Reduced kidney function in tenofovir disoproxil fumarate based regimen and associated factors: A hospital based prospective observational study in Ethiopian patients

Taklo Simeneh

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This is to certify that the thesis prepared by Taklo Simeneh, entitled "Reduced kidney function in tenofovir disoproxil fumarate based regimen and associated factors: A hospital based prospective observational study in Ethiopian patients" and submitted in partial fulfillment of the requirements for the degree of Master of Science in Pharmacology complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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External Examiner: Bruck Messele (PhD) Signature _____Date_____

Internal Examiner: Solomon Mequanente (PhD) Signature _____Date_____

Advisor: Tefera Abula (PhD, Professor) Signature _____Date_____

Advisor: Wondwossen Amogne (PhD) Signature _____Date_____

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Chair of Department

ABSTRACT

Reduced kidney function in tenofovir disoproxil fumarate based regimen and associated factors: A hospital based prospective observational study in Ethiopian patients

Taklo Simeneh

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Tenofovir Disoproxil Fumarate (TDF), a drug broadly used in combination antiretroviral therapy (cART), is associated with renal dysfunction but the prevalence varied from country to country and it is not known in Ethiopia. TDF has been in use in Ethiopia since 2014. The objectives of this study were to assess prevalence of renal dysfunction and risk factors associated with it and the mean change in estimated glomerular filtration rate (eGFR) in human immunodeficiency virus (HIV) infected patients receiving TDF based antiretroviral regimen at Tikur Anbessa Specialized Hospital (TASH). It was a hospital based prospective cohort study. The study participants were treatment naïve HIV infected patients initiating TDF containing combination antiretroviral therapy or switched to because of adverse events. Multivariable logistic analysis was used to identify variables which have significant association.

Total of 63 study participants were studied, 16 (25.4%) of whom had greater than 25% fall in eGFR relative to baseline. Only age greater than 50 years old, CD4 count less than 200cells/mm^3, and proteinuria were significantly associated with renal dysfunction in multivariable logistic regression. There was -8.4 ml/min/1.73m^2 mean change in eGFR relative to baseline at six month of study. In conclusion, the renal dysfunction (defined as greater than 25% decline in eGFR) was found in a quarter of the study population. The long term impact and the clinical implication of it are not clear.

Future prospective study is required with large sample size and long duration to ascertain the prevalence of acute kidney injury (AKI) and its progression to chronic kidney disease (CKD).

Key words: Tenofovir Disoproxil Fumarate (TDF), TDF based antiretroviral regimen, renal dysfunction.

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ABCC2----------ATP binding cassette subfamily C member 2

ABCC4----------ATP binding cassette subfamily C member 4

AHR----------Adjusted hazard ratio

AKI----------Acute kidney injury

AOR----------Adjusted odds ratio

ART----------Antiretroviral therapy

ATP----------Adenosine triphosphate

CD4----------Cluster of differentiation 4

CI----------Confidence interval

CKD----------Chronic kidney disease

CKDEPI----------Chronic kidney disease epidemiology collaboration

COR----------Crude odds ratio

DNA----------Deoxyribonucleic acid

eGFR----------estimated glomerular filtration rate

HAART----------Highly active antiretroviral therapy

HR----------Hazard ratio

IQR----------Inter quartile range

MDRD----------Modification of diet in renal disease

MRP 2----------Multidrug resistance protein 2

MRP 4----------Multidrug resistance protein 4

NSAIDs----------Non-steroidal anti-inflammatory drugs

RNA----------Ribonucleic acid

SD----------Standard deviation

SNPs----------Single nucleotide polymorphisms

SPSS----------Statistical package for social sciences

TASH----------Tikur Anbessa Specialized Hospital

TDF----------Tenofovir Disoproxil Fumarate

1. INTRODUCTION

1.1. Overview of TDF and HIV

A tenofovir disoproxil fumarate (TDF) is an oral prodrug of tenofovir, a nucleotide reverse transcriptase inhibitor with activity against retroviruses, including HIV-1 and HIV-2 (1). It is a widely used drug in combination with other antiretroviral drugs for the treatment of HIV owing to its favorable pharmacodynamics and pharmacokinetics properties that allow once daily administration to increase adherence to lifelong treatment (2). However, TDF induced nephrotoxicity was reported recently in nearly 41% of participants treated with TDF based regimen for 10 years that makes its continuous use questionable (3). Several predisposing factors were identified for TDF-induced nephrotoxicity such as lower body mass index, protease inhibitor (4) low CD4 count, old age (5) and black race (6).

The use of highly active antiretroviral therapy (HAART) as treatment for HIV infection has greatly improved mortality and morbidity for adults and adolescents living with HIV around the world (2), but recent report indicated that almost half of HIV infected patients were not accessing HAART. And still Human Immunodeficiency virus/Acquired Immune Deficiency Syndrome (HIV/AIDS) is a devastating disease across the world by increasing costs of health care (7, 8) and decreasing the manpower (8). In the world, a total of 36.7 million people were living with Human Immunodeficiency Virus (HIV) in 2015. It caused 1.1 million AIDS related deaths and there were 2.1 million new infections globally in 2015. The highest prevalence of HIV was reported in Eastern and Southern Africa compared to other regions of the world in 2015 (8). In our country, considerable numbers of people were living with HIV and it caused significant number of deaths. Federal ministry of health in 2014 reported that a total of 769,602 people are living with HIV and annual AIDS related death was 35,578 (9).

Studies on HIV virology and pathogenesis address the complex mechanisms that result in HIV infection of the cell and destruction of the immune system. HIV's envelope proteins glycoprotein 120 and glycoprotein 41 help the virus entry by binding to CD4+ cell receptors and co-receptors on the outside of CD4+ cells and macrophages. T-cell tropic viruses require chemokine receptor 4 (CXCR4) to bind whereas macrophage-tropic strains of the virus require chemokine receptor 5 (CCR5). The joining of the proteins, the receptors and co-receptors fuses the HIV membrane with the CD4+ cell membrane, and the virus enters the CD4+ cell and macrophage. Once HIV enters the host cell, it converts its ribonucleic acid (RNA) into viral deoxy ribonucleic acid (DNA) by HIV reverse transcriptase. Further, HIV uses the enzyme integrase, HIV protease and other molecules for its success of survival in the host cell; it mainly deplete the CD4+ cells (10, 11). Knowing the pathogenesis of HIV led to the discovery of several classes

of antiretroviral drugs. Currently available classes of antiretroviral drugs include the nucleoside/nucleotide reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, a fusion inhibitor, a chemokine receptor 5 (CCR5) antagonist, and integrase strand transfer inhibitors (12). Currently two drugs in nucleotide or nucleoside reverse transcriptase inhibitors class and one from one of the non-nucleoside reverse transcriptase inhibitors, protease inhibitors or integrase strand transfer inhibitors class are combined for the treatment of HIV (12, 13) and despite their importance for the control of HIV, their shortcomings in short and long term use is its potential to cause renal dysfunction and liver disease that may be life threatening (14, 15).

A TDF based antiretroviral regimen is the preferred regimen for treatment of HIV in adults, adolescents, and pregnant women across the world (12, 13). Following absorption, TDF is rapidly converted to tenofovir, which is metabolized intracellularly to its active tenofovir diphosphate. Tenofovir diphosphate is a competitive inhibitor of HIV-1 reverse transcriptase and it terminates the growing DNA chain (1). TDF based regimen was found to have better virological suppression effect than zidovudine based regimen in randomized controlled trials (16, 17, 18). Additionally, an open-label, phase II trial in Thailand HIV infected adult patients revealed that TDF based antiretroviral regimen is well tolerated and effective in viral load suppression (HIV RNA less than 50 copies/ml) both in treatment naïve and treatment experienced patients (19). And also a two year retrospective cohort study in Ethiopia showed TDF based antiretroviral regimen was more efficacious than zidovudine based antiretroviral regimen regarding with immunological recovery (20). However, studies in Zambia and Nigeria showed that TDF based antiretroviral regimen is associated with higher mortality and a strong predictor of virological failure compared to zidovudine based regimen, respectively. In Zambia participants in TDF based regimen had lower CD4 count and lower body mass index than participants in Zidovudine based regimen. And in Nigeria more participants in TDF based regimen had hepatitis C virus coinfection than participants in zidovudine based regimen. These imbalances between the treatment groups can be the possible reason for the differences of the treatment outcomes (21, 22). Moreover, a new drug, Tenofovir Alafenamide Fumarate has equivalent efficacy to TDF with favorable effect on markers of bone and renal health; this will make tenofovir alafenamide fumarate preferred in HIV treatment to TDF although it is not extensively studied in long term use in different countries (23). TDF use is generally considered safe in clinical trials (24) and a meta-analysis of 17 prospective studies (including 9 randomized controlled trials) showed that TDF based antiretroviral therapy result in a modest decline in renal function that does not restrict TDF use where regular monitoring of renal function is impractical (25); however, there are increasing numbers of TDF induced nephrotoxicity case reports in real clinical practice (26, 27) and it has

a claim to be a potential cause of both acute kidney injury and chronic kidney disease (12, 13). The disparity of TDF safety profile between clinical trials and studies in real clinical practice may be attributed to different socio demographic factors, presence of comorbidity and comedications in real clinical practice (28-30). While the exact mechanism of tenofovir nephrotoxicity remains unclear, in a rat model proximal renal tubule mitochondrial swelling and reduction in activities of the respiratory electron transport chain complexes were seen. Mitochondria toxicity has been claimed as the major target of tenofovir induced renal toxicity (31). In addition, it can also indirectly damages the renal tubule possibly by activating nuclear factor kappa B protein 65 which is a proinflammatory transcription factor that result in inflammation induced kidney damage (32).

TDF induced chronic kidney disease (CKD) was significant both in developed and resource limited countries. In a retrospective study that included 213 subjects on antiretroviral therapy for a period of greater than 12 months in Brazil with study end point of estimated glomerular filtration rate (eGFR) less than or equal to 60ml/min/1.73m^2 estimated by modification of diet in renal disease (MDRD) equation, CKD was diagnosed in 8.4% of the study population. In this study, risk factors for CKD were hypertension (relative risk (RR) = 3.88, 95% confidence interval (CI) 1.84-8.16), time on HAART (RR = 1.15, 95% CI 1.03-1.27) and tenofovir exposure (RR = 2.25, 95% CI 1.04-4.95) (33).
A similar study in Italy with comparable sample size diagnosed CKD in 2.4% of the study subjects who were treated with TDF containing nucleoside reverse transcriptase inhibitors. Significant predictors ($p<0.05$) of renal impairment were age greater than 50 year (hazard ratio (HR) = 2.94, 95% CI 1.57-4.91), diabetes mellitus (HR = 5.11, 95% CI 2.35-7.64), and arterial hypertension (HR = 3.32, 95% CI 1.47-5.97) (34).

A Prospective observational study with study end point of eGFR less than 60ml/min revealed CKD incidence of 5.7%, 4.2% in United States of America and Asia pacific by using MDRD and chronic kidney disease epidemiology collaboration (CKD-EPI) equation to calculate eGFR, respectively. Black race, older age (greater than 50 year versus less than or equal to 30 year: (HR = 5.39, 95% CI 2.52-11.50, $p<0.001$)) and using protease inhibitor based regimen (HR = 1.93, 95% CI 1.22-3.07, $p = 0.005$) were risk factors for developing CKD (6, 35).

Another a 12 year prospective observational study in Japan found CKD in 10.8% of study subjects by using equations validated in Japanese population (36). Studies in Africa also showed significant percent of study subjects who had CKD but they used a single serum creatinine measurement to estimate GFR that

may not fully indicate CKD. Studies in Lesotho and Zimbabwe found 17.9% and 4.7% of study participants who had CKD, respectively (37, 38).

In Ethiopia studies were done to assess the impact of antiretroviral therapy on renal function, but those studies were done before the widespread use of TDF based antiretroviral regimen. Therefore, they did not show the impact of TDF based antiretroviral regimen on renal function. A cross sectional study in HIV infected patients on HAART at Felege Hiwot Hospital in Bahir Dar showed 12.9% of study subjects had eGFR less than 60ml/min (39). In addition, A retrospective study that was done at Gondar Hospital indicated that 11.7% of study subjects had CKD (15). These prevalences may be underestimated due to low percent (38.3% and 16%) of study subjects who were on TDF based antiretroviral regimen in Felege Hiwot Hospital and Gondar Hospital, respectively.

AKI was also found significantly in HIV infected patients who took TDF based antiretroviral regimen. Retrospective and prospective observational studies with 2 and 10 year exposure to TDF based antiretroviral regimen in Japan found greater than 25% decline of eGFR in 22.1% and 40.8% of study subjects, respectively. TDF associated renal dysfunction was more evident in patients with lower baseline body weight by multivariate analysis (<60 kilogram (kg): (AHR = 2.771, 95% CI 1.494-5.139, p = 0.001) and <70 kg (AOR = 2.5, 95% CI 1.55-4.00, p<0.001)) in the respective studies (3, 40). Furthermore, a prospective case cohort study in South Africa showed that among admitted patients with AKI 61% were on TDF based antiretroviral regimen (41).

Tenofovir undergoes renal elimination via a combination of glomerular filtration and active tubular secretion. Uptake of tenofovir from blood into renal proximal tubular cells is mediated by organic anion transporter protein 1 (OAT1) and organic anion transporter protein 3 (OAT3) (42). Its excretion from proximal tubule cell into urine is achieved by multidrug resistance protein 4 (MRP4) and multidrug resistance protein 2 (MRP 2) (42, 43) . Single nucleotide polymorphisms in these transporter proteins are associated with renal toxicity of tenofovir.

Single nucleotide polymorphism (SNP) in adenosine triphosphate binding cassette subfamily C member 4 (ABCC4) was associated with increase in the mean tenofovir plasma concentration (43). In addition, SNPs in adenosine triphosphate binding cassette subfamily C member 2 (ABCC2) was associated with kidney tubular dysfunction (44) and decreased eGFR (45). Therefore, occurrence of TDF induced AKI may differ in different racial groups owing to difference in the presence of polymorphism of tenofovir transporters in renal proximal tubule cells (46).

Additionally, HAART adverse drug reactions in developing countries may differ from those in developed countries because of high prevalence of conditions such as malnutrition, tuberculosis, anemia and patients presenting with advanced HIV disease (47). Furthermore, studies indicated that black race is at increased risk of developing AKI (6, 48, 49).

It has long been recognized that AKI has the capacity to initiate CKD. However, the frequency of disease progression remains a subject of considerable debate (50). In a study with a mean follow up of 5.7 years, 11.4% of AKI were progressed to end stage renal disease (ESRD) and AKI was also associated with mortality (48).

In Italy 2023 (29%) out of 6984 HIV infected patients developed mild renal impairment on TDF. Among them,191(9.4%) progressed to CKD (51). Clinical practice guideline for the management of chronic kidney disease in patients infected with HIV: 2014 Update by the HIV Medicine Association of the Infectious Diseases Society of America recommends that TDF treated patients shall be switched to alternative drugs when eGFR decline is greater than 25% from baseline and to level less than 60 ml/min/1.73m^2 (52).

Generally, the occurrence of renal dysfunction is becoming common in HIV infected patients who received TDF based antiretroviral regimen and its burden on survival and quality of life is becoming worse (51). The condition of renal complication in HIV infected patients can be worst in low and middle income countries as the condition requires enough capital to manage renal complications. Developing countries do not have enough access to dialysis and kidney transplant procedures, this further can make worse the consequence of AKI (53).

1.2 Renal dysfunction in TDF based regimen

Renal dysfunction has become a common entity in HIV infected patients. It can be caused directly or indirectly by HIV and/or drug related effects (28). TDF based regimen is found to be more risky than non TDF based regimen for the development of reduced kidney function (25). A number of cohorts and clinical studies have associated tenofovir with the development of renal toxicity with few studies on the opposing end (54).

TDF induced nephrotoxicity was reported in 0.5-45 % of HIV-positive patients and the wide range of prevalence is attributed to different populations and definitions of TDF-induced nephrotoxicity and duration of follow-up (55). Several studies revealed greater than 25% fall and mean change in eGFR relative to baseline in TDF based antiretroviral regimen, which are presented as follows.

A retrospective and prospective cohort study in Thailand showed greater than 25% fall in eGFR from baseline in 19.3% of 405 participants treated with TDF based antiretroviral regimen for a median of 16 months. A greater degree of decrease in GFR was found during the first three months (4). Similarly, another retrospective cohort study in Japan found renal dysfunction in 19.6% of 495 participants treated with TDF based antiretroviral regimen for 2 years (56).

In a retrospective cohort study consisting of 199 HIV infected patients who received TDF based antiretroviral regimen, there was a mean fall of 9ml/min/1.73m^2 in eGFR (glomerular filtration rate estimated by MDRD) from baseline at 6 month after treatment initiation. In this study, greater than 25% decline in eGFR from baseline was found in 22.1% of study subjects and the median time to this event occurrence was 246 days (range 1–1,339 days). Changes in eGFR in those patients treated with TDF containing antiretroviral regimen were characterized by a rapid decline during the first 24 weeks of therapy, followed by a plateau until 96 weeks (40).

A prospective observational cohort study in Spain revealed that majority of cases of greater than 25% fall in eGFR from baseline appeared mainly in the first months of treatment with a median of 6 (3–16.5) months and greater than 25% fall occurred in 10% of study participants after one year follow up (57).

A 10 year prospective study which enrolled 422 HIV infected Japanese patients who received TDF based antiretroviral regimen, showed greater than 25% decline in eGFR in 40.8% of study subjects. In this study, there was a mean fall of 8ml/min/1.73m^2 in eGFR from baseline at 6 month of post treatment initiation and eGFR was calculated using equation that was validated in Japanese people (3).

In Vietnam, a prospective observational study with 18 months follow up showed greater than 25% fall in eGFR from baseline in 12.4% of participants (58). Moreover, a retrospective cohort study in Malaysia and a prospective longitudinal study in Korea showed greater than 25% fall in eGFR to baseline in 15.2% and 6% of participants, respectively (5, 59).

A retrospective analysis of 1282 patients showed a mean fall of 8ml/min/1.73m^2 in eGFR (GFR estimated by MDRD) from baseline at 6 month of post TDF based antiretroviral regimen initiation (60). A study that was done in Italy revealed mean decline in eGFR of -5.1ml/min relative to baseline (34). In contrast, an open label, randomized trial consisting participants from Senegal and Cameroon showed a mean increase of 1.9ml/min of eGFR (61). And also study conducted in South Africa revealed nonsignificant mean decline in eGFR (-0.5ml/min) (62).

1.3 Factors associated with TDF induced renal dysfunction

Several factors that are associated with TDF induced renal dysfunction were identified in different studies. By multiple logistic regression, lower body weight (OR = 1.15 per 5 kg, 95% CI 1.00-1.33), lower body mass index (OR = 2.26 per 1 kg/m^2, 95% CI 1.74-2.94), baseline GFR (OR = 1.62 per 10 ml/min/1.73m^2, 95% CI 1.39-1.88), protease inhibitor (OR = 2.12, 95% CI 1.15-3.92), and nephrotoxic drug (OR = 3.16, 95% CI 1.44-6.98) were statistically significant factors associated with a 25% decrease in GFR (4). A study showed greater effect of ritonavir boosted atazanavir based antiretroviral regimen on mean decline in eGFR after the 12 months follow-up of study subjects (-10.4 ml/min/1.73 m^2 fall) than in those receiving efavirenz (-5.1 ml/min/1.73 m^2; p = 0.002) or ritonavir boosted lopinavir (-4.8 ml/min/1.73 m^2; p = 0.003) (34).

Another study also found that subjects receiving concomitant ritonavir boosted lopinavir, were more likely to develop eGFR less than 60ml/min (AHR = 2.4, 95% CI 1.2-4.8, p = 0.01) (60). Older age is also found as risk factors for developing renal dysfunction. Age (per 10 years older: (AHR = 2.21 95% CI 1.6- 3.0, p < 0.0001)) (60) and age greater than 50 years (prevalence ratio (PR) = 3.4, 95% CI 1.7-6.8)) (44) were associated factors with TDF induced reduction of eGFR less than 60ml/min/1.73m^2. And a study in Malaysia found that age greater than 50 years old was associated with greater than 25% reduction in eGFR (AOR = 2.26, 95% CI 1.22-4.17, P = 0.009) (5).

Among tenofovir users, factors associated with a reduction in eGFR were female gender (p<0.001) and African American ethnicity (p = 0.003) (29**)**. Baseline CD4 cell count less than 200cells/ml (HR = 2.66, 95% CI 1.65-4.29) was also found to be associated with reduction in eGFR (24). With regard to race, black race was more likely to develop AKI (6, 48, 49).

The presence of hypertension (30, 37), diabetes mellitus (30, 38), pulmonary tuberculosis (38) with HIV also associated with TDF induced renal dysfunction.

The role of renal tubular cell transporter proteins in tenofovir transport and toxicity profile was shown in cell line and animal model studies. MRP4 overexpressing cells were found to be 2.0 to 2.5-fold less susceptible to cytotoxicity caused by TDF (42) whereas in mice disruption of organic anion transporter protein 1 (OAT1) activity prevents tenofovir toxicity but loss of MRP4 can lead to increased renal proximal tubular toxicity (63).

SNPs in various renal proximal tubule cell transporter proteins are associated with increased concentration of tenofovir, and higher tenofovir plasma exposure is associated with decline in kidney function (64). Interestingly, cross sectional study that was conducted in Thailand HIV infected patients showed **(**after

adjusting for weight, eGFR, and the concomitant use of ritonavir-boosted protease inhibitors) a 30% increase in the mean tenofovir plasma concentration in patients having the *ABCC4* 4131 TG or GG genotype (43).

In a cross sectional study that was done in United States of HIV infected women that underwent intensive 24 hour pharmacokinetics sampling, adenosine triphosphate binding cassette subfamily G member 2 (ABCG2) SNP rs2231142 (rare allele carriers of CA or AA) was associated with 1.51fold increase in tenofovir area under the concentration-time curve (AUC) (95% CI 1.26-1.81, $p = 1.7 \times 10^{-5}$) (65).

A study done in Madrid showed that among 115 HIV infected patients, 19(16.5%) had Kidney tubular dysfunction. And SNP in adenosine triphosphate binding cassette subfamily C member 10 (rs9349256 (A →G)) located in intron 4 was significantly associated with kidney tubular dysfunction (66).

A cross sectional study conducted in 190 Japanese HIV infected patients revealed the conversion of genotype (GG → AA) at position 1249 of ABCC2 was significantly associated with kidney tubular dysfunction (AOR = 16.21, 95% CI 1.630-161.1, P = .017) (44).

In addition, study done in United State and Canada showed SNP in ABCC2 (rs8187707) was significantly associated with decreased estimated glomerular filtration rate (45). Although SNPs in renal tubular cell transporter proteins are associated in renal dysfunction (44), there is no conclusive findings to develop and recommend personalized TDF based antiretroviral therapy.

Accumulation of tenofovir owing to inhibition of MRP2 in renal proximal tubule cells by protease inhibitors is also implicated in increased risk of tenofovir related nephrotoxicity (67).

Tenofovir measurements with an area under the concentration time curve over the dosing interval, maximum concentration, and concentration at the end of the dosing interval (Ct) were 32%, 15%, and 51% higher, respectively, when TDF was co administered with ritonavir boosted lopinavir (n = 24) (68). And patients receiving TDF and ritonavir boosted protease inhibitor based antiretroviral regimen was at higher risk of developing CKD over 4 year (6).

Studies including prospective cohort studies showed risk factors and the extent of greater than 25% fall in eGFR to baseline in participants treated with TDF based antiretroviral regimen for 1-10 years (3, 4, 57). The prevalence of greater than 25% fall in eGFR compared to baseline varied from countries to countries (3, 59). To my knowledge, there is no study in Africa as well as in Ethiopia to show the extent of greater than 25% fall in eGFR relative to baseline.

Our national guideline put TDF based antiretroviral regimen as preferred first line for treatment of HIV in adults, adolescents and pregnant women, and many patients are more likely to be exposed to TDF. In addition, there is no routine renal function monitoring in our clinical settings (69).

So, the present study was planned to assess prevalence of renal dysfunction and risk factors associated with it and the mean change in eGFR. This study uses as a guide for early detection of renal dysfunction. It helps health care providers to identify HIV infected patients who are at risk. It also helps policy makers to give more attention to TDF related renal dysfunction and the necessity of future large scale study in order to know the clinical impact of TDF on renal function.

2. OBJECTIVES

2.1 General objectives

- ✓ To assess prevalence of renal dysfunction and risk factors associated with it and the mean change in eGFR in HIV infected patients receiving TDF based antiretroviral regimen at TASH

2.2 Specific objectives

- ✓ To assess prevalence of renal dysfunction in TDF based antiretroviral regimen
- ✓ To identify factors associated with renal dysfunction
- ✓ To determine mean change in eGFR relative to baseline at the end of study

3. METHODS

3.1 Study area and Period

Tikur Anbessa Specialized Hospital (TASH) was opened in 1972 as specialized hospital; then, the hospital became the site for training Medical Doctors. In 1998, the TASH is the largest referral hospital in the country, with 700 beds, was transferred to the School by the Federal Ministry of Health and it has since become a University teaching hospital. The Tikur Anbessa Specialized Hospital is now the main teaching hospital for both clinical and preclinical training of most disciplines. It is also an institution where specialized clinical services that are not available in other public or private institutions are rendered to the whole nation. The various departments, faculties and residents under specialty training in the School of Medicine provide patient care in the hospital. Recent report covered from July to October in 2016 showed that there were 3336 adult HIV positive patients and 473 pediatric patients on highly active antiretroviral therapy. The study area was selected purposively after making sure that larger number of HIV infected patients who newly initiated with TDF based antiretroviral regimen were found compared to other governmental hospitals in Addis Ababa. The study period was from January 15 to September 5, 2017 in Gregorian calendar.

3.2 Study design

The study design was hospital based prospective cohort study. HIV infected treatment naïve patients who are going to start taking TDF based antiretroviral regimen and treatment experienced participants whose ART are going to be switched to TDF based antiretroviral regimen were enrolled in the study.

3.3 Source population

Source population was all adult HIV positive patients who were treatment naïve and treatment experienced patients and registered to have follow up at TASH, ART clinic. Both treatment naïve and treatment experienced participants were enrolled before their commencement of taking TDF based antiretroviral regimen. Treatment experienced patients were those who were taking non TDF based antiretroviral regimen before enrollment in the study.

3.4 Study subjects

Adult HIV positive treatment naïve patients who newly start taking TDF based antiretroviral regimen after enrollment. And also treatment experienced patients whose ART are going to be switched to TDF based antiretroviral regimen from non TDF based antiretroviral regimen after enrollment were included in the study.

3.5 Sampling method and recruitment procedures

Due to time constraints 2 month was selected conveniently as study subject recruitment period and all study participants who fulfilled the inclusion criteria in the 2 month recruitment period (from January 15 to March 23, 2017) were enrolled consecutively in the study. The recruitment of participants was done by data collectors in the ART clinic where HIV diagnosis was done and ART intake form was filled. Information sheet was read and explained to all potential participants. Potential participants were treatment naïve participants who are assigned to newly start TDF based antiretroviral regimen. And potential participants also included treatment experienced participants whose ART are going to be switched to TDF based antiretroviral regimen after enrollment. After enrollment, the study participants were followed for 6 month duration. The presence of community HIV screening program in Addis Ababa and the test and treat strategy of TASH ART clinic played a key role for the enrollment of 61 study participants who were assigned to start taking TDF based antiretroviral regimen in the 2 month period. Another 5 treatment experienced participants whose ART are going to be switched to TDF based antiretroviral regimen in the 2 month period, were also enrolled in the study. Therefore, finally a total of 66 study participants were enrolled. 63 participants were completed the study and included in the analysis but 3 study participants were lost from follow up.

3.6 Inclusion criteria

Inclusion criteria were as follows: 1) Individuals who were voluntary to participate in the study 2) Age $\geq$ 18 years 3) Treatment naïve patients who were assigned to start taking TDF based antiretroviral regimen after enrollment 4) Treatment experienced patients whose ART are going to be switched to TDF based antiretroviral regimen 4) Patients who had eGFR by CKD EPI equation greater than 60ml/min/1.73m^2 4) Patients who gave consent to complete the study follow up period.

3.7 Exclusion criteria

Exclusion criteria were: 1) pregnant women 2) Inpatient individuals 3) Individuals who took TDF based antiretroviral regimen previously

3.8 Data collection procedures

Data was collected by two senior nurses after getting half day training about the objective, methodology and data collection approach. Data was collected through face to face interview and by laboratory tests. Patients' socio demographic and clinical characteristics were recorded by face to face interview of each study participants in the structured questionnaire. In addition, for patients who had prior HAART exposure, their previous HAART and baseline CD4 count were recorded in the checklist from patient chart. For treatment naïve patients' baseline CD4 count was done in routine care and it was recorded in the checklist.

Weight and height were measured by using Seca 761 weight scales and height ruler (with meter reading) which is attached with it, respectively (made in Germany). Body mass index of participants was calculated as follows: Body mass index = Weight (in kg) ÷ (Height (in m)) ^{2}

After collection of urine by 30ml urine cap (HENSO Medica.co.Ltd., China), baseline proteinuria and glycosuria were determined by comber 10 strip (HENSO Medica.Co.Ltd., China). Serum creatinine was analyzed prospectively at baseline, then after 1 month, 2 months and at end of 6 months by chemistry analyzer (HITACHI 902) at TASH chemistry laboratory. The selection of each study visits (1, 2 and 6 month) for the determination of eGFR was based on previous studies (4, 40, 57).

A study consisting of 20132 HIV infected patients revealed findings that support the use of CKD-EPI in HIV-positive individuals against MDRD equation (70). Clinical Practice Guideline for the Management of Chronic Kidney Disease in Patients Infected with HIV: 2014 Update by the HIV Medicine Association of the Infectious Diseases Society of America recommends that for patients requiring dose adjustment, GFR should be estimated by CKD EPI equation. Moreover, MDRD equation was validated only in patients who have eGFR less than 60ml/min/1.73m^3 whereas CKD EPI equation was validated both in patients with less than 60ml/min/1.73m^3 and greater than 60ml/min/1.73m^3 (38). Therefore, CKD EPI equations were used to estimate GFR. GFR in black male and female was estimated by CKD EPI equations as follows:

- ✓ For female with serum creatinine $\leq$ 0.7mg/dl; GFR = $166 \times (Scr/0.7)^{-0.329} \times (0.993)^{Age}$ and with serum creatinine > 0.7mg/dl; GFR = $166 \times (Scr/0.7)^{-1.209} \times (0.993)^{Age}$
- ✓ For male with serum creatinine $\leq$ 0.9mg/dl; GFR = $163 \times (Scr/0.9)^{-0.411} \times (0.993)^{Age}$ and with serum creatinine > 0.9mg/dl; GFR = $163 \times (Scr/0.9)^{-1.209} \times (0.993)^{Age}$ (71).

  Where, age in year and serum creatinine in mg/dl.

3.9 Data quality assurance

The data collectors were trained for half day on the objective and methodology of the research, data collection approach. The questionnaire was translated to Amharic language and back translated into English by another person to check for consistency. During data collection, data was checked for its completeness and missing information at each point. Furthermore, data was checked during entry into the computer before analysis.

3.10 Operational definition

Renal dysfunction is defined as greater than 25% fall in eGFR relative to baseline (25).

3.11 Data analysis procedures

Mean (±standard deviation (SD)), median (interquartile range (IQR)), frequencies and percent (%) were used to describe patients' characteristics. The prevalence of greater than 25% decline in eGFR relative to baseline was calculated by dividing a number of patients with greater than 25% decline in eGFR by total number of patients and multiplied by 100. A repeated measures (within-subjects) analysis of variance (ANOVA) were used to compare means of eGFR obtained at different study visits and post hoc tests were performed by paired t-test using Bonferroni correction to pinpoint specific mean that significantly differ from other means. Univariate logistic regression was used to determine the factors associated with renal dysfunction. Clinically significant factors in the literature were entered in multivariable logistic regression without restriction by $p < 0.2$. Other independent variables that presented with $P < 0.20$ were considered in a multivariable logistic regression model. Adjusted odds ratio (AOR) and its 95% CI were estimated. A p-value <0.05 was considered statistically significant. All statistical analyses were performed using the 20 version of SPSS program.

3.12 Ethical consideration

The ethical clearance was obtained from Addis Ababa University, School of pharmacy (Ref. No. ERB/SOP/07/09/2016). After receiving ethical clearance, permission to conduct the research was obtained from Internal medicine department of TASH, Addis Ababa University. Information sheet was prepared and read to all eligible participants about the purpose of study. Informed consents were obtained from the study participants and were enrolled after they signed on informed consent paper voluntarily. Name of the participant was omitted from the questionnaire; instead medical record number was used to ensure confidentiality.

4. RESULT

4.1 Sociodemographic and clinical characteristics of study participants

Total of 66 HIV infected patients with 61 naïve to antiretroviral therapy and 5 treatment experienced whose ART are going to be switched to TDF based antiretroviral therapy because of adverse effects were enrolled in the study. Three treatment naïve participants were lost from follow up without having serum creatinine values after baseline visits and their age was around the median of study participants. These participants were reported non-adherence as the reason for their loss of follow up but they reported that they hated to take the drugs without adverse events. Among lost participants, 2 were males who had CD4 counts of 206, 76 which was lower than the median CD4 count of the study participants and 1 was female who had CD4 count of 606 that is higher than the median CD4 count of participants. Total of 63 participants were included in the final analysis. The mean ($\pm$ SD) age was 39.7 ($\pm$10), 43 (68.3%) of study participants were female. The mean ($\pm$SD) body mass index was 22.6 ($\pm$4.5) kg/m^2 and other sociodemographic characteristics are shown in Table 1.

Most of patients (58 patients, 92.1%) were treatment naïve at initiation of tenofovir containing antiretroviral therapy, and the remaining 5 (7.9%) had prior exposure to zidovudine based antiretroviral regimen. Majority of patients (56, 88.9%) were taking TDF + lamivudine + efavirenz whereas the remaining patients were taking TDF + lamivudine+ ritonavir boosted atazanavir regimen. Median (IQR) CD4 count was 241 (106-457), 42 (66.7%) of patients were found in world health organization (WHO) clinical stage I. The median (IQR) of duration of HIV infection since diagnosis was 3 month (0 – 60) month; 20 (31.8%) of study participants were with comorbidity of either hypertension (6.4%), type 2 diabetes mellitus (3.2%), cancer (12.7%), tuberculosis (12.7%) or kidney stone (3.2%). Opportunistic infections other than tuberculosis were found in 8 (12.7%) of participants.

Among study participants, 8 (12.7%) were taking isoniazid preventive therapy. In addition, angiotensin converting enzyme inhibitor + hydrochlorothiazide and metformin + glibenclamide were taken by 4 (6.3%) and 2 (3.2%) of study participants, respectively. and other clinical characteristics are shown in Table 2.

Table 1: Baseline sociodemographic characteristics of study participants in Tikur Anbessa Specialized Hospital (TASH), October, 2017. [n = 63]

Characteristics		Frequency (%)
Sex	Male	20 (31.7)
	Female	43 (68.3)
Marital status	Single	8 (12.7)
	Married	40 (63.5)
	Divorced	11 (17.5)
	Widowed	2 (3.2)
	Missing	2 (3.2)
Educational status	Illiterate	5 (7.9)
	Primary	18 (28.6)
	Secondary	28 (44.4)
	Diploma	5 (7.9)
	Degree	7 (11.1)
Ethnicity	Oromo	20 (31.7)
	Amhara	22 (34.9)
	Tigre	6 (9.5)
	Others	15(23.8)
Occupation	governmental employee	12 (19)
	private	34 (54)
	not employed	17 (27)
Age	$\leq$ 50 years old	56(88.9)
	>50 years old	7 (11.1)
Body mass index (in kg/m^2)	< 18.5	12 (19)
	$\geq$18.5	51 (81)

Note: Other ethnic groups include Mixed ethnicity, Gurage, Silte, Hadiya, Welayta, and Harari

Table 2: Baseline clinical characteristics of study participants in TASH. [n = 63]

Characteristics		Frequency (%)
WHO stage	Stage 1 Stage 2 Stage 3 Stage 4	42 (66.7) 3 (4.8) 6 (9.5) 12 (19)
Cotrimoxazole prophylaxis	Yes No	42 (66.7) 21 (33.3)
Duration of HIV infection since DX	Median (IQR) 3 (0-60) month	
	< 3 month ≥3 month	37 (58.7) 26 (41.3)
Baseline CD4 count (cells/mm^3)	Median (IQR) 241 (106-457)	
	< 200 CD4 count ≥ 200 CD4 count	29 (46) 34 (54)
Baseline serum creatinine (mg/dl)	Mean (±SD) 1.01 (± 0.16)	
	< 1 ≥1	18 (28.6) 45 (71.4)
Baseline eGFR (ml/min/1.73m^2)	Mean (±SD) 90.8 (±16.8)	
	< 90 ≥90	35(55.6) 28 (44.4)
History of cancer	Yes No	8 (12.7) 55 (87.3)
Antituberculosis drugs	Yes No	4 (6.3) 59 (93.7)
NSAIDs	Yes No	6 (9.5) 57 (90.5)
Proton pump inhibitor	Yes No	9 (14.3) 54 (85.7)
Chemotherapy	Yes No	4 (6.3) 59 (93.7)
Baseline proteinuria	+1 +2	9 (14.3) 4 (6.3)
Baseline glycosuria	+1 +3	2 (3.2) 1 (1.6)

WHO, World Health Organization; DX, Diagnosis; IQR, Inter quartile range; SD, Standard deviation; NSAIDs, Nonsteroidal anti-inflammatory drugs; eGFR, estimated glomerular filtration rate.

4.2 Renal dysfunction among study participants

Among study participants greater than 25% fall in eGFR were found in 16 (25.4%) of study participants during the entire study period and the majority of these cases were occurred in the first month of study follow up period. Out of 16 (25.4%) of study participants who were diagnosed with renal dysfunction, 7 (11.1%) and 9 (14.3%) were male and female, respectively. Renal dysfunction was occurred in 15 (23.8%) of participants who were taking TDF + lamivudine + efavirenz and in 1 (6.2%) of participants who were taking TDF + lamivudine + ritonavir boosted atazanavir regimen. In the study period, 11 (93.3%) of the cases were occurred in patients who had baseline eGFR equal or greater than 90ml/min/1.73m^2.

In the current study, CKD was confirmed by 2 consecutive measurement of eGFR < 60ml/min/1.73m^2 at 4 month interval and CKD was detected in 2 (3.2%) of study participants.

Table 3: Greater than 25% fall in estimated glomerular filtration rate of study participants in TASH, October, 2017. [n = 63]

		Greater than 25% decline in eGFR	
		New cases	Total cases
Months	0	-	-
	1	10 (15.9)	10 (15.9)
	2	2 (3.2)	9 (14.3)
	6	4 (6.3)	13 (20.6)

Note: Glomerular filtration rate were estimated by CKD EPI equation and events expressed in number (percent); eGFR, estimated glomerular filtration rate in ml/min/1.73m^2. New cases mean cases that occurred newly at each visit.Total cases mean sum of cases that occurred in previous study visit (should persisted) and new cases occurred at a given visit.

Table 4: Chronic kidney disease among study participants in TASH, October, 2017. [n = 63]

		eGFR < 60ml/min/1.73m^2 measured at 1 point of time	eGFR < 60ml/min/1.73m^2 measured at 4 month interval
		Number (%)	Number (%)
Months	0	0 (0)	-
	1	7 (11.1)	-
	2	4 (6.3)	-
	6	3 (4.8)	2 (3.2)

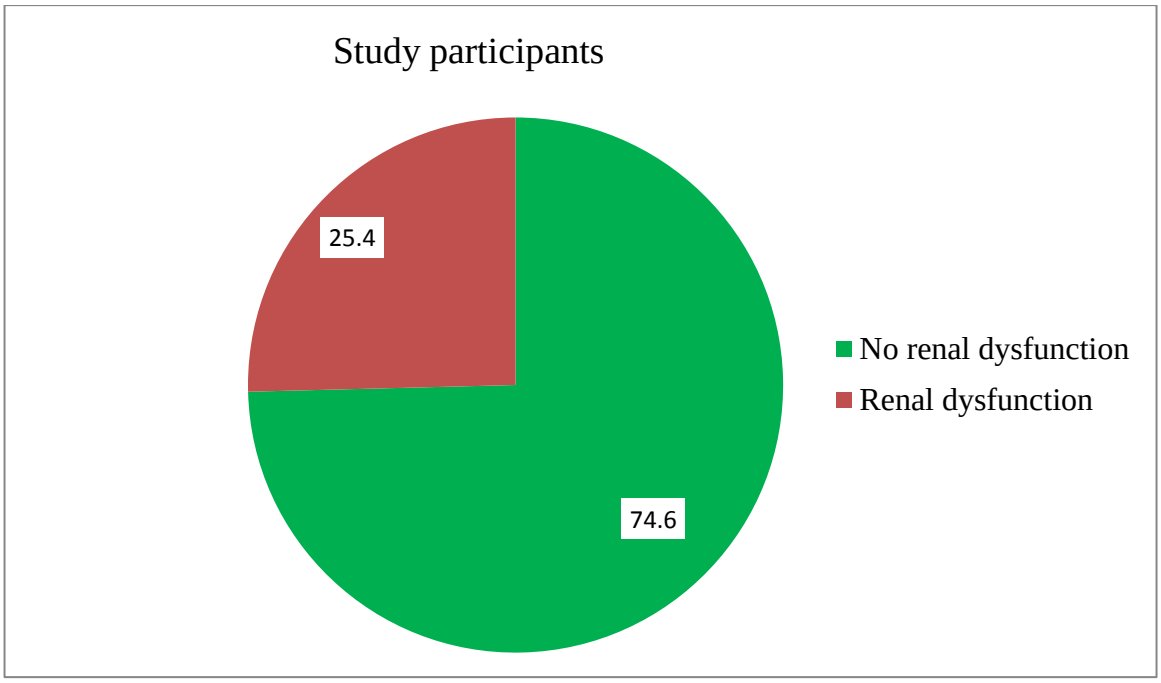

Figure 1. The overall prevalence of renal dysfunction of participants treated with TDF based antiretroviral regimen in TASH, October 2017, Addis Ababa, Ethiopia. [n = 63]

From the total of 63 participants who were treated with TDF based antiretroviral regimen, 25.4% of them had renal dysfunction and 74.6% of them did not have renal dysfunction.

4.3 Factors associated with renal dysfunction

Hypertension, type 2 diabetes mellitus, tuberculosis, kidney stone and prior exposure to antiretroviral drugs were not included in univariate logistic analysis because participants with these factors did not experience renal dysfunction. Clinically significant factors (body mass index, chemotherapy, age and protease inhibitor) were included in multivariable logistic regression without restriction by $p < 0.2$. Independent variables which were entered in univariate logistic regression were sex, ethnicity, age, body mass index, WHO clinical stage, baseline CD4 count, duration of HIV infection since diagnosis, current ART, cotrimoxazole prophylaxis therapy, isoniazid preventive therapy, history of cancer, presence of opportunistic infections, presence of chemotherapy, proton pump inhibitors, presence of proteinuria, presence of glycosuria, baseline serum creatinine and baseline eGFR. Among them, baseline CD4 count, baseline eGFR, presence of proteinuria, history of cancer and cotrimoxazole prophylaxis therapy were significantly associated with renal dysfunction.

In multivariable logistic regression, age greater than 50 years old, presence of proteinuria and baseline CD4 count less than 200 cells/mm^3 were significantly associated with renal dysfunction (AOR = 64.8, 95% CI 1.60-2707.70, P = 0.029; AOR = 51.3, 95% CI 1.80-1448.70, P = 0.021 and AOR = 63.2, 95% CI 2.02-1979.66, p = 0.018), respectively. However, other variables did not maintain their statistical significance in multivariable logistic regression (Table 5).

Table 5: Factors associated with greater than 25% fall in eGFR during the study period by univariate and multivariable logistic regression in TASH, October, 2017. [n = 63]

Variable	Category	Renal dysfunction		Univariate COR (95% CI)	Multivariable AOR (95% CI)
		Yes	No		
Sex	Male Female	7 9	13 34	1.0 0.5 (0.20, 1.64)*	1.0 19.0 (0.30,1338.12)
Ethnicity	Oromo Amhara Tigre Others	6 5 1 4	14 17 5 11	1.2 (0.27, 5.33) 0.8 (0.18, 3.69) 0.6 (0.05, 6.27) 1.0	
Age	≤ 50 year > 50 year	13 3	43 4	1.0 2.5 (0.49, 12.54)*	1.0 64.8(1.60,2707.70)**
Body mass index (kg/m^2)	< 18.5 ≥18.5	3 13	9 38	1.0 (0.23, 4.16) 1.0	0.08 (0.01, 3.40) 1.0
WHO stage	I II III IV	11 0 0 5	31 3 6 7	1.0 0.0 (0.00) 0.0 (0.00) 2.0 (0.50, 7.70)	
Duration of HIV infection since DX (in month)	< 3 ≥3	10 6	27 20	1.0 0.8 (0.25, 2.68)	
Baseline CD4 count	< 200 CD4 count ≥200 CD4 count	13 3	16 31	8.4 (2.10, 33.80)* 1.0	63.2(2.02,1979.66)** 1.0
Current ART	TDF+3TC+EFV TDF+3TC+ATV/r	15 1	41 6	1.0 0.5 (0.50, 4.10)	1.0 0.1 (0.01, 32.90)
Cotrimoxazole prophylaxis	Yes No	15 1	27 20	11.1(1.40,92.10)* 1.0	0.91 (0.04, 22.43) 1.0
Isoniazid preventive therapy	Yes No	1 15	7 40	0.4 (0.04, 3.40) 1.0	
History of cancer	Yes No	5 11	3 44	6.7 (1.40, 32.30)* 1.0	36.6 (0.50, 2699.88) 1.0
Opportunistic infections	Yes No	2 14	6 41	1.0 (0.18, 5.40) 1.0	
Chemotherapy	Yes No	2 14	2 45	3.2 (0.40, 25.00) 1.0	3.74 (0.02, 938.60)
Proton pump inhibitor	Yes No	2 14	7 40	0.8 (0.20 , 4.40) 1.0	
Baseline serum creatinine	< 1mg/dl ≥ 1mg/dl	7 9	11 36	2.6 (0.80, 8.40)* 1.0	0.35(0.01, 9.90) 1.0
Baseline eGFR (ml/min/1.73m^2)	< 90 ≥ 90	5 11	30 17	1.0 3.9 (1.15, 13.06)*	1.0 11.6(0.23, 587.30)
Proteinuria	Yes No	8 8	5 42	8.4 (2.20, 32.30)* 1.0	51.33(1.80,1448.70)** 1.0
Presence of glycosuria	Yes No	2 14	1 46	6.6(0.60, 78.00)* 1.0	53.0 (0.49, 5774.33) 1.0

COR, Crude odds ratio; AOR, Adjusted odds ratio; CI, Confidence interval; ART, Antiretroviral therapy; 3TC, Lamivudine; EFV, Efavirenz; ATV/r, ritonavir boosted atazanavir. *Indicates variables that were entered in univariate logistic regression; **Indicates variables which were significant in multivariate logistic regression (P < 0.05).

4.4 Mean change in estimated glomerular filtration rate

The mean (± SD) baseline eGFR of study participants was 90.8 (± 16.8)ml/min/1.73m^2 and 55.6% of them had baseline eGFR of less than 90ml/min/1.73m^2. A repeated measures one way ANOVA determined that means differed significantly between time points (F (2.63, 163.32) = 8.80, P < 0.005). Post hoc tests using bonferroni correction revealed nonsignificant mean increase of eGFR from post 1 month to post 2 month TDF based regimen initiation (p = 1) whereas there were nonsignificant mean reduction of eGFR from post 1month to post 6 month (P = 1) and from post 2 month to post 6 month TDF based regimen initiation (P = 1). Post hoc tests using bonferroni correction showed significant mean reduction of eGFR from baseline to post 1, 2, 6 month of TDF based regimen initiation (shown in table 6).

A repeated measures one way ANOVA confirmed that means of SCr significantly differed between time points (F (2.75, 170.33) = 8.58, P < 0.0005). Post hoc tests using bonferroni correction revealed that there was no significant difference between mean of SCr of post 1 month and 2 month, post 1 month and 6 month, and post 2 month and 6 month of TDF based regimen initiation. Post hoc tests using bonferroni correction showed significant mean increase of SCr from baseline to post 1, 2, 6 month of TDF based regimen initiation (shown in table 6).

Table 6: Changing patterns of estimated glomerular filtration rate and Serum creatinine over 6 months follow up of study participants in TASH, October, 2017. [n = 63]

	Months			
	0	1	2	6
Mean eGFR ± SD	90.81 ± 16.8	82.46 ± 17	82.93 ± 15.3	82.44 ± 15
Mean change in eGFR ± SD 95% CI of mean change in eGFR	- -	-8.35 ± 17.5*$^+$ -12.81, -3.8	-7.89 ± 15.10*$^+$ -11.70, -4.10	-8.37 ± 18.4*$^+$ -13.00, -3.70
Mean SCr ± SD	1.01 ± 0.16	1.10 ± 0.19	1.09 ± 0.17	1.10 ± 0.17
Mean change in SCr ± SD 95% CI of mean change in SCr	- -	0.09 ± 0.18*$^+$ 0.05, 0.14	0.08 ± 0.17*$^+$ 0.04, 0.12	-0.08 ± 0.19*$^+$ 0.04, 0.13

Note: eGFR, estimated glomerular filtration rate; SEM, standard deviation; CI, Confidence interval; SCr, Serum creatinine. P values by repeated measures one way ANOVA (* stands for P < 0.05) and Post hoc tests using paired t test with bonferroni correction ($^+$ stands for P < 0.05).

5. DISCUSSION

In the present study, renal dysfunction was detected in 16 (25.4%) of study participants and factors associated with renal dysfunction were age greater than 50 years old, baseline CD4 count less than 200 cells/mm^3 and proteinuria. In this study, there was significant mean reduction of eGFR at 1, 2 and 6 month of post TDF based regimen initiation compared to mean baseline eGFR (-8.35; P = 0.001, -7.89; P = 0.001 and -8.44; P = 0.002, respectively).

Among participants who were diagnosed with renal dysfunction at end of first month visit, 9.5% of them continued with renal dysfunction at 2 and 6 month study visits. However, none of them developed CKD. The result of this study was higher than from studies conducted in Thailand (19.3%), Malaysia (15.2%), Japan (19.6%, 22.1%), Spain (10%), Vietnam (12.4%), and Korea (4, 5, 40, 56-59). This discrepancy may be attributed to difference in genetic factors as some patients experience renal adverse effects of tenofovir more frequently than others. This issue may be related to genetic polymorphism in renal proximal tubule cell transporter proteins and consequently accumulation of TDF in proximal tubular cells may lead to reduction in glomerular filtration rate (55). Participants in Thailand, Spain, Vietnam and Korea had higher median baseline CD4 count (more than 320cells/mm^3) than the median CD4 count (241cells/mm^3) of the participants of the present study. Participants in Japan and Vietnam had younger median age (36 years) than participants (40 years) of the present study. Therefore, these differences in median CD4 count and age may partially explain the discrepancy between the result of the current study and the previous studies.

The finding of the present study was lower than the finding of another study done in Japan (40.8%) (3). This discrepancy may be attributed to difference in sociodemographic factors and number of study participants. A study done in Japan was a 10 year follow up study and most of patients (89%) compared to this study (11.1%) were taking protease inhibitor based antiretroviral regimen which is known to decrease eGFR greater than TDF regimen with non-nucleoside reverse transcriptase inhibitors (4). Therefore, these differences in duration of follow up and proportion of participants taking protease inhibitor based antiretroviral regimen may be another possible reason for the variation of the findings.

Even if assessment of CKD was not the objective of the present study, CKD was diagnosed in 2 (3.2%) of participants which was similar with other study (72). The result of this study was lower than the result of the study done in Japan. The difference might be due to difference in duration of study follow up (36).

The result of this study was lower than the results of the studies done in Africa. However, studies in Africa were cross sectional which diagnosed CKD at a point of time which might overestimate CKD (37, 38). The finding of this study was higher than the study finding in Italy. Participants in Italy did not receive protease inhibitors and had higher median baseline CD4 count, so these differences might be the reason for the discrepancy of the findings (34). The difference also might be attributed to sociodemographic factors because black race is more risky for developing CKD (6).

In the present study, age greater than 50 years old was associated with renal dysfunction (AOR = 64.8, 95% CI 1.60-2707.70, p = 0.029). This finding was in line with another finding that was done in Malaysia (5) and it is known that age greater than 50 years old is an established risk factor for tenofovir induced nephrotoxicity. This can be explained by age related structural and physiological deterioration of the kidney (13).

There was a significant association between baseline CD4 count less than 200cells/mm^3 and eGFR fall greater than 25% as compared to study participants who had baseline CD4 count equal or greater than 200cells/mm^3 in the present study (AOR = 63.2, 95% CI 2.02-1979.66, P = 0.018). This result was similar with the study in Maryland and United states of America (24, 29). Study done in Spain also revealed that patients who had lower baseline CD4 count were associated with AKI (57). This might be related to the fact that patients with advanced HIV infection are more risky to renal dysfunction owing to direct injury of HIV on renal cells (28).

The present study had also shown that there was association between proteinuria and renal dysfunction (AOR = 51.3, 95% CI 1.80-1448.70, P = 0.021) and this result was similar with study conducted in Canada (73). The study done in Japan also found that participants with proteinuria had significantly low eGFR compared to participants without proteinuria (74). In the current study body mass index was not associated with renal dysfunction in contrast to the finding of the study in Thailand (AOR = 2.26) (4). The discrepancy might be attributed to difference in number of study participants.

The present study by post hoc tests using bonferroni correction showed significant mean reduction of eGFR from baseline to post 1, 2, 6 month of TDF based regimen initiation. The mean decline in eGFR of -8.4ml/min/1.73m^2 at the end of the study relative to baseline was similar with other studies (3, 40, 60). This result was higher than the finding of the study done in Italy. The participants of the study in Italy had higher median baseline CD4 count than participants of this study and no participants receiving protease inhibitors. So, these differences might be the reason for the disagreement of the findings (34).

In addition, the finding of the current study was higher than results from study done in Africa (participants recruited from Senegal and Cameroon). Participants of the study in Africa did not take protease inhibitors and had no comorbidities in contrast to our study. These differences might partially explain the discrepancy of the findings (61). The result of the study in Africa showed a mean increase of 1.9ml/min in eGFR, which is in contrast to findings from several studies (3, 40, 60). How tenofovir increases glomerular filtration rate in African HIV infected patients is not yet clear.

The result of the current study was also higher than the result of the study from South Africa. The discrepancy of the findings might be partially due to difference in inclusion criteria. The median age of South African participants was 35.4 years whereas median age of the participants of this study was 40 years (62). The mechanism by which tenofovir reduces glomerular filtration rate is not well understood. In fact, tenofovir is known to cause renal tubular dysfunction (44), which might subsequently result in significant reduction in glomerular filtration rate (45, 74).

6. STRENGTH OF THE STUDY

- ✓ This is a prospective study which assessed the impact of tenofovir on renal function more than at a point of time.
- ✓ Greater than 95% of study participants completed the study follow up period.
- ✓ More independent variables were assessed to identify their association with renal dysfunction.

7. LIMITATION OF THE STUDY

- ✓ The study was conducted by enrolling consecutively all participants who fulfilled the inclusion criteria in the two month period, so mean change in eGFR may not be generalized to the whole target population.
- ✓ The sample size is small.
- ✓ The duration of follow up is relatively short.
- ✓ Renal tubular dysfunction was not assessed in this study, so tenofovir associated renal dysfunction may be underestimated.

8. CONCLUSION

Greater than 25% fall in eGFR was occurred in a quarter of participants. Among 10 (62.5%) of the cases that were occurred at the end of one month follow up period, 9.5% of them continued with renal dysfunction at 2 and 6 months of study visits.

In multivariable logistic regression, age greater than 50 years old, CD4 count less than 200cells/mm^3 and proteinuria were significantly associated with renal dysfunction.

There was similar mean change in eGFR relative to baseline after one, two and six months of follow up period. And also there was significant reduction in mean of eGFR at 1, 2 and 6 months of study visits compared to mean of baseline eGFR.

9. RECOMMENDATION

As renal dysfunction was occurred in a considerable number of participants and it was persisted throughout the study visits in 6(9.5%) of participants, which may progress to CKD. Therefore, renal function monitoring is recommended in patients receiving tenofovir. Patients who have age greater than 50 years old, CD4 count less than 200cells/ mm^3 and proteinuria are at risk of developing tenofovir associated renal dysfunction, so renal function monitoring is especially recommended for these patients.

A prospective long term study with large sample size should be performed to better ascertain TDF based antiretroviral regimen induced renal dysfunction and factors associated with it. And the progression of greater than 25% fall in eGFR to kidney disease should be ascertained in long term study.

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Annex 1: Information sheet in English

Hello. My name is Taklo Simeneh and I am a master student at Addis Ababa University, College of Health science. I am conducting this research for partial fulfillment of master's degree in pharmacology. The study needs study participants who are going to be newly initiated with TDF based regimen. I would very much appreciate your participation in this study. The study period is 6 months and participants are required to be volunteer in filling required questionnaire and allowing their medical chart to be reviewed and /or to give blood and urine sample if your routine laboratory monitoring not match with the study schedule. If required participants will give blood sample at least 3 times in the full course of the study. Your Height and weight will be measured and you will be asked about your socio demographic profiles for a period of 10 – 15 minutes. For any cost you pay for this study will be compensated and any laboratory fee for the purpose of study will be covered by me.

The objective of this study is to assess renal dysfunction and factors associated with it and the mean change in eGFR in 6 month study period.

Whatever information regarding you will be kept strictly confidential, and will not be shared with anyone other than members of our research team. Participation in this study is voluntary; you can withdraw any time from this study even if your full course participation is necessary.

At this time, do you want to ask me anything about the study? May I begin registering your name now?

Signature of the researcher: ---------- Date: ----------/--------/--------

1. Agree to participate if yes, enroll
2. Agree to participate if no, leave

For more information and questions here is the contact address of investigator:

Taklo Simeneh

You can call any time via 0948854469

Annex 2፡ Information sheet in Amharic

<u>ስለምርምሩ መረጃ</u>

እኔ ታክሎ ስሜነህ በአዲስ አበባ ዩኒበርስቲ የሁለተኛ ዲግሪ የፋርማኮሎጅ ተማሪ ስሆን የመመረቂያ ፅሁፌን የምሰራው የቴኖፎቪር የጎንዮሽ ጉዳት በሚል ሲሆን በኩላሊት ላይ ያለውን የጎንዮሽ ጉዳት ያተኩራል፡፡ ጥናቱ ላይ እንዲካተቱ የሚፈለጉ ሰወች ቴኖፎቪር ጥምር አዲስ የሚወስዱ ሰዎች ናቸው፡፡

በጥናቱ ላይ ብትሳተፉ በጣም ደስተኛ ነኝ፡፡ የጥናቱ ዐላማ ቴኖፎቪር በኩላሊት ጤነኛ ስራ ላይ ያለውን ጉዳት ሲሆን የሚወስደው ጊዜ 6 ወር ነው፡፡ ከተሳታፊዎች የሚጠበቁት፡-- 10 አስከ 15 ደቂቃ ላልበለጠ ጊዜ ቃለመጠይቅ መደረግ፣ ካርዳቸው ላይ ለጥናቱ የሚረዱ መረጃወች እንዲታዩ ፈቃደኛ መሆን፣ አስፈላጊ ከሆነ የሽንት ናሙና መስጠት እና 3 ጊዜ የደም ናሙና መስጠት፣ የሰውነት ክብደትዎን ለማወቅ ይረዳ ዘንድ ክብደትዎን ለመለካት ፍቃደኛ መሆን ናቸው፡፡

ማንኛውም ከእናንተ የተገኘ መረጃ ጥናቱን ከሚያደርጉት ሰወች ውጭ ለማንም ተላልፎ እንደማይሰጥ ለጥናት ስነ ምግባር ኮሚቴ ቃል ገብቻለሁ፤ አሁን ለናንተም ቃል እገባለሁ፡፡ ጥናቱ በጥናቱ ለመሳተፍ ፍላጎት ያላቸውን ብቻ ያካትታል፣ በጥናቱ አለመሳተፍ ምንም ጉዳት አያስከትልም፣ የእናንተ መሳተፍ ግን ለጥናቱ የላቀ ድርሻ አለው፡፡ ማንኛውም የጥናቱ ተሳታፊ በፈለገው ጊዜ ጥናቱን ማቋረጥ ይችላል፡፡ ጥያቄ ካላችሁ ጠይቁ፤ አሁን ስማችሁን መመዝገብ እችላለሁ፡፡

የተመራማሪው ፊርማ፡- ቀን፡-

1. ለመሳተፍ ፈቃደኛ የሆነ.....ለጥናቱ ይመዘገባል
2. ፍቃደኛ ያልሆነ..... ይታለፋል

ለበለጠ መረጃ በሚከተለው ስልክ ደውሉ
ታክሎ ስሜነህ፤ 0948854469

Annex 3: Consent form in English

I_____ am informed on study to be conducted by Masters Student in AAU, college of health sciences on tenofovir exposure and reduced kidney function. My participation in this study is voluntary, no obligation to participate. There is no harm by not participating and no special benefit by participating and also the study period takes 6 months. I heard all the information mentioned above and willing to participate in the study.

1. Study participant signature_____ date_____/_____/____

2. Researcher name and signature……

Annex 4: Consent in Amharic

በጥናቱ ለመሳተፍ ፈቃደኛ መሆንዎን የሚገልፅ የስምምነት ውል

እኔ.....የተባልሁ ሰው ከላይ ቴኖፎቢር ኩላሊት ላይ የሚያደርሰው ጉዳት በሚለው የ ሁለተኛ ዲግሪ የመመረቂያ ጥናት ርዕስ የተነገረውን ሁሉ ሰምቸ ፣ትረጉሙ ገብቶኝ ፣እንድሳተፍ ግፊት ሳይደረግብኝ ፣ ጥናቱ ለ6 ወር በጥቁር አንበሳ ሆስፒታል የሚደረግ መሆኑን ተረድቸ፣ በጥናቱ ባለመሳተፍ የሚቀር ምንም ጥቅም አለመኖሩ ተነግሮኝ ተስማምቻለሁ፡፡

የተሳታፊ ፊርማ.....ቀን.....

ጥናቱን የሚያደርገው አካል ስም.....ፊርማ.....

Annex 5: Questionaire and checklist in English

Questionnaire and checklist to assess patient characteristics (sociodemographic, diseases, drugs and laboratory values).

I. Identification and demographic data

1. First name (includes father name)?……..

2. Chart number?……

3. Sex (as given)?…….

4. Age (as given)?……

5. Weight (BMI (as measured)) and height?……

6. Marital status? 1. Single 2. Married 3. Divorced 4. Widow/er 5. Missing

7. Education 1. Illiterate 2. Primary school 3. Secondary school 4. Diploma 5. University graduate

8. Ethnicity? 1. Oromo 2. Amhara 3. Tigray 5. Others (specify)……

9. Occupation (as given)?…….

II. Medical History

1. When was HIV diagnosed (as given)? .....
2. Prior exposure/experience with antiretroviral therapy? 1. Yes 2. No
3. If yes, review the records (list the drugs).

   ……

   ……
4. History of Hypertension? 1. Yes 2. No 3. Doesn't know
5. If yes to question # 4, specify the duration and the medication being taken.

   ……

   …….
6. History of diabetes mellitus? 1. Yes 2. No 3. Doesn't know
7. If yes to question # 6, specify the duration and the medication being taken.

……

……

8. History of liver disease? 1. Yes 2. No 3. Doesn't know
9. If yes to question # 8, specify the duration and the medication being taken.

……

……

10. History of renal disease? 1. Yes 2. No 3. Doesn't know
11. If yes to question # 10, specify when the diagnosis was made and indicate current medications for the renal disorder.

……

……

12. History of other diseases? 1. Yes 2. No 3. Doesn't know
13. If yes to question # 12, mention the disease name, duration and the medication being taken.

……

……

14. Which WHO clinical stage of HIV /AIDS? 1. Primary HIV infection 2. Stage I 3. Stage II 4. Stage III 5. Stage IV

III. Current diseases and drugs

1. History of current tuberculosis on treatment? 1. Yes 2. No
2. If yes to question # 1, specify the drugs and duration on treatment?

.....

3. Mention other diseases if you are suffering from and name the medication you are taking.

……

……

……

4. Type of ART combination being taken currently and start date?

…….....

5. Are you taking currently cotrimoxazole prophylaxis? 1. Yes 2. No
6. If yes to question # 5, determine dosage regimen, start and stop date.

……

……..

7. Are you taking currently isoniazid (INH) preventive therapy? 1. Yes 2. No

8. If yes to question # 7, determine dosage regimen, start and stop date. ……
……..

III. Laboratory values

1. Hemoglobin (Hgb)……..date……..…..

2. WBC count……..date……..….

3. Platelet……..date……

4. Urine analysis:

- ✓ Glucose…….date……..…..
- ✓ Protein……...date…….……..…
- ✓ Specific gravity……...date……
- ✓ Microscopy……..date……

5. Liver function tests

- ALT…….date……..…
- AST……. date..…….
- ALP……..date……..

6. Bilirubin (T) (D)……date……

7. CD4 count……..date…….….

8. Serum creatinine……date……..

9. Glomerular filtration rate as estimated by CKD EPI equation……..date……..

10. HBsA……date…….

11. HCV Antibody…….date……..

12.VDRL……date…….

13. Others (laboratory tests)…….……

Laboratory values after therapy initiation

After one month:

1. SCR…….……..date…….…...

2. eGFR by CKD EPI equation……..……date…….

3. Others (laboratory tests)……

After two month:

1. SCR……..date…….

2. eGFR by CKD EPI equation ……..date……..……..

3. Others (laboratory tests)……..……..

After six month:

1. SCR……date……..….

2. eGFR by CKD EPI equation…….date……

3. Urine analysis:

- ✓ Glucose…….date……..……...
- ✓ Protein……...date…….…….…
- ✓ Specific gravity……...date……
- ✓ Microscopy……..date……....

4. Weight (BMI)…….date……..……..

5. CD4 count…….……date……..……..

6. Others (laboratory tests)……..……

- Diseases (events) occurred after ART initiation. Write start and stop date of events. ……
- Drugs added after ART initiation. Write start and stop date.…….

Annex 6: Questionaire in Amharic

ቃለ መጠይቅ

ሀ) የጥናቱን ተሳታፊ መለያ እና ስነ-ህዝባዊ መረጃዎች

1. ስም ከነአባት?----------

2. የህክምና ቁጥር?----------

3. ፆታ?----------

4. ዕድሜ?----------

5. ክብደት?---------- እና ቁመት?----------

6. የጋብቻ ሁኔታ? ሀ. ያላገባ/ች ለ. ያገባ/ች ሐ. የፈታ/ች መ. የሞተበት/የሞተባት ሠ. ተለያይተው የሚኖሩ

7. የትምህርት ደረጃ? ሀ. ማንበብም ሆነ መጻፍ አልችልም ለ. አንደኛ ደረጃ ያጠናቀቅሁ (1 -8) ሐ. ሁለተኛ ደረጃ ያጠናቀቅሁ (9-12) መ. ከሁለተኛ ደረጃ የበለጠ ከድግሪ ያነሰ ያጠናቀቅሁ ሠ. ዩንቨርስቲ ያጠናቀቅሁ

8. ብሔርዎ ምንድን ነው? ሀ. አማራ ለ. ትግሬ ሐ. ኦሮሞ መ. ሌላ ይግለፁ-------

9. ከሚከተሉት የስራ መደቦች የትኛው እርስዎን ይገልፃል? ሀ. የመንግስት ተቀጣሪ ለ. የግል ተቀጣሪ ሐ. ስራ የሌለው

ለ) የበፊት የህክምና መረጃዎች

1. ቫይረሱን መቼ ተመርምረህ አወቅህ (በወር ይገለፅ)?----------

2. ፀረ-ኤች ኤይቪ ወስደው ያውቃሉ? ሀ. አዎ ለ. የለም

3. ለጥያቄ ተራ ቁጥር 2 መልሱ አዎ ከሆነ የመዳኒቶች ዐይነት ከህክምና ካርድ ይታይ

4. የደም ግፊት አለብዎ? ሀ. አዎ ለ. የለም ሐ. አላውቅም

5. ለጥያቄ ተራ ቁጥር 4 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

6. የስኳር ህመም አለብዎ? ሀ. አዎ ለ. የለም ሐ. አላውቅም

7. ለጥያቄ ተራ ቁጥር 6 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

8. የጉበት ህመም አለብዎ? ሀ. አዎ ለ. የለም ሐ. አላውቅም

9. ለጥያቄ ተራ ቁጥር 8 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

10. የኩላሊት ህመም አለብዎ? ሀ. አዎ ለ. የለም ሐ. አላውቅም

11. ለጥያቄ ተራ ቁጥር 10 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

12. ሌላ ህመም አለብዎ? ሀ. አዎ ለ. የለም ሐ. አላውቅም

13. ለጥያቄ ተራ ቁጥር 12 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

14. በአለም ጤና ድርጅት መስፈርት የኤች አይቪ/ኤድስ የህመም ደረጃ ሀ. ጉንፋን መሰል በኤችአይቪ የመያዝ ምልክት ለ. ደረጃ 1 ሐ. ደረጃ 2 መ. ደረጃ 3. ደረጃ 4

ሐ) በጥናቱ መጀመሪያ ወቅት ስለተከሰቱ በሽታዎች ና መዳኒቶች መረጃ

1. የሳንባ ነቀርሳ አለብዎ? ሀ. አዎ ለ. የለም

2. ለጥያቄ ተራ ቁጥር 1 መልሱ አዎ ከሆነ የመዳኒቱ ዐይነት ና ለምንያህል ጊዜ እንደተወሰደ ይገለፅ

---

3. ሌላ አሁን ያለብዎ ህመም ና የሚወስዱት መድሃኒት ይጠቀስ

---

4. አሁን የምትወስዱት የፀረ-ኤች አይቪ መድሃኒት ዐይነት? ----------የጀመረበት ቀን----------

5. በሽታ መከላከያ ባክትሪም ይወስዳሉ? ሀ. አዎ ለ. የለም

6. ለጥያቄ ተራ ቁጥር 5 መልሱ አዎ ከሆነ የመድሃኒቱ መጠን ፣ የተጀመረበት ቀን ና የቆመበት ቀን ይጠቀስ----------
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7. የሳንባ ነቀርሳ መከላከያ ክኒን ይወስዳሉ? ሀ. አዎ ለ. የለም

8. ለጥያቄ ተራ ቁጥር 5 መልሱ አዎ ከሆነ የመድሃኒቱ መጠን ፣ የተጀመረበት ቀን ና የቆመበት ቀን ይጠቀስ----------
---

Annex 7: Serum creatinine and eGFR follow up profile of study participants

Code	Sex	Age	Baseline SCr	SCr at 1 month	SCr at 2 month	SCr at 6 month	Baseline eGFR	eGFR at 1 month	eGFR at 2 month	eGFR at 6 month
1	2	32	0.8	0.9	1	1	112.82	97.84	86.14	86.14
2	2	41	1.1	1.3	1.2	0.9	72.06	58.88	64.87	91.85
3	2	45	1	1.2	1	1	78.62	63.07	78.62	78.62
4	2	40	0.8	1	1	0.9	106.65	81.43	81.43	92.5
5	2	50	0.9	1.3	1.2	1.3	86.22	55.28	60.89	55.28
6	2	65	0.9	1.2	1.2	1	77.6	54.8	54.8	68.32
7	1	45	0.9	1.1	1	1.1	118.82	93.23	104.61	93.23
8	1	42	0.9	1.1	1	1.1	121.35	95.21	106.84	95.21
9	1	35	1	1.1	1	1	112.23	100.01	112.23	112.23
10	2	40	0.9	0.9	0.9	0.9	92.5	92.5	92.5	92.5
11	1	42	1	1.3	1.2	1.6	106.84	77.8	85.7	60.53
12	2	43	0.8	0.9	1	1.1	104.43	90.57	79.74	71.06
13	2	18	0.9	0.8	1	0.9	107.95	124.48	95.04	107.95
14	2	38	0.7	0.8	0.8	1	127.11	108.16	108.16	82.59
15	2	40	1	1	1.2	1	81.43	81.43	65.32	81.43
16	1	38	1.5	1.5	1.4	1.5	67.3	67.3	73.16	67.3
17	2	30	1	1	1.2	1.2	87.36	87.36	70.08	70.08
18	1	55	1	1.3	1	1.4	97.52	71.01	97.52	64.92
19	1	35	1.3	1.4	1.2	1.2	81.72	74.72	90.02	90.02
20	2	34	1.1	1.1	0.9	1.2	75.69	75.69	96.48	68.14
21	2	45	1.1	1	1.3	0.9	70.07	78.62	57.25	89.3
22	2	38	0.9	1.3	1.3	1.3	93.8	60.14	60.14	60.14
23	2	33	1	1.1	1.1	1	85.54	76.23	76.23	85.54
24	1	26	1.5	1.2	1.2	1.1	73.22	95.9	95.9	106.54
25	2	60	1.1	1.2	1.2	1.2	63.06	56.76	56.76	56.76
26	2	60	1.1	1.2	1.2	1.2	63.06	56.76	56.76	56.76
27	1	39	1	1.3	1.1	1	109.12	79.46	97.24	109.12
28	2	45	1.1	1	1	1.1	70.07	78.62	78.62	70.07
29	1	38	1.3	1.3	1.4	1.2	80.02	80.02	73.16	88.15
30	1	38	1.1	1.5	1.5	1.4	97.93	67.3	67.3	73.16
31	2	28	1	1.2	1.1	1.2	88.6	71.07	78.95	71.07
32	2	32	1	0.7	0.9	0.9	86.14	132.58	97.84	97.84

Annex 7. Continued

Code	Sex	Age	Baseline SCr	SCr at 1 month	SCr at 2 month	SCr at 6 month	Baseline EGFR	eGFR at 1 month	eGFR at 2 month	eGFR at 6 month
33	2	39	1	1.1	0.9	1	82.01	73.08	93.15	82.01
34	2	40	0.8	1	0.8	1	106.65	81.43	106.65	81.43
35	1	33	1	1.1	1.1	1.1	113.81	101.43	101.43	101.43
36	2	23	1	1	1.3	1.3	91.76	91.76	66.82	66.82
37	2	32	1.1	1.2	1.2	1.2	76.77	69.1	69.1	69.1
38	2	45	0.9	0.9	1	1.2	89.3	89.3	78.62	63.07
39	2	28	1	1.2	1.1	1	88.6	71.07	78.95	88.6
40	2	35	0.8	1	1	1	110.46	84.34	84.34	84.34
41	2	48	0.6	0.8	0.8	1	124.65	100.82	100.82	76.98
42	1	64	1.1	1.4	1.4	1.4	81.58	60.95	60.95	60.95
43	2	27	0.8	1	0.9	1	116.85	89.22	101.34	89.22
44	1	40	1	1.5	1.4	1.3	108.35	66.37	72.14	78.9
45	1	40	1	1.4	1.4	1.4	108.35	72.14	72.14	72.14
46	2	40	1	1	1	1	81.43	81.43	81.43	81.43
47	2	40	1	0.9	1	0.9	81.43	92.5	81.43	92.5
48	1	39	1	1	1.1	1.1	109.12	109.12	97.24	97.24
49	2	32	1	0.9	1	1	86.14	97.84	86.14	86.14
50	2	50	1.2	1.3	0.9	1	60.89	55.28	86.22	75.91
51	2	49	1.2	0.8	1	0.9	61.32	100.12	76.44	86.83
52	2	40	1.1	1.3	1	1.1	72.57	59.3	81.43	72.57
53	2	29	1	1	1.1	1	87.98	87.98	78.4	87.98
54	1	30	1.3	1	1.1	1.1	84.64	116.24	103.59	103.59
55	2	42	1	0.9	1.1	1.1	80.3	91.21	71.56	71.56
56	2	28	1	1.2	1.1	1	88.6	71.07	78.95	88.6
57	2	60	1	1	0.8	1	70.76	70.76	92.67	70.76
58	1	60	1	1	1	1.1	94.15	94.15	94.15	83.9
59	1	33	1.2	1.2	1.1	1	91.3	91.3	101.43	113.81
60	2	26	0.9	1	0.9	0.8	102.05	89.85	102.05	117.67
61	2	40	1	1	1.4	1.1	81.43	81.43	54.22	72.57
62	1	37	1.1	1.2	1.2	1.2	98.62	88.77	88.77	88.77
63	2	40	0.9	1	1	0.9	92.5	81.43	81.43	92.5

Code is a number given to participants in order to keep their confidentiality; SCr, Serum creatinine; eGFR, estimated glomerular filtration rate. In sex column 1 stands for male and 2 stands for female.