by 2.

Principle

Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia from this reaction combines with 2-oxoglutarate and NADH in the presence of glutamate-Dehydrogenase (GLDH) to yield glutamate and NAD+. The test has been optimized so that the GLDH is the rate limiting enzyme. The decrease in absorbance is proportional to the urea concentration within the given time intervals. As the kinetics is very fast this test is preferably designed for analyzer application.

$$\text{Urea} + 2H_2O \xrightarrow{\text{Unease}} 2NH_4^+ + CO_3^{2-}$$



References for the laboratory test procedures:

1. Tietz NW, editor. Text book of Clinical Chemistry.3rd ed. Philadelphia: WB Saunders, 2001
2. Chernecky & Berger: Laboratory Tests and Diagnostic Procedures, 5th ed.2008
3. Lothar Thomas: Clinical Laboratory Diagnostics, use and assessment of Clinical Laboratory Results 1st ed.1998,
4. Chemistry analyzer Operator Manual and leaflet for Urea determination