Risk factors of short Telomere length and decreased mitochondrial DNA in HIV patients

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CROI 2016: Boston
Poster No. 670

Background

Premature aging is one of the important issues in HIV/AIDS. *Telomere length (TL) shortening* and *alterations of mitochondrial biogenesis* are recognized as markers of cellular aging and correlated with age-related disorders such as insulin resistance.

In HIV infection, *TL shortening* is caused by chronic immune activation, inflammation, and oxidative stress. HIV proteins down modulate the expression of telomerase activity, and nucleoside reverse transcriptase inhibitors (NRTIs) may also contribute to short TL through inhibition of telomerase reverse transcriptase activity. *Mitochondrial toxicity* is induced by loss of mitochondrial membrane potential, by increase of reactive oxygen species, and through different mechanisms of the viral proteins Vpr, Tat and HIV protease. NRTIs also induce mitochondrial toxicity by inhibition of the catalytic subunit of the mtDNA polymerase γ .

In this study, we measured the leukocyte TL and mitochondrial DNA copy number to nuclear DNA (mtDNA/nDNA) in cART treated HIV patients and uninfected healthy controls. We investigated the association between TL and mtDNA/nDNA, and examined which clinical parameters determined TL and mtDNA/nDNA in young and middle-aged HIV patients.

Methods

Subjects:

Three hundred and sixty-two HIV patients on cART for > 6 months, who achieved a viral load <40 copies/ml, and 141 HIV-uninfected volunteers (controls) were enrolled. We divided these subjects into two groups; young subjects (<40years old) and middle-aged subjects (≥40years old). All patients and volunteers provided written informed consent and studies were approved by KMC ethics.

Measurements of leukocyte TL and mtDNA:

Leukocyte TL was measured as telomere repeat copy number relative to single gene copy number (β2 microgloblinx1/2) (T/S ratio), and mtDNA quantity was measured as the mitochondrial gene human cytochrome-*c* oxidase subunit I (CCOI) relative to the nuclear gene for the human polymerase γ accessory subunit (ASPOLG). A standard curve was included in each run, and were used for gene quantifications.

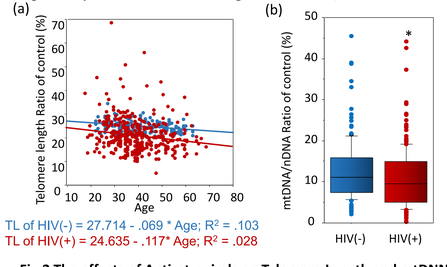
Statistical Analysis *:*

Data were analyzed using the two-tailed Mann-Whitney U-test for comparing continuous variables in two groups and *Bonferroni–Dunn test* for more than three groups. Linear regression analysis was used to determine the factors that associate with TL and mtDNA/nDNA. We assessed several variables associated with HIV infection (CD4, HIV-RNA before cART), cART (duration, regimen), and other factors (age, smoking, BMI, hypertension, HOMA-IR, etc.). For categorical variables, "No" was scored 0, and "Yes" was scored 1. Variables found to be important in univariate analysis were multivariate model candidates.

Table 1 Characteristics of the study participants with HIV positives and controls

HIV positives	***Total***	***Young***	***Middle-age***
n	362	187	175
Sex (M/F)	350/12	182/5	168/7
Age	39.9 (18-69)	32.1 (18-39)	48.3 (40-69)
BMI (kg/m^2)	22.8 (1.8-39.8)	23.1 815.6-39.8)	22.6 (12.8-33.9)
Smoking (past history)(Yes/No)	176/126 (58.2%)	87/57(60.4%)	89/67(57.1%)
Smoking (current)(Yes/No)	126/172(42.3%)	66/75(46.8%)	60/96(38.4%)
Hypertension (Yes/No)	45/275	12/145	33/129
nadirCD4+cell count	191.2 (1-517)	208.8 (1-577)	173.8 (1-493)
currentCD4+cell count	461.4(22-1300)	480.2(52-1300)	448.0(22-1020)
HIV-RNA (log_{10}c/mL)	4.74 (2.60-6.95)	4.71 (3.13-6.95)	4.76 (2.6-6.23)
Duration of cART (months)	44.22 (6-218)	40.2 (6-218)	47.85 (6-199)
Key drug (PI/NNRTI/INSTI)	160/39/163	82/26/79	78/13/84
Backbone			
(Thymidine analog/EPZ/TVD/sparing)	18/104/223/17	8/38/130/11	10/66/93/6
Telomere length	19.9±0.38	21.0±0.55	18.7±0.49
mtDNA/nDNA	10.1±0.34	10.1±0.49	10.0±0..49
Controls	***Total***	***Young***	***Middle-age***
n	147	64	83
Sex (M/F)	138/9	60/4	78/5
Age	41.8 (22-63)	30.9 (22-39)	50.1 (40-63)
Telomere length	24.8±0.20	25.4±0.32	24.4±0.24
mtDNA/nDNA	12.6±0.62	12.7±0.61	12.4±0.98

Fig.1 Comparison of Telomere length and mtDNA/nDNA between HIV(+) and HIV(-)



TL of HIV(-) = 27.714 - .069 * Age; R^2 = .103
TL of HIV(+) = 24.635 - .117* Age; R^2 = .028

Fig.1 TL in PBMCs was measured using real-time quantitative PCR. Data shown are normalized to the data of control (MOLT cells) (a) Decline in TL with age, (b) Box plot of mtDNA/nDNA (*p<0.0001, *Mann-Whitney U-test*: compared with HIV(-))

Fig.2 The effects of Antiretrovirals on Telomere Length and mtDNNA/nDNA (n=362)

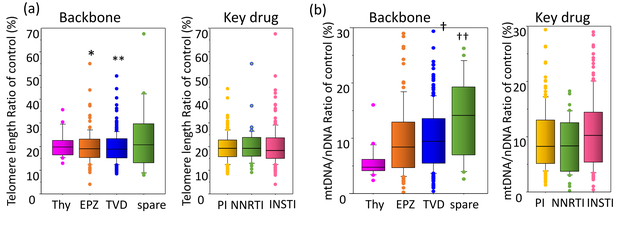


Fig.2 TL (a) and mtDNA/nDNA (b) in PBMCs was measured using real-time quantitative PCR; Data shown are normalized to the data of control (MOLT cells) . *Thy:Thymidine analogue, EPZ:ABC/3TC, TVD: TDF/FTC, Spare: NRTI sparing, INSTI:integrase strand transfer inhibitor (Bonferroni–Dunn test: *EPZ vs. spare: p=0.0085, **TVD vs. spare:p=0.0031, †TVD vs. Thy: p=0.0034, ††spare vs. thy :p=0.0005)*

Table 2. Correlation between Telomere length / mtDNA copy number and various parameters

	Telomere length Single regression analysis				***Telomere length*** Multiple regression analysis				***mtDNA/nDNA*** Single regression analysis				***mtDNA/nDNA*** Multiple regression analysis				
HIV positives	***Young***		***Middle-age***		***Young***		***Middle-age***		***Young***		***Middle-age***		***Young***		***Middle -aged***		
	r	*p value*	*r*	*p value*	*r*	*p value*	*r*	*p value*	*r*	*p value*	*r*	*p value*	*r*	*p value*	*r*	*p value*	
mtDNA/nDNA	-.188	0.0108	-.091	0.2307	-.176	0.0326			-	-	-	-					
Sex (M/F)	-.156	0.0328	-.116	0.1280	-.082	0.3145			.036	0.6285	.044	0.5652					
Age	-.128	0.0797	-.018	0.8127	-.151	0.0672			-.145	0.0501	-.132	0.0805					#1
BMI (kg/m^2)	-.015	0.8407	-.129	0.0887					.036	0.6417	.163	0.0319			.120	0.1484	
Smoking (past history)	.034	0.6840	-.038	0.6407					.024	0.7764	-.034	0.6769					#1
Smoking (current)	.027	0.7514	-.038	0.6369					.055	0.5166	-.158	0.0494			-.171	0.0411	
Hypertension	.046	0.5663	-.008	0.9229					-.100	0.2150	.096	0.2231					
FBS	-.090	0.2350	-.006	0.9339					-.039	0.6158	-.079	0.3120					
HOME-IR	.075	0.7085	-.037	0.7755					-.285	0.1498	.168	0.1879					
TG	.117	0.1181	-.081	0.2935					-.083	0.2745	.014	0.8556					
LDL-c	.063	0.4309	.091	0.2635					.051	0.5257	.086	0.2934					#1
HDL-c	-.059	0.4642	.113	0.1687					.005	0.9539	.183	0.0245			.266	0.0016	
nadir CD4+cell count	.149	0.0501	.004	0.9616	.175	0.0331			-.053	0.4893	.011	0.8857					
current CD4+cell count	.070	0.6968	.038	0.7538					.288	0.1040	.288	0.0148					
HIV-RNA (log_{10}c/mL)	-.099	0.1943	.013	0.8695					.010	0.8925	-.008	0.9151					
Duration of cART (months)	-.050	0.4993	.123	0.1041					-.010	0.8989	-.029	0.7045					
PI use (past)	.061	0.4116	-.018	0.8113					-.126	0.0914	.065	0.3963					
PI use (current)	-.021	0.7773	.154	0.0423			.109	0.1452	0.153	0.0386	-.022	0.7727	.149	0.0435			#2
NRTI use	-.077	0.2991	-.271	0.0003			-.253	0.0008	-.079	0.2888	-.166	0.0284			-.168	0.0245	#2
Thymidine ana use(past)	-.002	0.9791	.019	0.8047					-.036	0.6340	-.165	0.0292			-.166	0.0586	#2
Thymidine ana use (cur)	.034	0.6488	-026	0.7259					-.151	0.0427	-.159	0.0359	-.147	0.0457	-.063	0.4718	
Controls	***Young***		***Middle-age***		***Young***		***Middle-age***		***Young***		***Middle-age***		***Young***		***Middle-age***		
mtDNA/nDNA	.415	0.0008	.399	0.0002			.379	0.0001	-	-	-	-					
Sex (M/F)	-.005	0.9664	.132	0.2366					.056	0.6658	.162	0.1460					
Age	-.167	0.1865	-.0409	0.0001			-.375	0.0002	.284	0.0251	-.099	0.3766					

r: Regression coefficient, BMI: Body Mass Index, FBS : Fasting blood sugar, LDL-c: Low -density lipoprotein-cholesterol, HDL: High density lipoprotein, PI: Protease inhibitor, NRTI: Nucleoside Analogue Reversetranscriptase Inhibitor, Thymidine ana: Thymidine analogue

#1 Model1:adjusted for BMI, smoking and HDL-C
#2 Model2: adjusted for NRTI use and Thymidine analog use

Summary

- TL and mtDNA decreased in middle-aged generation than in young both in HIV patients and controls.(Table1)
- TL was significantly shorter and the rate of decline by age was greater than in controls.(Table1, Fig1)
- mtDNA decreased significantly in HIV patients than in controls. (Table1, Fig1)
- NRTI use was associated with short TL, and thymidine analogue use was associated with decreased mtDNA/nDNA (Fig.2)
- TL was positively associated with mtDNA in young and middle-aged controls, but not in HIV patients (Table2), probably because various factors were associated with TL and mtDNA in HIV patients.
- In simple linear regression analysis, many factors were associated with decreased mtDNA in middle-aged HIV-patients, including traditional aging risk factors and ARVs.

Multiple linear regression analysis showed that in middle-aged patients, NRTI use was independent factor of short TL (p=0.0008) and decreased mtDNA (p=0.0245). Other clinical parameters associated with traditional aging risk factors (smoking, low HDL-C) were independent factor of decreased mtDNA/nDNA in middle-aged patients.

Conclusion

NRTI use was a common risk factor of *short TL* and *decreased mtDNA*. To minimize the premature aging of HIV patients, we must optimize the cART. NRTI sparing regimen is one of the candidates, especially in middle-aged patients.

Conflict of Interest (COI) of the Principal Presenter: No potential COI to disclose