Impact of Antiretroviral Treatment Containing Tenofovir Difumarate on the Telomere Length of Aviremic HIV-Infected Patients

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Objective: To evaluate the in vivo relevance of the inhibitory effect of tenofovir on telomerase activity observed in vitro.

Design: Cross-sectional study of HIV-infected patients with suppressed virological replication (HIV RNA <50 copies/mL for more than 1 year).

Methods: Telomere length in whole blood was measured by quantitative real-time polymerase chain reaction. We performed a multivariate analysis to elucidate variables associated with telomere length and also evaluated the association between telomere length and use of tenofovir difumarate (TDF) adjusted by significant confounders.

Results: 200 patients included, 72% men, median age 49 (IQR 45–54.5), 103 with exposure to a TDF containing antiretroviral treatment (ART) regimen (69.9% for more than 5 years) and 97 never exposed to a TDF containing ART regimen. In the multivariate analysis, significant predictors of shorter telomere length were older age ($P = 0.008$), parental age at birth ($P = 0.038$), white race ($P = 0.048$), and longer time of known HIV infection (10–20 and ≥20 years compared with <10 years, $P = 0.003$ and $P = 0.056$, respectively). There was no association between TDF exposure and telomere length after adjusting for possible confounding factors (age, parental age at birth, race, and time of HIV infection). Total time receiving ART and duration of treatment with nucleoside reverse transcriptase inhibitors were associated with shorter telomere length, but these associations were explained by time of known HIV infection.

Conclusions: Our data do not suggest that telomerase activity inhibition caused by TDF in vitro leads to telomere shortening in peripheral blood of HIV-infected patients.

Key Words: HIV infection, antiretroviral therapy, telomerase, telomere

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INTRODUCTION

HIV-infected patients have an increased risk for several "non-AIDS" complications such as cardiovascular disease, cerebrovascular events, malignancy, liver disease, kidney disease, bone disease, and neurocognitive decline that are classically associated with the normal aging process.[1]

There is a continuous debate about if the higher risk of these complications in HIV-infected patients is the expression of an "accelerated" aging process—complications occurring prematurely—, or an "accentuated" aging process—higher prevalence of complications at every age strata. The Danish HIV-cohort study has not found evidence to suggest accelerated aging in the HIV-infected population.[2] In contrast, 2 recent studies using epigenetic biomarkers of aging have found that HIV-infected patients have an age advancement of approximately 5 years compared with HIV uninfected controls.[3,4]

Proposed mechanisms for the abnormal aging of HIV-infected patients are the proinflammatory state and immune activation associated to even well-controlled HIV infection,[5] traditional risk factors (such as smoking) that are more prevalent among HIV-infected people, or other still unknown causes.

Another potential cause of accelerated or accentuated aging in HIV-infected patients could be telomere attrition.

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There is a close association between shortened telomere length in peripheral blood mononuclear cells and diseases of aging, including cardiovascular diseases, dementia, and cancer.[6]

Interestingly, multiple studies have reported shorter telomeres in-HIV-infected patients compared with HIV negative controls.[7–12] In HIV-infected patients, telomere attrition could be caused by inhibition of human telomerase by antiretroviral drugs, more specifically nucleos(t)ide reverse transcriptase inhibitors [N(t)RTIs].[13–15]

Two recent studies have reported that tenofovir (TFV) at therapeutic concentrations is a potent inhibitor of telomerase activity[16,17] causing telomere shortening in vitro. Of the currently recommended N(t)RTIs, TFV is a more potent inhibitor of telomerase than abacavir, lamivudine, or emtricitabine. In contrast, certain protease inhibitors (PIs) such as saquinavir can upregulate telomerase activity in vitro.[18] Although the inhibition of telomerase caused by N(t)RTIs has been repeatedly demonstrated in vitro, there are very limited data about the in vivo impact of different antiretroviral treatment (ART) regimens on telomere shortening. Indeed, to the best of our knowledge, there is no study that has explored the impact of TFV containing regimens on telomere length of HIV-infected patients receiving ART. This is a relevant issue because TFV administered as tenofovir difumarate (TDF) or tenofovir alafenamide is recommended as a preferred treatment option for initial treatment of HIV infection in all expert guidelines.

To try to determine the impact of TFV on telomere length of HIV-infected patients receiving ART, we have compared telomere length in a cohort of virologically suppressed, HIV-infected patients who were receiving antiretroviral regimens including and not including TDF. Our research hypothesis was that exposure to TDF would be associated with shorter telomere lengths.

PATIENTS, MATERIAL AND METHODS

Study Design and Population

A total of 103 HIV-infected patients exposed to treatment with TDF ("TDF exposed") and 97 HIV-infected patients who had never received TDF ("Non-TDF exposed"), aged >18 years old were included in the study. All patients were recruited from Hospital Universitario La Paz (Madrid, Spain) between March 2014 and March 2015. Main inclusion criteria included the following: HIV antibody positive, stable ART (defined as ART without changes in regimen for at least 12 months) and plasma HIV RNA of less than 50 RNA copies per milliliter for at least 1 year before recruitment. We offered participation in the study to all the patients who met inclusion criteria in our database.

Exclusion criteria were detectable viral load in the last 3 months before the inclusion (a unique viral load above 50 but below <200 RNA copies per milliliter was allowed during the 3 months before recruitment), current or previous treatment with chemotherapy or biologic treatments, acute infection with systemic repercussion during the 3 weeks before the inclusion, former or active alcoholism, pregnancy and type 2 HIV infection. Relevant demographics, parental age at birth of the participant, clinical,and behavioral data were also collected.

Variables

Patient's information regarding age, sex, race, chronic hepatitis C status, and HIV-related variables (Nadir CD4 count, transmission route, and AIDS stage) was collected retrospectively from clinical records. Researchers interviewed participants to self-report about parental age at birth, financial income, educational level, lifelong use tobacco, alcohol, and nonprescription drugs. Income <12,000 €/yr represented the median income cut-off in the Spanish region where our hospital is located.[19]

For cigarette smoking, data were recorded on a yes (active or former)/no basis, number of cigarettes per day and years. Given that the effect of tobacco over the telomere length would be cumulative and potentially irreversible,[20] cumulative exposure in "pack-years" was calculated as (cigarettes/d × years smoking)/20. Cumulative exposure to alcohol in "gr-years" was calculated as grams of alcohol/d × years drinking. Alcoholism was defined as daily alcohol consumption >70 gr/d in men and >40 gr/d in women.

Ethics Statement

The study was approved by the Ethics Committees of Hospital Universitario La Paz (Madrid, Spain). Written informed consent was obtained from all patients.

Sample Preparation: DNA Extraction

Genomic DNA was extracted from 1 mL of whole blood using MagPurix Blood DNA Extraction Kit 1200 according to manufacturer's instruction (Zinexts Life Science Corp., New Taipei City, Taiwan). DNA concentration was quantified using Qubit dsDNA BR assay kit (Life Technologies, Carlsbad, CA).

Telomere Length Determination by Quantitative Real-Time PCR

Relative telomere length was determined by monochrome quantitative multiplex polymerase chain reaction (PCR) assay[21] with minor modifications. Briefly, PCR reactions were performed on a CFX96 Touch real-time PCR detection system (Bio-Rad, Hercules, CA) in a final volume of 20 μL containing 20 ng of genomic DNA, 1X PowerUp SYBR Green Master Mix (Applied Biosystem, Foster City, CA), and 900 nM final concentration of each telomere primers (telg, 5′-ACACTAAGGTTTGGGTTTGGGTTTGGGTTTGGGTTAGTGT-3′ and telc, 5′-TGTTAGGTATCCCTATCCCTATCCCTATCCCTATCCCTAACA-3′) and albumin primers (albu, 5′-CGGCGGCGGGCGGCGCGGGCTGGGCGGAAATGCTGCACAGAATCCTTG-3′ and albd, 5′-GCCCGGCCCGCCGCGCCCGTCCCGCCGGAAAAGCATGGTCGCCTGTT-3′). The thermal cycling profile was stage 1: 2 minutes at 50°C and 10 minutes at 95°C; stage 2: 2 cycles of 15 seconds at 94°C and 1.30 minutes at 49°C; and stage 3: 32 cycles of

15 seconds at 94°C, 15 seconds at 62°C, 15 seconds at 74°C with signal acquisition, 15 seconds at 84°C, and 15 seconds at 88°C with signal acquisition. A standard curve was prepared with genomic DNA from an HIV-negative patient by serial dilution and was included in each run in duplicate to assess amplification efficiency and linearity. All samples were run in triplicate and those with a SD of the threshold cycle (Ct) greater than 0.20 were reanalyzed.

Statistical Analysis

Characteristics of the sample were described using percentages for categorical variables, and mean (SD) or median (interquartile range) for continuous variables with normal or nonnormal distribution, respectively. χ^2, Student's t and Kruskal–Wallis were used accordingly for group comparisons.

Generalized linear models with log link function were fitted to evaluate the association of independent factors with telomere length. Variables independently associated with telomere length were evaluated using a backward stepwise procedure until all variables in the model had $P < 0.10$. Models were fitted separately for the full sample and the TDF exposed and nonexposed. Evaluated factors included: current age and sex, paternal and maternal age at birth, race, income, education, alcohol and tobacco consumption, use of injected drugs, HIV transmission route, hepatitis C virus coinfection and previous treatment with interferon, level of C-reactive protein, time with HIV infection, time on ART, time on nucleoside reverse transcriptase inhibitors (NRTI) and time on TDF (only for the group exposed to TDF), treatment with PIs, CD4 cell count nadir, and AIDS stage.

The specific effects of exposure to TDF (Yes/No), the time on NRTI (per 5 years) and for the group exposed to TDF, the total time on TDF (<5; 5–10; and >10 years) on telomere length were evaluated using an estimative approach, adjusting for age and evaluating the confounding introduced by the rest of independent variables. Finally, as an exploratory analysis, the association of telomere length and history of receiving PIs and time on PIs was evaluated with a similar approach.

Wald test was used to derive *P*-values. Analyses were conducted using STATA (V.13.0 MP; Stata Corporation, College Station, TX).

RESULTS

Characteristics of Study Participants

The characteristics of the participants exposed and nonexposed to TDF are listed in Table 1. Patients were on average predominantly men. There were no differences in sex, race, income, or educational level. TDF treated patients had a significantly higher consumption of alcohol, (58.3% vs 33%, $P = 0.001$), whereas we did not find differences in smoking habit or history of intravenous drug use.

HIV had been acquired mainly by sexual contact in both groups. More than 80% in each group had known their HIV infection for more than 10 years. Duration of known HIV infection was significantly longer in patients exposed to TDF. Patients nonexposed to TDF had been receiving ART for more than 10 years less frequently than patients exposed to TDF, but this difference did not reach statistical significance. All participants had ever received a regimen that contained an N(t)RTI, with longer duration of N(t)RTI exposure in the TDF exposed group, where more than 70% of the patients had received an N(t)RTI containing regimen for more than 10 years. Time of virological suppression was similar in both groups. Patients never exposed to TDF were more frequently receiving triple therapy, whereas patients exposed to TDF had more frequent use of boosted PI monotherapy. Current PI treatment and time on a PI regimen were similar in both groups. There were no differences in current CD4 count or nadir CD4 count.

Univariate Analyses of the Association Between Possible Predictors and Leukocyte Telomere Length

Younger age (<45 vs ≥50 years) and race different than white were significantly associated with longer telomere length among all participants (Fig. 1). No associations with sex, parental age at birth, educational level, or income were seen overall. Cumulative exposure to tobacco was associated with shorter telomere length, whereas history of intravenous drug abuse and cumulative alcohol consumption were not associated.

Longer time of known HIV infection was associated with shorter telomere length. Compared with patients who have known their HIV infection for less than 10 years, patients with known HIV infection for 10–20 and ≥20 years had telomeres that were 18% and 15% shorter ($P < 0.001$ for both).

Longer time on ART and longer time receiving N(t)RTI infection were associated with shorter telomere length. Exposure to TDF was not associated with telomere length, but among the exposed, those with longer exposure had longer telomeres, an effect of borderline statistical significance ($P = 0.060$). Also, those treated with PIs had shorter telomeres but only in the group not treated with TDF. Lower nadir CD4 cell counts, CD4 count, and AIDS stage were not associated with shorter telomere length (Table 1, Supplemental Digital Content, http://links.lww.com/QAI/B6).

Multivariate Analyses of the Association Between Possible Predictors and Leukocyte Telomere Length

Table 2 shows the results from the multivariate analysis overall and separately for the group exposed and nonexposed to TDF. Significant predictors of shorter telomere length, overall and in patients nonexposed to TDF, in a multivariate linear regression model included older age ($P = 0.008$), paternal age at birth ($P = 0.038$), and white race ($P = 0.048$). In addition, longer time of known HIV infection was associated with shorter telomere length (10–20 and ≥20 years compared with <10 years, $P = 0.003$ and $P = 0.056$, respectively).

TABLE 1. Participant Characteristics

	Total, n (%)	Non-TDF Group, n (%)	TDF Group, n (%)	*P*
N	200 (100)	97 (48.5)	103 (51.5)	
Sex				
Men	144 (72)	74 (76.3)	70 (68.0)	0.190
Women	56 (28)	23 (23.7)	33 (32)	
Age, median (IQR), yrs	49 (45–54.5)	49 (45–55)	49 (46–54)	0.993
<45	42 (21.0)	24 (24.7)	18 (17.5)	0.279
45–50	65 (32.5)	27 (27.8)	38 (36.9)	
≥50	93 (62.5)	46 (47.4)	47 (45.6)	
Paternal age at birth, mean (SD)	32.1 (6.7)	32.2 (7.4)	32.0 (6.1)	0.898
Maternal age at birth, mean (SD)	29.5 (6.1)	29.7 (6.4)	29.3 (0.56)	0.710
Race				
White	189 (94.5)	89 (91.8)	100 (97.1)	0.098
Other	11 (5.5)	8 (8.2)	3 (2.9)	
Income				
Lower (≤12,000 €/yr)	95 (47.5)	45 (46.4)	50 (48.5)	0.761
Higher (>12,000 €/yr)	105 (52.5)	52 (53.6)	53 (41.5)	
Education				
Primary	86 (43.0)	41 (42.3)	45 (43.7)	0.323
Secondary	61 (30.5)	26 (26.8)	35 (34.0)	
University	53 (26.5)	30 (30.9)	23 (22.3)	
Alcohol	91 (45.5)	32 (33)	59 (58.3)	0.001
Years, median (IQR)	29 (20–35)	28 (19–35)	30.5 (23–36)	0.172
Alcohol gr/wk, median (IQR)	45 (20–120)	50 (30–140)	40 (10–100)	0.104
Smoking	106 (53)	47 (48.5)	59 (57.3)	0.211
Years smoking, median (IQR)	31 (24–37)	33 (27–38)	30 (21–37)	0.264
Cigarettes per day, median (IQR)	15 (10–20)	15 (8–20)	15 (10–20)	0.818
Ever IDU	61 (30.5)	24 (24.7)	37 (35.9)	0.086
HIV transmission route				
Sexual	124 (62.0)	65 (67.0)	59 (57.3)	0.167
Parenteral	70 (35.0)	28 (28.9)	42 (40.8)	
Unknown	6 (3.0)	4 (4.1)	2 (1.9)	
HCV coinfection	40 (20.0)	14 (14.4)	26 (25.2)	0.097
Previous interferon treatment	40 (20.0)	16 (16.5)	24 (23.3)	0.229
Time with HIV infection, yrs, median (IQR)	18.48 (14.18–22.5)	16.9 (11.98–21.94)	19.39 (15.72–23.59)	0.007
<10	23 (11.5)	15 (15.5)	8 (7.8)	0.070
10–20	99 (49.5)	51 (51.6)	48 (46.6)	
≥20	78 (39.0)	31 (32.0)	47 (45.6)	
Time on ART, yrs, median (IQR)	14.92 (10.28–17.92)	14.34 (10.02–17.12)	15.0 (11.08–18.52)	0.09
<10	40 (20.0)	22 (22.7)	18 (17.5)	0.206
10–20	138 (69.0)	68 (70.1)	70 (68.0)	
≥20	22 (11.0)	7 (7.2)	15 (14.6)	
Time on NRTI, yrs, median (IQR)	11.95 (9.01–16.16)	11.07 (8.21–15.89)	12.68 (9.51–16.38)	0.2
<5	23 (11.5)	14 (14.4)	9 (8.7)	0.495
5–10	42 (21.0)	22 (22.7)	20 (19.4)	
10–15	71 (35.5)	31 (32.0)	40 (38.8)	
≥15	64 (32.0)	30 (30.9)	34 (33.0)	
Time on TDF, yrs, median (IQR)	—	—	8.48 (3.88–10.37)	—
<5	—	—	31 (30.1)	—
5–10	—	—	41 (39.8)	
≥10	—	—	31 (30.1)	
Time suppressed, (yrs) median (IQR)	6.79 (4.56–7.68)	6.89 (3.90–7.72)	6.70 (5.57–7.53)	0.99
Current ART regimen				
Triple therapy	128 (64.0)	69 (71.3)	59 (57.28)	<0.001
Boosted PI monotherapy	65 (32.5)	21 (21.25)	44 (42.7)	
NRTI-sparing regimen	7 (3.5)	7 (7.22)	—	

(*continued on next page*)

TABLE 1. (*Continued*) Participant Characteristics

	Total, n (%)	Non-TDF Group, n (%)	TDF Group, n (%)	*P*
Current NRTI back-bone	128 (64)	69 (71.3)	59 (57.28)	0.041
TDF/FTC	57 (44.53)	—	57 (96.61)	<0.001
ABC/3TC	65 (50.78)	64 (92.7)	1 (1.69)	
Other combinations	6 (4.68)	5 (7.25)	1 (1.69)	
Current boosted PI	100 (50.0)	50 (51.45)	50 (50.0)	0.671
Time on boosted PI	7.87 (4.82–11.0)	8.92 (5.43–11.0)	6.07 (4.20–11.12)	0.18
Ever exposed to PIs as part of ART	161 (80.5)	77 (79.4)	84 (81.6)	0.698
Time on PIs, yrs, median (IQR)	7.9 (4.8–11.0)	8.9 (5.4–11.0)	7.0 (4.2–11.1)	0.1802
<5	41 (25.5)	18 (23.4)	23 (27.4)	0.232
5–10	62 (38.5)	27 (35.1)	35 (41.7)	
≥10–15	31 (19.3)	20 (26.0)	11 (13.1)	
≥15	27 (16.8)	12 (15.6)	15 (17.9)	
CD4 count, cells/μL, median (IQR)	776 (551–1037)	801 (575–1080)	733 (519–1005)	0.21
Nadir CD4 count, cells/μL, median (IQR)	186 (92–276)	193 (93–289)	179 (87–246)	0.22
<100	52 (26)	24 (24.7)	28 (27.2)	0.278
100–200	81 (40.1)	36 (37.1)	45 (43.7)	
≥200	58 (29)	34 (35.1)	24 (23.3)	
Unknown	9 (4.5)	3 (3.1)	6 (5.8)	
Previous AIDS stage	106 (53.0)	51 (52.6)	55 (53.4)	0.907
Comorbidities				
Chronic kidney failure	8 (4.0)	6 (6.2)	2 (1.9)	0.126
High blood pressure	37 (18.5)	20 (20.6)	17 (16.5)	0.454
Diabetes mellitus	28 (14.0)	15 (15.5)	13 (12.6)	0.563
Treatment with statins	61 (30.5)	35 (36.1)	26 (25.2)	0.096
Blood inflammation biomarkers				
Reactive C protein, mg/L, median (IQR)	1.10 (0.37–3.79)	1.25 (0.42–4.46)	0.885 (0.29–2.99)	0.105
D-Dimer, ng/mL, median (IQR)	224 (<170–321)	230 (<170–321)	208 (171–319)	0.87
Fibrinogen, mg/dL, median (IQR)	330.5 (290–386.5)	332 (294–380)	329 (289–389)	0.92
Telomeres length (PCR), median, mean (IQR)	0.760, 0.782 (0.668–0.861)	0.749, 0.797 (0.685–0.861)	0.772, 0.768 (0.664–0.863)	0.962

IDU, injection drug user; IQR, interquartile range.

In the group nonexposed to TDF, shorter telomere length was associated with white race ($P = 0.016$) and longer time with HIV-infection (10–20 and ≥20 years compared with <10 years, $P = 0.048$ and $P = 0.067$, respectively).

In the group exposed to TDF, longer telomere length was associated with high educational level, lower income, and total time on ART, but not with other predictors, with the association with age in the limit of statistical significance.

Impact of Treatment Containing TDF on Telomere Length

Patients exposed to TDF had telomeres that were 4% shorter than those of patients who have never received TDF, but this difference did not reach statistical significance [exp(b) = 0.96; 95% confidence interval (CI): 0.90 to 1.03, $P = 0.238$], nor any other independent variable was identified as a confounder of this effect.

In the group of patients exposed to TDF, in the crude analysis, compared with those with less than 5 years of exposure, those with 5–10 years had 8.7% longer telomere length [exp(b) = 1.087; 95% CI: 0.997 to 1.185, $P = 0.060$] and those with over 10 years had 6.4% longer telomere length [exp(b) = 1.064; 95% CI: 0.970 to 1.167, $P = 0.189$], although the global significance of this association was far from statistical significance ($P = 0.163$), no confounders were identified.

Impact of Time Receiving N(t)RTIs on Telomere Length

Telomere length decreased with time on N(t)RTI, with 4% attrition for every 5 year of treatment with N(t) RTIs [exp(b) = 0.96; 95% CI: 0.93 to 0.99, $P = 0.01$]. However, after adjusting for age, the relationship between shorter telomere length and longer time on NRTI decreased to 3% and did not reach statistical significance (95% CI: 0.95 to 1.00, $P = 0.076$).

Impact of Time Receiving PIs on Telomere Length

At the crude level, in patients with previous exposure to PIs, telomere length was 7.6% shorter than in those not previously exposed [exp(b) = 0.924; CI 95%: 0.857 to 0.996; $P = 0.039$]. However, further adjustment by age and

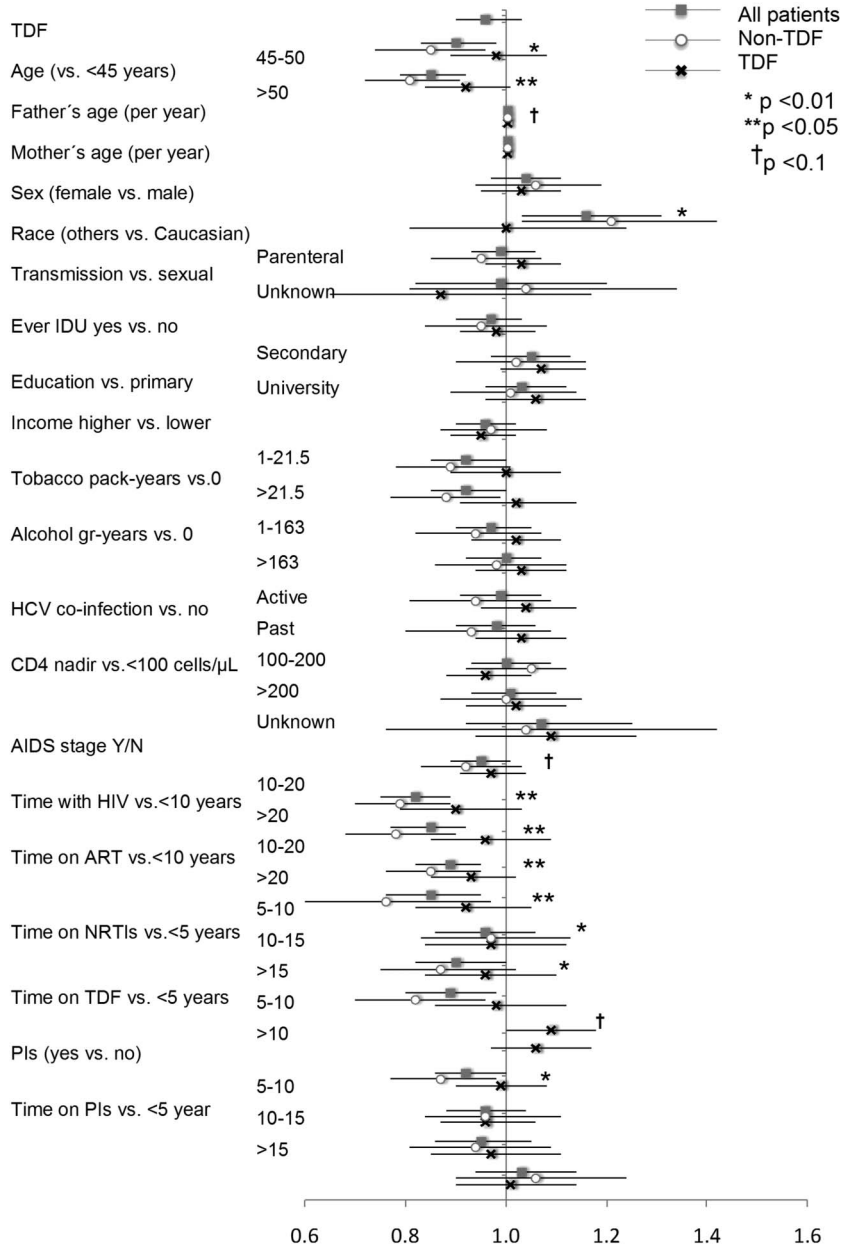

FIGURE 1. Univariate analyses of the association between possible predictors and leukocyte telomere length.

total time on ART made this association disappear [exp(b) = 0.953; CI 95%: 0.884 to 1.027; $P = 0.204$]. In the group of patients exposed to PI, no association was evident between length of exposure and telomere length, and no confounders were identified.

DISCUSSION

In our study, we have shown that ART including TDF does not seem to have an intrinsic negative impact on telomere length in peripheral blood of HIV-infected patients with virological suppression. Compared with patients who have never received TDF, patients who have received TDF for a prolonged period of time—70% for more than 5 years—did not have shorter telomeres in a multivariate analysis looking specifically at the impact of TDF on telomere length.

Ours is the first study that has looked specifically for an in vivo effect of TDF on telomere length in HIV-infected patients. For this reason, we ought to compare a group of patients who have received long-term treatment with TDF with a group of patients who have never been treated with TDF. Both groups were highly comparable in terms of factors that in previous studies have been associated with telomere length: sex distribution, age, income, and smoking status.[6] Besides, it has been shown that HIV by itself can down-regulate telomerase activity.[22–24] Importantly, all patients in both groups have prolonged virological suppression. Consequently, our results are not affected by differences between

TABLE 2. Multivariate Analyses of the Association Between Possible Predictors and Leukocyte Telomere Length

	All		Non-TDF Exposed		TDF Exposed	
Variable	**Exp(coef.) CI (95%)**	***P***	**Exp(coef.) CI (95%)**	***P***	**Exp(coef.) CI (95%)**	***P***
Age (Ref. <45 yrs)						
≥45/50	0.95 (0.87 to 1.03)	0.216	0.91 (0.79 to 1.04)	0.169	0.99 (0.90 to 1.10)	0.908
≥50	0.90 (0.83 to 0.97)	0.008	0.88 (0.77 to 1.01)	0.065	0.92 (0.83 to 1.01)	0.074
Father's age at birth (per yr)	1.005 (1.000 to 1.009)	0.038	1.006 (0.999 to 1.013)	0.080	—	
Race (Ref. Caucasic)						
Other	1.13 (1.00 to 1.28)	0.048	1.22 (1.04 to 1.43)	0.016	—	
Education (Ref. Primary)						
Secondary	—		—		1.12 (1.03 to 1.22)	0.006
University	—		—		1.10 (1.00 to 1.21)	0.044
Income (Ref. Low)						
High					0.92 (0.86 to 0.99)	0.031
Time with HIV infection (Ref. <10 yrs)						
≥10–20	0.87 (0.79 to 0.95)	0.003	0.86 (0.74 to 1.00)	0.048	—	
≥20	0.91 (0.82 to 1.00)	0.056	0.86 (0.74 to 1.01)	0.067	—	
Time on ART (Ref. <10 yrs)						
≥10–20	—		—		0.89 (0.82 to 0.98)	0.017
≥20	—		—		0.91 (0.80 to 1.03)	0.120

groups in virological control. Finally, both groups were comparable with regard to total time receiving ART or time receiving N(t)RTIs, 2 factors that theoretically could affect telomere length.[16,17] It is important to highlight that our participants treated with TDF had a longer duration of N(t) RTI exposure and despite this fact, TDF exposure was not associated with shorter telomeres.

There are very few previous studies that have explored in vivo the impact of different types of ART regimens on telomere length and none that have specifically focused on the impact of TDF. Leeansyah et al[17] in a cross-sectional study with a small sample of just 53 patients found in a univariate analysis that duration of N(t)RTI-containing ART was inversely associated with telomere length and that there was no association with telomerase activity. However, in a multivariate analysis, duration of N(t)RTI-containing ART was no longer significantly related to telomere length. In a substudy of the MONET clinical trial, comparing darunavir/ritonavir monotherapy versus darunavir/ritonavir and 2 N(t)RTIs for maintenance of virological suppression, there was no significant association between telomere length and the duration of previous N(t)RTI treatment.[25] Besides, in MONET there were no significant differences between the 2 arms after 3 years of follow-up in telomerase activity or mean change per year of telomere length. Finally, in a cohort of 229 HIV-infected patients, Zanet et al[7] found no evidence of a relationship between telomere length and antiretroviral therapy exposure, or current type of antiretroviral therapy, although in this study the prevalence of treatment with TDF was not reported.

In our multivariate analysis, longer time of HIV infection was associated with shorter telomere length. Compared with patients with less than a decade of known HIV infection, patients with longer durations of known HIV infection had telomeres that were 9%–13% shorter. Time with HIV infection had substantial colinearity with time receiving ART and time receiving NRTIs. When we included these 3 variables in our model, the effect of total time on ART and time receiving NRTI on telomere shortening was explained by total time with known HIV infection. Our study adds further evidence that HIV by itself or by causing persistent inflammation, immune activation, or immune senescence seem to be the predominant factors causing telomere shortening and not the use of specific antiretrovirals such as TDF.[7–9,11,12,26]

The other variables associated with telomere shortening were as expected, older age[20] and parental age at birth.[27] In our study white race was also associated with shorter telomere length. This finding has to be considered with caution because the impact of race on telomere length is controversial with conflicting findings depending on the study and the sample analyzed.[28]

Our study is limited by its cross-sectional nature that leads to unmeasured bias in the distribution of different ART regimens. However, we think that our results are inconsistent with a large effect of TDF on telomere shortening at least in peripheral blood in HIV-infected patients. Despite the fact that TDF is the strongest inhibitor of telomerase activity in vitro, this effect seems to be compensated by an unknown mechanism in vivo. One possible explanation is that because HIV-Tat protein by itself can downregulate telomerase expression and activity,[24] the negative effect of TDF seen in vitro is compensated by its antiviral activity in vivo. If the inhibition of telomerase caused directly by HIV is substantially higher than the inhibition caused by TDF, then the net effect of TDF on telomere shortening could be positive and similar to other antiretrovirals. We explored also if treatment with PIs could antagonize the negative effect of TDF because an in vitro study has shown that saquinavir can upregulate telomerase activity.[18] However, results of our analysis do not support this hypothesis. Additional mechanisms by which telomerase inhibition may lead to abnormal

aging in the absence of shorter telomeres include DNA damage induced by deprotection of telomeres that may finally result in premature senescence.[29]

Another limitation of our study is that we did not determine telomere length on $CD4^+$ or $CD8^+$ T cells, specific subsets of T cells or non–T-cell population or in high replicating tissues. It remains possible that the effect of TDF on the telomerase of these cell subsets could be different from its overall effect on whole blood. Beside, TDF levels could be different depending on the body compartment, and its impact in the different tissues should be studied. In addition, time of HIV infection was no precisely measured in our study because this variable was calculated from the time of diagnosis and not from the actual time of seroconversion. Finally, because ours was a retrospective study using medical records, and for certain variables such as complete antiretroviral history, not all the data were available.

Our results do not completely rule out that there are in vivo differences among antiretrovirals in its ability to produce telomere shortening in vivo. We recognize that a better control group would be HIV-infected patients never exposed to nucleosides, because other nucleosides such as abacavir have an impact on telomerase activity.[16,17] The issue of a different impact of various types of ART on telomere length would not be definitively answered if this endpoint were not measured in a randomized clinical trial comparing different ART regimens. In particular, it would be very interesting to compare in a prospective randomized study the impact on telomere length in different cells and tissues of N(t) RTI-containing and N(t)RTI-sparing regimens in naive patients who start ART.

In summary, we have found that despite its confirmed ability to inhibit telomerase in vitro, ART including TDF does not seem to lead to telomere shortening in the peripheral blood of HIV-infected patients.

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