Table of contents

Abstract in English

Background: Limited studies assessed the effect of nutritional supplementation on serum 25-hydroxyvitamin D (25(OH)D) level in sub-Saharan Africa. Moreover, estimated glomerular filtration rate estimating (eGFR) equations using serum creatinine are not validated in most African settings and various results are reported on the effect of TDF on kidney function.

We assessed the serum 25 (OH)D level and renal function of HIV positive and negative adults in Ethiopia, the performance of eGFR equations compared to 24-hour creatinine clearance in HIV positive adults and investigated the role of antiretroviral treatment (ART) and nutritional intervention in the serum 25 (OH)D and the role of ART especially tenofovir disoproxil fumarate (TDF) on renal function changes in HIV positive adults.

Method: A randomized nutritional supplementation trial was conducted on HIV positive adults who were eligible for ART initiation in Ethiopia. The trial compared 200 g/d of lipid-based nutrient supplement with no supplementation during the first three months of ART. The supplement provided an amount of vitamin D twice the recommended daily allowance (10µg/200g).

Blood sample for serum 25(OH)D and creatinine measurement to estimate glomerular filtration rate and spot urine samples for urine dipstick analyses were collected at baseline and after 3, 6 and 12 months of ART. The performance of eGFR equations ((Cockcroft and Gault (CG), Modification of Diet in Renal Disease (MDRD), and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)) compared to 24-hour creatinine clearance in sample of HIV positive adults that volunteered for 24-hour urine collection.

Result: A total of 348 HIV positive and 100 HIV negative persons were recruited. Median baseline serum 25(OH)D level was higher in HIV positive than HIV negative persons. The supplemented group had a 4.1 (95% CI: 1.7; 6.4) nmol/l increase in serum 25(OH)D whereas the non-supplemented group had a 10.8 (95% CI: -13.9; -7.8) nmol/l decrease in serum 25(OH)D level after 3 months of ART.

We found no difference in eGFR between HIV positive and negative adults. For all eGFR equations, the correlation between eGFR and 24-hr creatinine clearance was 0.45-0.53 and the

accuracy within 30% of 24-hr creatinine clearance was 24–46%. Removing ethnic coefficient reduced the bias and improved accuracy of the CKD-EPI and the MDRD estimates.

About 82.3% of HIV positive adults were initiated on a TDF-based ART regimen. The median (IQR) change in eGFR with 12 months of ART was 0.8 (-11.1; 10.0) ml/min/1.73m^2. About 41% and 26.9% of patients had a drop greater than 3 and 10 mL/min/1.73 m^2 eGFR at 12 month, respectively. However, none of the patients had declined eGFR to < 60ml/min/1.73m^2 within 12 months. There was no difference in change of eGFR from the baseline to 12 months between patients initiating TDF- based regimen and non TDF-based regimen. Moreover, none of the patients had persistent proteinuria or glycosuria.

Conclusion: Nutritional supplementation which contained vitamin D prevented a reduction in serum 25 (OH) D levels in HIV positive persons initiating ART. Ethiopian HIV positive adults in the present study had relatively good kidney function at the initiation of ART and it remained stable over 12 months of ART. Moreover, no difference in renal function was observed between HIV positive adults treated with TDF and non TDF-based regimen. However, older HIV positive adults and patients with unsuppressed viral load deserve special focus on renal monitoring and also data on long-term safety of TDF is still needed in this population. All eGFR equations overestimated 24-hr creatinine clearance in the study population.

Resume på dansk

Baggrund: Kun få studier har undersøgt effekten af ernæringstilskud til HIV smittede på serum 25-hydroxyvitamin D (25(OH)D) niveau i Afrika syd for Sahara. Desuden er vurdering af nyrefunktionsniveau ved bestemmelse af den estimerede glomerulære filtration rate e (eGFR) ved brug af serum kreatinin generelt ikke valideret i afrikanske lande, og studier af effekten af det antiretrovirale stof, tenofovir disoproxil fumarate (TDF) på nyrefunktion har vist inkonsistente resultater.

Vi undersøgte serum 25(OH)D og nyrefunktion blandt Hiv- positive og negative voksne Etiopiere, og sammenlignede eGFR resultater med 24-timers kreatinin clearance bestemmelser blandt HIV positive voksne. Desuden undersøgte vi betydningen af antiretroviral behandling (ART) og ernæringstilskud på serum (OH)D, samt betydningen af ART, specielt TDF, på nyrefunktion blandt Hiv-positive voksne.

Metoder:

Et randomiseret ernæringsstudie blev gennemført blandt Hiv-positive voksne som opfyldte kriterierne for påbegyndelse af ART i Etiopien. Studiet sammenlignede 200 g/dag af et fedtbaseret ernæringstilskud med intet tilskud over de første tre måneder under ART. Ernæringstilskuddet indeholdt en mængde af vitamin D svarende til to gange det anbefalede daglige indtag (10µg/200g). Blodprøver til bestemmelse af serum 25(OH)D ved baseline og 3 måneder efter ART og kreatinin, til estimering af den glomerulære filtration rate, samt spot urinprøver til urin-dipstick analyser ved baseline samt efter 3, 6 og 12 måneder på ART blev indsamlet. Resultaterne af formlerne for eGFR ((Cockcroft and Gault (CG), Modification of Diet in Renal Disease (MDRD), og Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI)) blev Bundersøgt og sammenlignet med 24-timers kreatinin clearance i en stikprøve af Hiv-positive voksne, som var villige til at deltage i 24-timers urin opsamling.

Resultater:

I alt 348 Hivpositive og 100 Hiv-negative personer blev rekrutteret. Median baseline serum 25(OH)D var højere blandt Hiv-positive end Hivnegative. Den gruppe, der modtog

ernæringssupplement havde 43% (95% CI: 31%; 55%) højere serum 25(OH)D end den ikke-supplementerede gruppe ved 3 måneder. Den supplementerede gruppe fik en 4.1 (95% CI: 1.7; 6.4) nmol/l stigning i serum 25(OH)D, mens den ikke-supplementerede gruppe havde et 10.8 (95% CI: -13.9; -7.8) nmol/l fald i serum 25(OH)D efter 3 måneders ART. Vi fandt ingen forskel i eGFR mellem Hiv-positive og negative. Korrelationen mellem eGFR og 24-h kreatinin clearance var 0.45-0.53 og nøjagtigheden inden for 30% af 24-hr kreatinin clearance var 24–46%. Bias blev reduceret og nøjagtigheden forbedret af CKD-EPI og MDRD estimaterne hvis den etniske coefficient blev udeladt.

Ca. 82.3% af Hiv-positive voksne påbegyndte TDF-baseret ART. Den mediane (IQR) ændring i eGFR efter 12 måneders ART var 0.8 (-11.1; 10.0) ml/min/1.73m^2. Der var ingen forskel i ændring af eGFR fra baseline til 12 måneder mellem patienter som påbegyndte TDF-baserede sammenlignet med ikke-TDF-baserede regimer. Ingen af patienterne havde persisterende proteinuri eller glykosuri.

Konklusion: Ernæringstilskud med vitamin D forebyggede en reduktion i serum 25(OH)D blandt Hiv-positive voksne som påbegyndte ART. Etiopiske Hiv-positive voksne i dette studie havde en god nyrefunktion ved påbegyndelsen af ART og nyrefunktionen forblev stabil gennem 12 måneders ART. Desuden var der ingen forskel i nyrefunktionen mellem Hivpositive patienter behandlet med TDF- og ikke-TDF-baserede regimer. Det skal dog påpeges at alle eGFR ligninger overestimerede 24-hr kreatinin clearance.

List of Papers

1. Yilma D, Kæstel P, Olsen MF, Abdissa A, Tesfaye M, Girma T, Krarup H, Mølgaard C, Michaelsen KF, Ritz C, Kirk O, Andersen ÅB, Friis H. **Change in serum 25(OH)D with antiretroviral initiation and nutritional intervention in HIV-positive adults** Br J Nutr. 2016 Nov 8:1-8.

2. Yilma D, Abdissa A, Kæstel P, Tesfaye M, Olsen MF, Girma T, Ritz C, Friis H, Andersen ÅB, Kirk O, **Serum creatinine and estimated glomerular filtration rates in HIV positive and negative adults in Ethiopia** PLoS One. 2019 Feb 12;14(2)

3. Yilma D, Abdissa A, Kæstel P, Tesfaye M, Olsen MF, Girma T, Ritz C, Friis H, Andersen ÅB, Kirk O **Renal function in Ethiopian HIV-positive adults on antiretroviral treatment with and without tenofovir** (Submitted for publication)

1. Introduction

Human immunodeficiency virus (HIV) infection has been pandemic for the last 30 years and continues to be a major global public health issue (1). In 2017, 1.8 million people were newly infected with HIV and an estimated 36.9 million people were living with HIV worldwide (2). Sub-Saharan Africa is at the epicenter of the epidemic and continues to carry the health and socioeconomic impact of the HIV epidemic, with 25.7 million people living with HIV in Africa in 2017 (2).

Treatment for cure or an effective vaccine has not been yet found for HIV. However, there is a significant advance in HIV treatment since the first antiretroviral drug, zidovudine (AZT), was approved for treatment of acquired immunodeficiency syndrome (AIDS) in 1987 (3). The development of a number of antiretroviral (ARV) drugs and the transition from a one drug treatment to combination of three drugs treatment concept for HIV infection has brought impressive benefit in reducing HIV related morbidity and mortality (4). Moreover, the better understanding of benefits of early initiation of combination antiretroviral treatment (ART) has led to an improvement in ART coverage (5). For the first time ever in 2017, more than 50% of the global population living with HIV were receiving ART (2).

Expanded access to ART has led to a decline in AIDS-related deaths globally (6). In 2017, there was a 38% reduction in death annually relative to year 2000 (2). However, an increase in non-AIDS related morbidity and mortality due to non-AIDS malignancies, cardiovascular, pulmonary, chronic liver and kidney disease was reported in HIV patients when compared to the general population (7–12). A number of reasons have been reported for an increase in non-AIDS morbidities in HIV patients like aging, drug toxicities, coagulation activation, persistence inflammation and immune dysfunction (13–15).

Vitamin D is important for regulation of the immune system and reduction of inflammation in the body (16,17). Studies have indicated that HIV Positive persons with low Vitamin D levels have been associated with disease progression and mortality (18,19). Moreover, some ARV drugs affect vitamin D metabolism and may lead to further reduction (20,21). The current direction pointed to restoration of vitamin D to normal values during treatment with ART in HIV positive

persons with vitamin D supplementation (22). This underlines the need to study further vitamin D levels and the effect of vitamin D supplementation on serum vitamin D level during ART in HIV positive persons living in different geographical location and climates.

ARV drug toxicity in HIV positive persons is one of the contributing factors for an increase in non-AIDS morbidity (23). Most ARV drugs can result in kidney injury with long term use and when provided with combination of other drugs (24,25). In contrast, ART is the mainstay treatment for HIV associated nephropathy (HIVAN) (26). HIVAN is common in black race and it is the third leading cause of nephropathy in the population of African descent (27). Hence, this could indicate the need for appropriate screening and monitoring of kidney function pre and post ART in HIV positive persons. However, the limited diagnostic facilities for determination of renal function and the lack of validated equation for estimated glomerular filtration rate (eGFR) in most low and middle income countries may hamper early diagnosis of renal disease in HIV patients (28). Besides, there are limited facilities for managing end stage renal disease (ESRD) in low and middle income countries which may further reduce survival time (29–31). Therefore, studies that show the extent of renal disease in ART naïve and ART experienced African HIV positive persons and identify factors that worsen renal disease or result in kidney injury have paramount importance. Such data will not only help to develop strategies to early identify and prevent kidney injury in HIV positive persons but also for efficient use of renal function tests for screening and monitoring in resource limited settings.

This PhD thesis is based on data from a cohort of HIV positive adults enrolled in a nutritional intervention trial in ("ARTfood study") in Ethiopia.