Role of Plasmatic and Urinary Concentration of Tenofovir Disoproxil Fumarate in a Cohort of Patients Affected by Chronic Hepatitis B

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Abstract

The aim of this study was the evaluation of plasmatic and urinary therapeutic drug monitoring (TDM) of tenofovir disoproxil fumarate (TDF) in a cohort of patients affected by chronic hepatitis B (CHB). In 68 enrolled patients eGFR was 68 ml/min in naive, while in adefovir dipivoxil (ADV) pretreated patients was 55.5 ml/min (p<0.001). The HBV E genotype was related to lower TDF level (β= -0.698, p<0.001). Urinary TDF concentration was related to ADV pretreatment (β=0.829, p<0.001). TDF urinary concentrations maybe useful in clinical management of CHB patients with older age and previous treatment with ADV.

Introduction

Hepatitis B infection affects millions of people worldwide causing annually a large number of deaths for cirrhosis and hepatocellular carcinoma [1].

Two different strategies are currently available as first line therapy: a finite course treatment with pegylated alpha interferon (PEG-IFN) or long term nucleos(t)ide analogues (NAs); several factors such as age, comorbidity, cirrhosis and hepatocellular carcinoma familiarity, extra hepatic manifestations, virological and serological response predictors, HDV coinfection should be considered before treatment initiation [2].

However, since IFN is not always indicated and response rate is low, NAs are widely used in the majority of patients with previous failure to PEG-IFN treatment. Undetectable HBV-DNA after 24–48 weeks and HBsAg reduction or loss during treatment are response predictors, valuable both in HBeAg positive and negative patients [3].

Among NAs we distinguish drugs with low genetic barrier of resistance (lamivudine, telbivudine and adefovir) and high genetic barrier (entecavir, tenofovir disoproxil fumarate, tenofovir alafenamide) [2].

Despite its high genetic barrier and high effectiveness, tenofovir disoproxil fumarate (TDF) has been largely reported to cause kidney failure, hypophosphatemia, osteoporosis, bone fractures, especially in older patients and while taken for lifelong period [4] [5]. Maggi et al. [6] quantifies TDF toxicity by using estimated glomerular filtration rate (eGFR), proteinuria (> 200 mg/24 h), hypophosphatemia (phosphate < 2.5 mg/dL), increased parathyroid hormone (> 65 pg/mL), plasmatic levels of vitamin D, DEXA scanning. Authors recommend the routine monitoring of eGFR and phosphatemia in TDF treated patients, with the need of improvement in the knowledge on kidney biomarkers to identify an early impairment.

Recently, tenofovir alafenamide (TAF) has been proposed as a safer alternative to TDF in HBV infection treatment in older patients (age > 60 years) and in patients with bone or kidney disease [7]. In fact, TAF is a prodrug of TDF with higher plasmatic stability and a longer plasmatic half-life thus allowing a dose-reduction resulting in lower kidney excretion and bone accumulation than TDF [8] A novel approach using the therapeutic drug monitoring (TDM) of TDF was applied in the clinical management of patients affected by HIV; the role of plasmatic and urinary levels of TDF was related to the risk of renal damage

and treatment failure [9]; no data were currently available about this possible approach in patients affected by CHB and treated with TDF.

The aim of this study was the evaluation of the role of plasmatic and urinary levels of TDF in a cohort of chronic HBV according to clinical outcomes (virological, serological, biochemical) and toxicity (renal failure, treatment interruption).

Materials And Methods

We performed a retrospective cross-sectional study at Infectious Disease Unit, “Amedeo di Savoia Hospital”, Torino, Italy. We included all HBV HBeAg positive and negative, naïve or experienced patients treated with TDF from March 2019 and June 2019. Patients have been tested for HDV antibodies (HDV IgG) and HDV-RNA. All HIV-coinfected patients were excluded from the study.

The study was approved by our Local Ethic Committee (number 002360, January, 15th, 2015); it was conducted in agreement with Helsinky Declaration and all patients delivered their own written informed consent.

Serum HBV-DNA levels were quantified with Real Time method PCR COBAS AmpliPrep/COBAS TaqMan HBV Test 2.0 (Roche Molecular Systems, NJ, USA). HBV genotyping was performed using the INNOLIPA method (Innogenetics, Belgium). HBsAg, HBeAg, anti-HBe have been evaluated using the Elecsys Instrumental Platform method (Roche Diagnostics, Italy), instead; the quantification of ‘S’ antigen was carried out thanks to the ARCHITECT method (Abbott Diagnostics, Ireland) with a range of 0.05–250.0 IU/ml; values of qHBsAg > 250.0 IU/ml were subsequently diluted and retested. The pharmacokinetic analysis was made on samples collected 24 hours before drug administration ($C_{through}$) at the last visit, according to the TDF plasmatic dosage method published by De Nicolò et al. [10]. The ratio [urinary TDF concentration / TDF plasmatic concentration] was also calculated. Therapeutic drug monitoring on TDF plasma and urinary $C_{through}$ was performed.

We collected the following baseline data: age, sex, weight and height, BMI, geographical origin, HBV genotype (A, B, C, D, E), level of education, current job, probable route of transmission according to medical history (unknown, sexual, IDU, vertical, familiar, iatrogenic). Concomitant drugs and comorbidities were also collected.

We divided our population in two groups: naive and experienced patients. For experienced patients we collected data on previous treatments including PEG-IFN, lamivudine, adefovir, entecavir, tenofovir, telbivudine and on resistance associated mutations as appropriate.

We collected data on alanine aminotransferase (ALT) and aspartate aminotransferase (AST), platelets, qHBsAg, HBV-DNA, HBeAg and HDV coinfection at baseline (treatment initiation) and at their last four visits of follow up. Tolerability was evaluated at the same time points by plasmatic creatinine and eGFR calculated by MDRD formula (Modification of Diet in Renal Disease).

Hepatic staging data were collected according to liver stiffness at transient elastography (Fibroscan) or by liver biopsy whereas available. At transient elastography 9 KPa was the cut-off to identify patients with cirrhosis. As concerns liver biopsy, the Ishak score has been reported. Indirect signs of cirrhosis at ultrasound scan and the most recent alpha-fetoprotein (AFP) value have also been recorded.

Continuous variables for descriptive analyses have been summarized as medians (interquartile range (IQR): from the 25th to the 75th percentile). Categorical variables are described as frequencies and percentages. All variables were compared using the Shapiro-Wilk test. Categorical variables were compared using Mann-Whitney and Kruskal-Wallis tests. Continuous variables have been evaluated with Spearman's correlation. Associations have been assesses using the $\chi 2$ test. Univariate and multivariate analyses for plasmatic and urinary TDF levels were performed with linear regression model. Multivariate analysis has been adjusted for the following variables: age, gender, BMI, baseline qHBsAg, HBV-DNA baseline, HBV genotype, eGFR, liver stiffness, presence of HBV resistance, being naïve versus experienced.

Results

We enrolled 68 patients treated with TDF. Plasmatic and urinary TDF data were available for 29 patients out of 68, whereas 28 patients were lost at follow up. In the Table 1 the baseline characteristics of the study population were reported: 32 (47.1%) patients were Italians, 14 (20.6%) Africans and 12 (17.6%) Chinese. The geographical origin of the rest of the population is divided between 9 (13.3%) Europeans and 1 (1.5%) South American.

Table 1
Baseline characteristics of study population

Study population (N = 68)	**N(%) or median [IQR]**
Duration of therapy (years)	8.7 [7–11]
Age (years)	49.50 [34.25–62.25]
Sex	
M	51 (75)
F	17 (25)
Geographical origin	
Italy	32 (47.1)
Europe, other than Italy	9 (13.3)
Africa	14 (20.6)
Cina	12 (17.6)
South America	1 (1.5)
HBV genotypes	
A	8 (11.8)
B	3 (4.4)
C	9 (13.2)
D	37 (54.4)
E	11 (16.2)
Employment status	
Unemployed	23 (33.8)
Workers	45 (66.2)
Educational level	
None	8 (36.4)
Junior high school	10 (45.5)
High school	2 (9.1)
University	2 (9.1)
BMI	24 [22.65–26.38]
Route of trasmission	

Study population (N = 68)	N(%) or median [IQR]
Sexual	4 (5.9)
Intravenous drug use	6 (8.8)
Perinatal	1 (1.5)
Familiar	17 (25.5)
Health-care associated	7 (10.3)
Unknown	33 (48.5)
Naive	32 (47.1)
Experienced	36 (52.9)
HBeAg	
Positive	24 (35.3)
Negative	44 (67.7)
HDV coinfection	
IgG positive	4 (5.9)
IgG negative	64 (94.1)
HBV baseline drug resistance	
Present	18 (26.9)
Absent	49 (73.1)
qHBsAg (Log IU/ml)	3.27 [2.61–3.99]
HBV-DNA (Log IU/ml)	2.72 [1.3–5.44]
Basal liver stiffness (KPa)	7 [6-10.43]
Presence of liver cirrhosis	14 (20.6)
ALT (IU/ml)	43.5 [22-81.75]
AST (IU/ml)	33 [22.50-81.75]
eGFR (ml/min)	80.15 [69.25–88.87]
Plasmatic TDM (ng/ml)	45 [34-57.5]
Urinary TDM (ng/ml)	17490 [12307.5-24858]
Urinary TDF/Plasmatic TDF ratio	393.66 [254.44-622.99]

The median age was 50 years [IQR 34–62], 51 (75%) of them were males. The distribution of genotypes was: 8 (11.8%) A, 3 (4.4 %) B, 9 (13.2%) C, 37 (54.4%) D, 11 (16.2%) E. Overall, 45 (66.2%) of the subjects had a current occupation, while 23 (33.8%) were unemployed. 18 (81.8%) of patients had no educational qualifications. Median BMI was 24 Kg/m^2 [IQR 22.65–26.38]. The analysis of the route of transmission shows that the infection had an unknown origin in 33 (48.5%); sexual in 4 (5.9%); IDU in 6 (8.8%); vertical in 1 (1.5%); familiar in 17 (25%); iatrogenic in 7 (10.3%). At baseline, 32 (47.1%) subjects were naive for antiviral treatment and 36 (52.9%) experienced a previous antiviral treatment. Among experienced patients 10 (27.7%) received PEG-IFN as first treatment, 1 (2.7%) as second; 15 (41.7%) lamivudine as first, 2 (5.6%) as second; 2 (5.6%) patients took ADV first, 7 (19.4%) second, 2 (5.6%) third; 7 (19.4%) patients took ETV first, 5 (13.9%) second and 1 (2.7%) third; 2 (5.6%) patients took telbivudine as first treatment; 4 (11.1%) took TDF as second treatment and 1 (2.7%) as third. Reason for switch of regimen was due to the onset of resistance, which occurred overall in 18 (27%) patients. Median duration of therapy was 8.7 years [IQR 7–11]. All patients have been screened for HDV coinfection at baseline and a positive serology for HDV IgG was present in 4 (5.9%) patients with negative HDV-RNA. At baseline HBeAg was positive in 24 (35.3%) of the 68 patients, negative in 44 (64.7%).

In this population, median baseline qHBsAg was 1850 IU/ml [IQR 404.75–9850], 3.27 Log IU/ml [IQR 2.61–3.99]. Median HBV-DNA baseline was 375 IU/ml [IQR 20–260800.5], 2.72 Log IU/ml [IQR 1.3–5.44]. Median stiffness was 7 KPa [IQR 6–10.43]: 35 (51.5%) patients showed less than 7.3 kPa stiffness, 14 (20.6%) patients 7.3–9.4 kPa, 5 (7.4%) patients 9.5–11.7 kPa, 14 (20.6%) patients had more than 11.7 kPa stiffness.

Median ALT and AST values were 43.5 IU/ml [IQR 22–81.75] and 33 IU/ml [IQR 22.5–56.75], respectively. The median eGFR was 80.15 ml/min [IQR 69.25–88.86]. Median plasma concentration of TDF ($C_{through}$) was 45 ng/ml [IQR 34–57.5], whereas median urinary concentration of TDF ($C_{through}$) was 17490 ng/ml [IQR 12307.5–24858]; the median ratio urinary TDF concentration/plasma TDF concentration was 393.66 [IQR 254.44–622.99].

Median baseline eGFR in naïve patients was 68 ml/min [IQR 59.5–76], while in ADV pretreated was significantly lower: 55.5 ml/min [IQR 51.75- 60] ($p < 0.001$).

Median eGFR decline observed was 4 ml/min [IQR 1–6] in naïve patients, while 15.5 ml/min [IQR 13.25-21] in subjects with previous treatment with ADV ($p < 0.001$).

Median value of TDF urinary concentration in ADV pretreated patients was 26590 [IQR 18360- 33871.75] and resulted statistically higher compared to naïve subjects: 6650 ng/ml [IQR 5552.25–8700] ($p < 0.001$) (Fig. 1).

Linear regression shows a direct correlation between the urinary concentration of TDF (ng/mL) and the decrease of eGFR compared to baseline (Z-coefficient − 6,434) ($y = 1666.6x + 3.894$ ($r^2 = 0.510$; $p < 0.001$) (Fig. 2).

Median liver stiffness (kPa) value was higher in ADV pretreated patients: 18.5 kPa [IQR 12–30] in comparison to naïve patients:7.05 kPa [IQR 6-8.43] ($p < 0.001$).

Median treatment duration was 12 years [8–14] for naïve patient and 8 years [6–12] for experienced, but the difference was not statistically significant ($p = 0.017$).

Considering patients' genotypes, TDF median plasmatic concentration (ng/mL) for E genotype patients was 26 [IQR 22- 31.5] and was statistically lower compared to patients with a different genotype 54.5 [IQR 45–66] ($p < 0.001$).

In the multivariate analysis the liver stiffness resulted predictive of higher plasmatic TDF concentration (β = 0.894, DS = 0.319, $p = 0.009$), while HBV E genotype was related to lower TDF level (β= -0.698, DS = 0.050, $p < 0.001$). Pretreatment with ADV was not associated with plasmatic TDF concentration (β= -0.124, DS = 0.390, $p = 0.122$).

In the multivariate analysis higher urinary TDF concentration was related to pretreatment with ADV (β = 0.829, DS = 0.202, $p < 0.001$).

Discussion

In this real-life study we have for the first time investigated the role of TDF plasmatic and urinary concentrations in patients with CHB; TDF is widely used in the treatment of HIV and HBV infections, with global good effectiveness in the viral suppression and low rate of drug-resistance. Although TDF is generally well tolerated, several studies reported potential kidney and bone toxicity during a long-course therapy [5]: mild and moderate renal failure were frequently associated with TDF treatment, while severe kidney injury is rare and more often related to other comorbidities or previous kidney diseases. Major risk factors related to the renal failure occurrence were: older age, HIV infection, elevated baseline creatinine levels and longer exposure to TDF [11]. In more detail, TDF leads to tubular toxicity with an involvement of mitochondrial alterations due to the oxidative stress; tubular proximal cells are responsible for the excretion of intracellular TDF, but the mitochondrial failure due to higher exposure to TDF leads to cell damage and nephrotoxicity [12].

The observation of TDF related toxicity is more frequent in patients affected by HIV, with prolonged TDF exposure, other potential drug-drug interactions with higher risk of reduced tubular secretion, occurrence of proteinuria, bone mineral density decline and proximal tubulopathy [13]. Conversely, in the treatment of CHB TDF is less frequent related to moderate/severe nephrotoxicity, and only moderate eGFR reduction was observed [6]. This aspect maybe explicated by the different immune-response during the CHB compared to HIV, and the lower chance of drug interaction with higher risk of toxicity. Despite the propose of different biomarkers, the TDM of TDF is not yet used in current clinical practice in CHB, although the TDF plasma concentration resulted predictive of TDF discontinuation due to renal toxicity in a cohort of HIV treated patients [9]. Based on these data, we conducted in our study an evaluation of plasmatic/urinary levels of TDF in patients affected by CHB; interestingly, the urinary concentrations of

TDF are related to eGFR reduction from baseline, with greater impact in patients with older age and previous treatment with ADV. For this reason, ADV pretreated patients have a higher risk of developing chronic kidney failure, unlike naïve patients under TDF treatment. The role of ADV on nephrotoxicity was previous deepened in some studies with the evidence of interstitium fibrosis due to the drug accumulation outside the cells, when the tubular uptake was inhibited [14, 15]. For this reason the interstitial damage due to ADV long term treatment can become chronic and irreversible, with a progressive reduction of eGFR in a large part of patients and the frequent evolution to interstitial nephritis and Fanconi syndrome [16].

These data suggest that ADV pretreated patients with older age may have more benefit switching to TAF. This aspect could be useful in clinical practice because, although the use of ADV was abandoned, a large part of patients who take TDF were previously treated with ADV and were at high risk of renal function quick worsening.

Finally, the role of HBV E genotype on plasmatic TDF concentration could be an interesting and novel data, but the explication and the clinical significance of this finding should be further assessed in other studies.

In conclusion, our study provides novel evidence about the predictive role of urinary TDF concentrations on the renal injury in patients affected by CHB and focuses the attention mainly on the subjects with older age and previously treated with ADV as at major risk of nephrotoxicity and quick need of switch to TAF.

Declarations

Conflicts of interest:

all Authors disclose no conflicts

Funding:

none

Ethics approval statement:

this study was approved by the local Ethic Committee as “HBV-Analogues Study” (Prot. N°002360; 26/1/2015) and all included subjects provided written informed consent.

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Figures

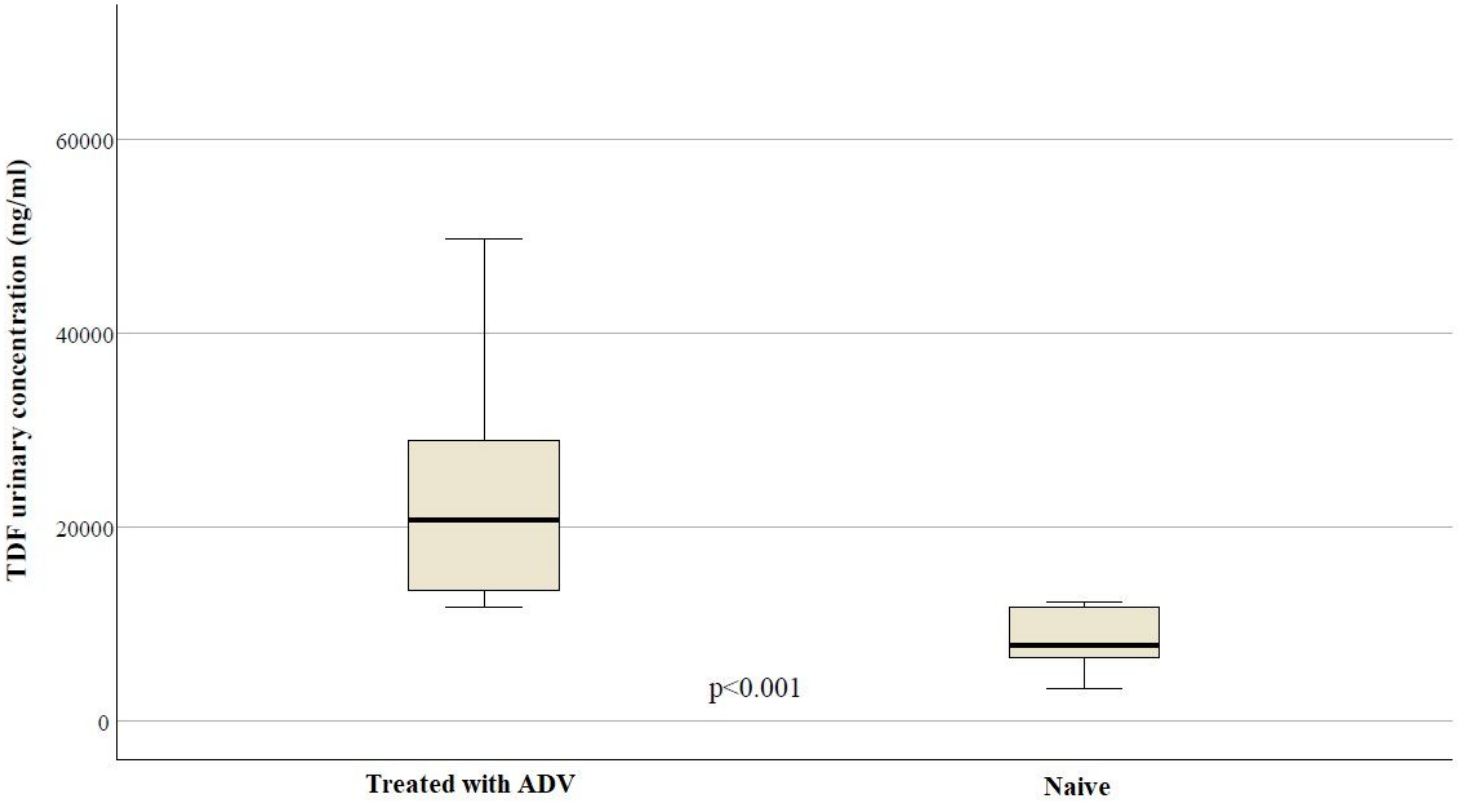

Figure 1

TDF urinary concentration in the study population according to previous ADV treatment.

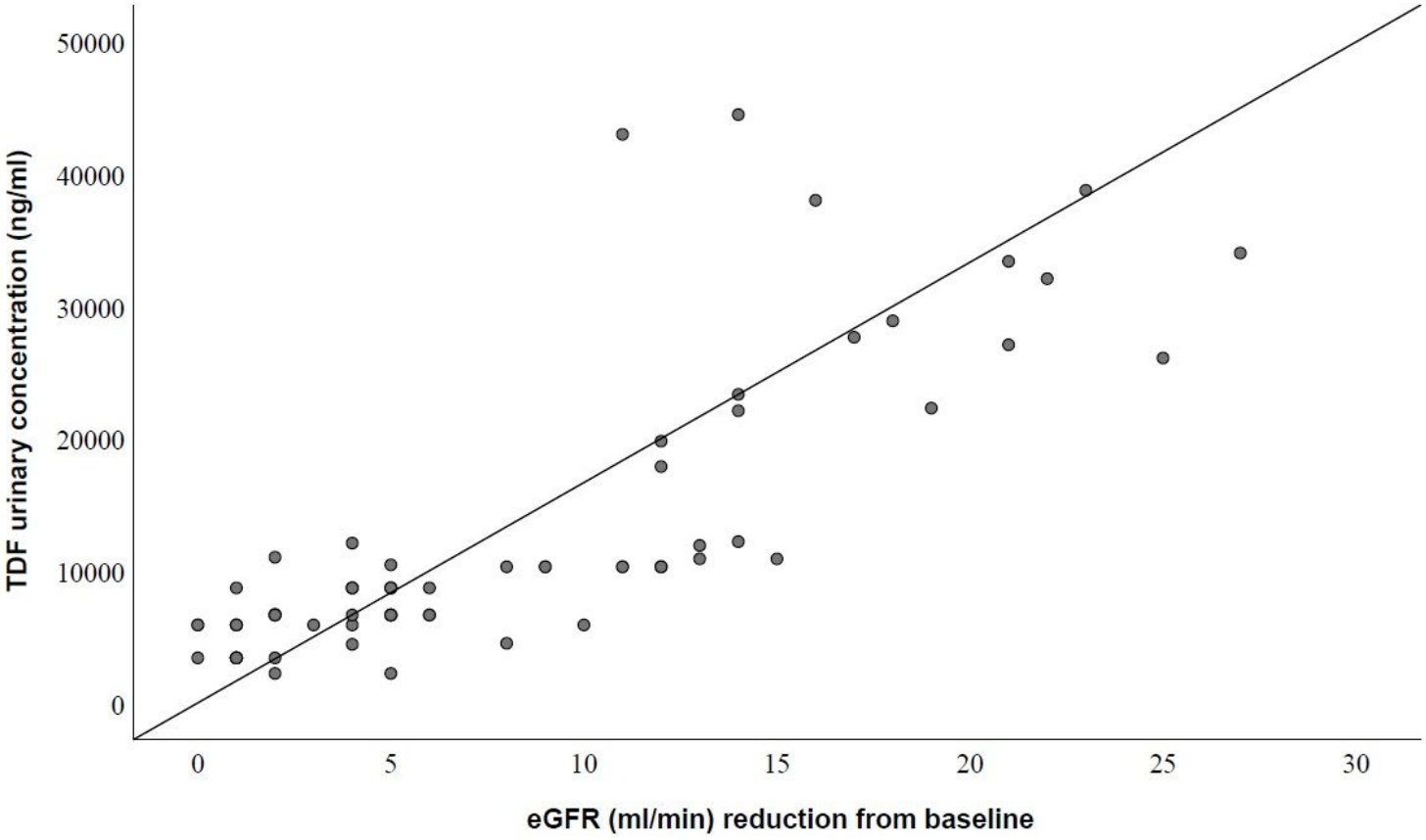

Figure 2

Relationship between TDF urinary concentrations and eGFR reduction from baseline in the study population.