

Original Research

Analysis of Leukocyte Telomere Length in Brazilian People Living with HIV with and Without Cancer

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Abstract

Advances in antiretroviral therapy (ART) have normalized the life expectancy of people living with HIV (PLWH) but have been linked to a premature presentation of age-related comorbidities, including cancer. Telomere length (TL) is a marker of cellular aging and was investigated in blood leukocytes from 78 PLWH on ART, compared with 163 HIV-uninfected controls. The PLWH group was stratified into three subgroups: HIV-only ($n = 57$), HIV with AIDS-defining cancer (ADC, $n = 9$), and HIV with NADC ($n = 12$). Quantitative polymerase chain reaction (qPCR) was used to measure the Relative Telomere Length (RTL), expressed as a T/S ratio. The mean RTL was significantly longer in PLWH ($p = 0.0002$) and in the HIV/Cancer group ($p = 0.0125$) than in the control group ($n = 163$). In addition, the mean RTL was significantly



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longer in the PLWH group with non-AIDS-defining cancers (NADCs) compared to the control group ($p = 0.03$). However, no statistical difference between the HIV-only versus the HIV/cancer groups concerning RTL was observed. PLWH with a longer time since diagnosis of HIV infection (>13 years) had a trend towards longer RTL, showing a borderline statistical significance ($p = 0.06$). Analysis by cancer type showed ADCs were mainly Kaposi's sarcoma (44.5%) and cervical cancer (33.3%), while NADCs were most commonly anal cancer (25%) and breast cancer (16.7%). These findings support the thesis that long-term ART may be associated with telomere elongation in PLWH, challenging the general perception of telomere shortening in this population. The significantly longer RTL in the NADC group suggests that telomere elongation confers greater cellular replicative potential, which might contribute to the elevated cancer risk in PLWH.

Keywords

HIV infection; cancer; telomere; antiretroviral therapy

1. Introduction

Advances in antiretroviral therapy (ART) have significantly increased the life expectancy of people living with HIV/human immunodeficiency virus (PLWH), being equivalent to the uninfected population [1]. However, several age-related comorbidities have been reported in PLWH, such as metabolic syndrome, neurocognitive disorders, cardiovascular disease, bone abnormalities, and non-AIDS-defining cancers (NADCs) at younger ages compared to uninfected adults [2-5].

Telomeres are genomic structures at the ends of chromosomes characterized by the repetitive DNA sequence (TTAGGG) $_n$, which play an essential role in chromosomal stability [6]. Telomere length (TL) measurements have been used as a biological marker to assess replicative exhaustion and senescence associated with numerous human diseases, including HIV pathogenesis [7-10]. Several studies have investigated the relationship between TL in PLWH and comorbidities, opportunistic diseases such as tuberculosis, co-infections (mainly hepatitis), and use of ART [9, 11-14]. In relation to cancer, alterations in telomere dynamics may increase disease risk, either through progressive shortening associated with genomic instability or through telomere elongation that enhances cellular replicative potential and promotes subsequent alterations. However, the proliferative capacity, a key characteristic of cancer, is strongly associated with longer telomeres [15-17].

The impact of long-term ART on TL and cancer in PLWH is a matter of debate. Therefore, this study investigated the relationship between ART administration and relative telomere length (RTL) in PLWH, including those without a cancer history, those with non-AIDS-defining cancer (NADC), and those with AIDS-defining cancer (ADC). Although telomere shortening is well documented in untreated HIV cases, it is still not fully understood how long-term antiretroviral therapy (ART) may alter this process and how these changes may be specifically linked to oncogenesis.

2. Materials and Methods

2.1 Ethics Statement

This study was conducted following the Declaration of Helsinki and approved by the Ethics Committee of the HUPE/UERJ (N° 14189113.2.0000.5259 approved in 2019). Written informed consent was obtained from all participants.

2.2 Study Participants

Seventy-eight PLWH were included in this study. They were monitored regularly every 6 months and used an ART regimen for at least 6 months. All of them were ≥ 18 years. Participants were divided into 2 groups: 57 (73%) in the HIV-only group and 21 (27%) in the HIV/Cancer group. Within the HIV/Cancer group, 12 participants had non-defining cancer (NADC), and 9 had AIDS-defining cancer (ADC).

All participants were recruited between February 2019 and August 2022 at the Infectious and Parasitic Diseases outpatient clinic of the Pedro Ernesto University Hospital (HUPE), in Rio de Janeiro, Brazil. Relevant demographic and clinical information were collected from each patient, as follows: age, gender, duration of ART or combined ART (cART) regimen, CD4⁺ T-cell counts, history of cancer, and previous co-infection. HIV-uninfected adult participants ≥ 18 years old ($n = 163$) from both genders and without sexually transmitted infections or cancer, were included in this study as a control group (mean age: 51.2 years). All participants signed the Informed Consent Form and answered the questionnaire voluntarily. Individuals with mental illness or disorders or with inherited genetic disorders were excluded from the study. They also consented to the collection of a blood sample for molecular assays.

2.3 Relative Telomere Length (RTL)

RTL was measured in blood leukocytes from all participants by quantitative polymerase chain reaction (qPCR). Briefly, peripheral blood samples were collected in EDTA tubes, and DNA was extracted from white blood cells using the Phenol-Chloroform method [18]. All DNA samples were stored at -20°C . The DNA was quantified in the Qubit® fluorometer 3.0 (Invitrogen, Life Technologies) using the Qubit dsDNA HS Assay Kit (Invitrogen, Waltham, MA, USA), according to the manufacturer's protocol. RTL was measured by qPCR and expressed as a T/S ratio, defined as the ratio of telomere repeat copy number (T) to single-copy gene (S), as previously described by Cawthon [19]. The qPCR reactions were performed in a final volume of 15 μL using DNA samples (20 ng), primers (Table 1), and Fast SYBR™ Green PCR Master Mix (Life Technologies, Carlsbad, CA) in a platform StepOnePlus™ Real-Time PCR System (Life Technologies, Carlsbad, CA). The qPCR reactions were carried out in duplicate. The thermocycling conditions and the primers for telomere (TEL) and *human beta-globin* (*Hbg*) used in qPCR reactions were determined as previously described [20]. To evaluate the quality of the qPCR assay, a five-point standard curve was constructed using 10-fold serial dilutions, with all reactions performed in triplicate (Figures S1-S8). High linearity was observed, with R^2 values of 0.98 for the *Hbg* gene and 0.99 for Tel.

Table 1 Primers sequences used in the qPCR reactions.

Regions (T/S)	Primers sequences	Annealing temperature (°C)
Tel 1	CGGTTTGTTTGGGTTTGGGTTTGGGTTTGGGTTTGGGTT	56°C
Tel 2	GGCTTGCCTTACCCTTACCCTTACCCTTACCCTTACCCT	
Hbg 1	GCTTCTGACACAACCTGTGTTCACTAGC	58°C
Hbg 2	CACCAACTTCATCCACGTTCCACC	

T = telomeric DNA repetitive region; S = single-copy gene (*Hbg*). Joglekar *et al.*, 2020 [20].

2.4 Statistical Analysis

The χ^2 , Fisher's exact, T-test, and Mann-Whitney tests were adopted to evaluate the statistical significance of the association between RTL and clinical parameters in PLWH. The Kruskal-Wallis nonparametric test was used to assess the significance of differences in RTL between the evaluated groups (PLWH, PLWH with cancer, and controls). Linear regression was used to evaluate the correlation between age and RTL in evaluated groups (PLWH, PLWH with cancer, and controls). The survey data were processed in GraphPad, version 9.4.1. In all statistical tests, a 5% significance level was considered. Thus, statistically significant associations were considered as those with *p*-values < 0.05.

3. Results

3.1 Participant Characteristics

A total of 78 PLWH on ART were included in this study. The male gender was the majority (56.4%). All PLWH participants reported HIV infection by sexual contact. The mean CD4⁺ T-cell count was 654 cells/ μ L (range: 128-1397 cells/ μ L). Demographic and clinical characteristics are shown in Table 2. The mean age of PLWH was 51.8 years (range: 27-71 years), similar to that of the control group (51.2 years; range: 25-75 years).

Table 2 Demographic and clinical characteristics of PLWH groups in this study.

Variable	<u>HIV/Cancer</u> (<i>n</i> = 21) N% of total		<u>HIV</u> (<i>n</i> = 57) N% of total		<u>Total</u> (<i>n</i> = 78)
Age, years (mean; SD)	54 (±9.6)		51 (±10.9)		51.5 (±10.5)
Gender <i>n</i> (%)					
Female	7	33.3	27	47.4	34
Male	14	66.7	30	52.6	44
Time from HIV diagnosis (mean, years)	14.7		15.7		14.1
Time between HIV and cancer diagnosis (mean, years)	10.1		-		-
Family history of cancer (%)					
Yes	15	71.4	37	65.0	52
No	6	28.6	20	35.0	26
Coinfection (%)					
STIs	12	57.1	23	40.3	35
RTIs	4	19.0	18	31.6	22
nRTIs	6	28.6	9	15.8	15
cART regimen					
NRTI	1	4.8	2	3.5	3
NRTI + NNRTI	6	28.6	20	35.1	26
NRTI + PI	2	9.5	10	17.5	12
NRTI + PI + II	3	14.3	6	10.5	9
NRTI + II	8	38.1	17	29.8	25
NRTI + NNRTI + II	0		1	1.8	1
Not informed	1	4.8	1	1.8	2
CD4 ⁺ cell/mm ³ (mean; range)	527.7 (282-1204)		701.6 (128-1397)		667.4 (128-1397)
CD4 ⁺ /CD8 ⁺ cell/mm ³ (mean; range)	0.7 (0.2-2.4)		1.0 (0.1-2.5)		0.9 (0.1-2.5)

HIV/cancer = HIV infection with previous cancer; HIV = HIV infection without previous cancer; STIs = Sexually Transmitted Infections; RTIs = Respiratory tract infections; nRTIs = Nonrespiratory tract infections; cART = combination antiretroviral therapy; NRTI = nucleoside reverse transcriptase inhibitor; NNRTI = non-nucleoside reverse transcriptase inhibitor; PI = protease inhibitor; II = Integrase inhibitor.

In the analysis of the HIV/cancer group, 9 participants (42.9%) had AIDS-defining cancers (ADC) (4 men and 5 women with a mean age of 48.8 years) (Table 3), while 12 participants (57.1%), 10 men and 2 women with a mean age of 57.8 years had non-AIDS-defining cancers (NADC) (Table 4). There was a statistically significant difference in age between these groups ($p = 0.02$), demonstrating that individuals in the ADC group are approximately 10 years younger than those in the NADC group, consistent with the distinct pathogenic pathways of these two cancer types. No association was found between the time of HIV diagnosis and cancer diagnosis ($p = 0.08$) or CD4⁺ cell count ($p = 0.21$), between the HIV only and HIV/Cancer groups, demonstrating that both groups had robust immune systems, thereby minimizing the risk of opportunistic infections. However, a decline in the

CD4⁺ count and CD4⁺/CD8⁺ ratio was observed in individuals in the HIV/cancer group compared to those in the HIV group (Table 3).

Table 3 Possible cofactors and family history associated with NADC tumors.

Case	Age	Time from HIV diagnosis to an NADC (years)	Gender - M or F	CD4 ⁺ cell/mm ³	NADC Tumor	Coinfection	Family history of cancer Yes or No - which
1	42	0	M	NI	Anal cancer	hepatitis B and syphilis	no
2	57	14	M	907	Anal cancer	syphilis	yes- bladder; bone; pancreatic cancer
3	58	NI	M	317	Anal cancer	no	no
4	49	11	F	842	Breast cancer	hepatitis C	yes - cervical cancer
5	66	3	F	NI	Breast cancer	herpes zoster	yes - bone cancer
6	60	23	M	453	Hodgkin lymphoma	no	no
7	68	11	M	1204	Melanoma	pneumonia	yes - lung cancer
8	58	17	M	386	Melanoma	hepatitis C and tuberculosis	yes - lung; colorectal cancer
9	44	2	M	721	Colorectal cancer	no	yes - colorectal; breast cancer
10	53	10	M	575	Prostate cancer	hepatitis A and syphilis	yes - mouth; cervical cancer
11	68	10	M	778	Liver cancer	hepatitis C	yes - prostate cancer
12	71	33	M	397	Bladder cancer	herpes zoster	yes - liver; melanoma; breast cancer
average	57.8	12.2	10M/2F	658.0	-	-	-

NADC = Non-AIDS-defining cancer. NI = not informed.

Table 4 Possible cofactors and family history associated with ADC tumors.

Case	Age	Time from HIV diagnosis to an ADC (years)	Gender - M or F	CD4 ⁺ cell/mm ³	ADC Tumor/survival (years)	Coinfection	Family history of cancer Yes or no - which
1	40	7	M	602	Kaposi sarcoma	hepatitis B and syphilis	yes - bladder cancer
2	57	5	M	332	Kaposi sarcoma	pneumonia	yes - stomach cancer
3	58	13	F	445	Kaposi sarcoma	no	yes - stomach; breast cancer
4	58	32	M	772	Kaposi sarcoma	syphilis	no
5	41	0	F	535	Cervical cancer	no	yes - breast cancer
6	43	0	F	282	Cervical cancer	no	yes - lung cancer
7	46	0	F	426	Cervical cancer	neurotoxoplasmosis and tuberculosis	no
8	45	3	F	715	non-Hodgkin lymphoma	no	yes - breast cancer
9	51	1	M	392	non-Hodgkin lymphoma	no	no
average	48.8	6.8	4M/5F	500.1	-	-	-

ADC = AIDS-defining cancer.

Among the individuals in the AIDS-defining cancer group (Table 3), 4 participants (44.5%) had Kaposi's sarcoma, 3 participants (33.3%) had cervical cancer, and 2 participants (22.2%) had non-Hodgkin's lymphoma. All cases of cervical cancer had a time from HIV to cancer diagnoses equal to 0 (zero) because both diagnoses were simultaneous, and the tests are conducted in parallel.

Among the non-AIDS-defining cancers, the most common was anal cancer (3 cases; 25%), followed by breast cancer (2 cases; 16.7%) and melanoma (2 cases; 16.7%). There was one case each of Hodgkin lymphoma, colorectal cancer, prostate cancer, liver cancer, and bladder cancer (Table 4). The "case 1" had the time from HIV diagnosis to cancer equal to 0 (zero) because both diagnoses were simultaneous (parallel tests). All the participants in the group of PLWH who had cancer were in remission at the time of collection; however, there is limited information about staging or remission time because they were treated in other institutions.

Other cancer-related co-factors, such as alcohol consumption and tobacco use, were not associated with cancer type, although they may influence disease progression. Case 9 (a colorectal cancer) is likely familial, as the participant reported a strong family history of the same cancer, including his mother, sister, grandmother, and uncles.

3.2 RTL Measurement of Study Participants

As shown in Figure 1, the mean RTL measurement (T/S) was significantly longer in PLWH groups ($n = 78$; T/S HIV: 1.04; T/S HIV/Cancer: 1.04) compared to individuals in the control group ($n = 163$; T/S: 0.96) ($p = 0.0002$ and $p = 0.0125$, respectively). No significant differences in mean RTL were observed among PLWH groups.

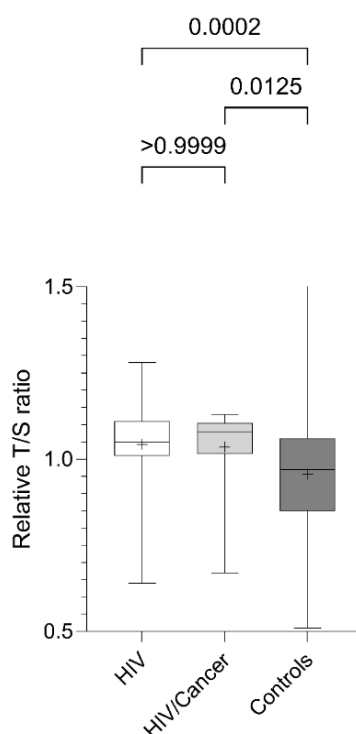


Figure 1 Comparison of RTL measurement between the study groups. Legend: RTL = relative telomere length. T/S ratio = amount of telomere repeat amplification product (T) to a reference single-copy gene signal (S). p -values are shown at the top; $p < 0.05$ is considered significant. Kruskal-Wallis non-parametric test.

In addition, a trend towards longer mean RTL was observed in PLWH with a longer time since diagnosis of HIV infection (above the mean of 13 years of infection) compared to the group with a shorter time since infection (≤ 13 years), showing a borderline statistical significance ($p = 0.06$).

In the PLWH group with cancer, the mean RTL was significantly longer in the PLWH group with NADCs than in the HIV-uninfected control group (1.05 *versus* 0.96; $p = 0.03$). However, no significant difference was found in the mean RTL between the control group and the PLWH group with AIDS-defining cancers (ADCs) (0.96 *versus* 1.01) or between NADCs and ADCs in PLWH groups (1.05 *versus* 1.01) (Figure 2).

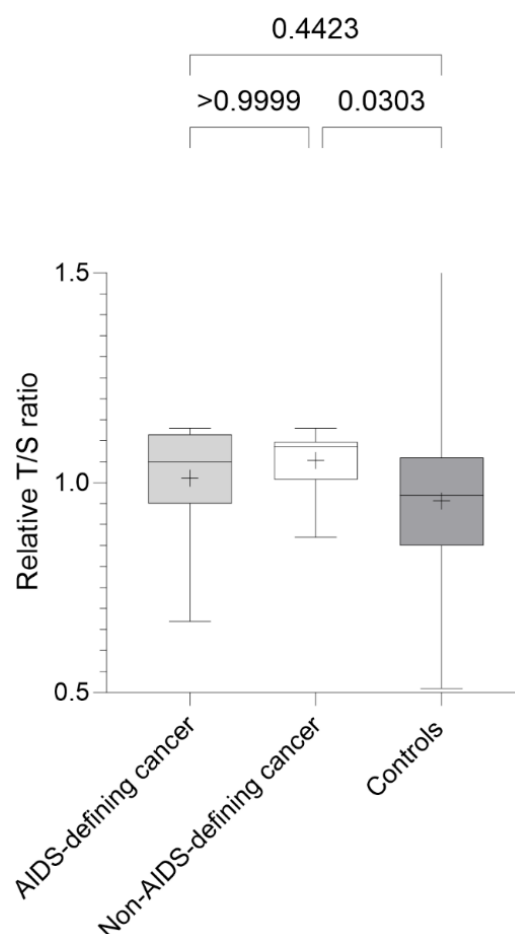


Figure 2 Comparison of RTL measurement between groups according to types of cancers. Legend: RTL = relative telomere length. T/S ratio = amount of telomere repeat amplification product (T) to a reference single-copy gene signal (S). p -values is shown on the top, p -values is are shown on the top, $p < 0.05$ is considered significant. Kruskal-Wallis non-parametric test.

By analyzing RTL measurements by gender, we found that the mean RTL was significantly longer in women from the PLWH group without cancer than in women in the control group (1.03 *versus* 0.91; $p = 0.0002$). No difference in mean RTL was found among males in the studied group ($p > 0.999$).

Other relevant factors were also investigated in the study for association with RTL. However, these factors were not significantly associated with RTL: age, use of different cART regimens, CD4 counts, CD4/CD8 ratio, and presence of co-infection, although 57% of individuals with HIV/cancer

have a sexually transmitted infection (Table 2). It was not possible to analyze the relationship between relative telomere length, viral load, and race because the Brazilian population is a product of mixing different ancestral backgrounds [21].

4. Discussion

4.1 Telomere Length

The long-term consequences of ART administration in PLWH on TL remain controversial. The introduction of ART has increased the life expectancy of PLWH; however, its use remains a paradox regarding cellular aging in this group, even though it improves survival and quality of life. Although it improves the immunological condition of these individuals, some studies point to a higher risk of illness and death when compared to HIV-negative individuals in the same age group, due to the premature aging process in this group and non-AIDS-defining illnesses, such as some neoplasms, also associated with aging [22-24]. It has also been suggested that premature and accelerated aging in PLWH may be related to the adverse effects of ART, as some nucleoside reverse transcriptase inhibitor (NRTI) drugs have been shown to inhibit telomerase activity in vitro, leading to accelerated telomere shortening [25, 26].

Auld *et al.* found that HIV-infected participants had significantly shorter telomeres compared to HIV-negative individuals [27]. However, no significant association was found between TL and new diagnosis or ART use among the HIV-infected participants. Additionally, the authors found no significant correlation between TL and tuberculosis diagnosis, characterized as HIV-associated opportunistic infection. In the present study, we also did not find a significant association between RTL and co-infection ($p = 0.77$). Ganesin *et al.* demonstrated an association between antiretroviral treatment (ART) and telomere length in HIV-positive children. They observed that children initiating ART (TARv) had significantly longer telomeres compared to HIV-positive children who had not received treatment, suggesting a tendency for telomere lengthening following the introduction of ART [28].

Schoepf *et al.* observed that significantly shorter telomeres were found in PLWH during untreated HIV infection. In contrast, individuals on suppressive ART showed no change in TL, though a visual trend towards an increase in TL was observed in men. The authors also described that no TL change was found during long-term suppressive ART (almost 10 years). Although there was no statistical significance, our study observed a trend towards increased telomeres in PLWH under the ART regimen with a longer HIV diagnosis (>13 years of infection). This prolonged use of ART may be linked to telomere lengthening, which could confer a protective effect on the genome of these individuals [29]. Accordingly, Raffenberg *et al.* observed that delaying ART start was significantly associated with TL shortening [30].

Our current findings support the telomere elongation hypothesis, showing significantly longer RTL in PLWH than in HIV-uninfected participants. However, due to the diversity of antiretroviral therapy regimens used by participants, it is not possible to associate a specific ART regimen with TL elongation. However, another study compared RTL between a PLWH group receiving dual therapy (DT) with dolutegravir + lamivudine and another group that maintained the standard triple therapy (TT). After 48 weeks, the within-group analysis showed a significant RTL gain in the DT group but not in the TT group, suggesting that DT therapy has a positive effect on TL [31]. This finding suggested that the specific ART regimen might influence TL.

Montejano *et al.* observed a trend towards longer TL among patients with prolonged exposure to tenofovir disoproxil fumarate (TDF) [32]. In their study, Montejano *et al.* [32] evaluated 200 individuals (72% men and 28% women), with a mean age of 49 years and an average of 18.5 years living with HIV. The mean duration of ART administration was 14.9 years, and the authors reported a trend toward longer TL in individuals with longer TDF exposure. However, this effect reached only borderline statistical significance ($p = 0.06$) [27]. These findings are consistent with those of our study (78 PLWH; mean age of 51.8 years), in which the majority of participants were men (56.4%), and the mean duration of HIV infection was 13 years. In our analysis, we also observed a trend toward increased TL, with borderline statistical significance ($p = 0.06$), when comparing the group with longer HIV infection and ART duration (>13 years) to the group with shorter infection time (<13 years).

In an observational study of 128 people living with HIV, with a mean duration of HIV infection of 23.6 years and receiving diverse ART regimens for at least 12 months, shorter TL was observed compared with matched controls. However, an overall increase of 2.9% in mean RTL among PLWH was reported after six years of follow-up, suggesting that ART may exert a positive effect on TL [33]. The authors also reported that 58.4% of participants exhibited greater RTL at the end of the follow-up period compared with baseline. Although we were unable to measure TL across multiple time points or years in our cohort to directly evaluate the dynamics of ART and RTL, our findings are consistent with those reported by Cadiñanos *et al.* [33], who observed telomere lengthening in PLWH with a mean duration of infection of 13 years.

Bukic *et al.* observed no significant difference in RTL of PLWH among those receiving nucleoside reverse transcriptase inhibitors (NRTIs) as background therapy in combination with an integrase inhibitor, a protease inhibitor, or a non-nucleoside reverse transcriptase inhibitor (NNRTI) ($p = 0.76$). In contrast, statistical analysis confirmed that ART regimens containing NNRTIs only significantly affected RTL ($p = 0.018$) [34]. These findings highlight the potential influence of specific ART classes (particularly NNRTIs) on telomere dynamics and emphasize the need to consider drug class effects when interpreting biomarkers of biological aging in people living with HIV. In our study, various ART combinations were administered over time, making each group too small to apply statistical tests.

Cells with longer telomeres have increased replicative potential and may be in a more cancer-prone state [35]. PLWH have a higher risk of developing cancers, defined as ADCs and NADCs. ADCs are commonly represented by Kaposi sarcoma, non-Hodgkin lymphoma, and cervical cancer. In contrast, NADCs usually include anal cancer, Hodgkin lymphoma, oropharyngeal cancer, hepatocellular carcinoma, and non-small cell lung cancer [36, 37]. An increased incidence of NADCs has been observed in the PLWH population. Moreover, individuals co-infected with the hepatitis C virus (HCV) exhibit a higher cumulative incidence of developing NADCs, even when hepatocellular carcinoma is excluded [38]. In the present study, the most prevalent ADCs were Kaposi sarcoma ($n = 4$), cervical cancer ($n = 3$), and non-Hodgkin lymphoma ($n = 2$). In contrast, NADCs were represented by anal cancer ($n = 3$), breast cancer ($n = 2$), Hodgkin lymphoma ($n = 1$), melanoma ($n = 2$), colorectal cancer ($n = 1$), prostate cancer ($n = 1$), liver cancer ($n = 1$), and bladder cancer ($n = 1$). We found that RTL was significantly higher in the PLWH with NADCs group than in the HIV-uninfected control group (1.05 versus 0.96; $p = 0.03$). However, we did not observe a statistically significant difference in RTL between the ADC groups and the control group (1.01 versus 0.96; $p = 0.44$), nor between the NADC and ADC groups (1.05 versus 1.01; $p = 0.99$).

Although the use of ART has reduced the incidence of some AIDS-defining cancers by improving immune function, the risk of several non-AIDS-defining neoplasms remains elevated, suggesting that chronic immune dysregulation, inflammation, and age-related mechanisms may contribute to cancer susceptibility in people living with HIV. Several mechanisms may be associated with cancer in people living with HIV, such as the activation of DNA damage response mechanisms that can be triggered by the presence of nucleic acids of viral origin, causing genetic instability [39, 40] and by oxidative stress originating reactive oxygen species that are also characteristic of HIV infection and that cause DNA damage [41, 42]. Some studies have also associated HIV infection with epigenetic alterations, with changes in the expression of genes important for homeostasis, mainly tumor suppressor genes such as *p16^{INK4A}* and *FOXP3* [43, 44].

4.2 Study Limitations

This study has some limitations, including the small sample size, large variability in ART regimens, and absence of RTL analysis during untreated HIV infection, since all patients were under an ART regimen. The sample for this study was a convenience sample. Data collection was limited exclusively to patients seen during the recruitment period at the Infectious and Parasitic Diseases Outpatient Clinic at Pedro Ernesto University Hospital (HUPE) in Rio de Janeiro, Brazil. HUPE is a public institution that provides care to low-income patients who often reside in distant regions. These patients typically attend follow-up appointments only every six months. Recruiting participants for the project proved to be very difficult. Patients frequently lacked the time or willingness to remain at the hospital for voluntary research participation after long hours of travelling and waiting for their Doctor's appointment; most preferred to return home immediately after their doctor's visit.

Another significant limitation is the absence of HPV testing in the study population because HPV is associated with the development of several kinds of cancer [45]. An additional constraint was the inability to associate telomere length with participants' races, which would be particularly challenging given the high levels of miscegenation in the Brazilian population [21]. Other studies have highlighted the relationship between telomere length and ethnicity [46, 47]. Our findings on telomere length and gender are consistent with those reported by Diez Roux *et al.*, who also observed longer telomeres in women compared to men in a multi-ethnic study [46].

On the other hand, the strength of this work is the long period of successful treatment of the study population, combined with the observed results. Larger, preferably longitudinal studies are warranted to corroborate our findings. Therefore, recognizing the initial changes in telomere length in PLWH and the long-term effects of ART regimens is crucial for monitoring and preventing further genomic damage that can lead to age-related or inflammatory diseases.

5. Conclusion

Both AIDS-defining and non-AIDS-defining cancers are more common in PLWH. This elevated incidence underscores the critical need for a deeper understanding of the association between TL and chromosome instability, oncogenesis in this population. Our primary finding was the significantly longer RTL in the overall PLWH cohort on ART compared to the HIV-uninfected control group, suggesting a potentially protective mechanism against cellular aging and elevating the susceptibility to cancer in this population at the same time. Although this study did not demonstrate

a statistically significant association between RTL and a specific ART regimen, our data suggest a possible trend toward TL maintenance or modest TL recovery among individuals receiving ART. It is plausible that stable and sustained treatment may influence TL through immune reconstitution. However, this interpretation should be approached with caution. Longitudinal studies with extended follow-up are required to better elucidate the relationship between type-specific ART administration and RTL in PLWH.

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Author Contributions

MHO, DBL, RTS, GA: original draft, formal analysis, writing. MC-J, MAB, PLC: methodology. LD ran the statistical tests. All authors have read and agreed to the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

AI-Assisted Technologies Statement

Artificial intelligence (AI) tools were used solely for basic grammar correction and language refinement in the preparation of this manuscript. Specifically, OpenAI's ChatGPT was employed to improve the readability and linguistic clarity of the English text. All scientific content, data interpretation, and conclusions were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the content of the manuscript.

Additional Materials

The following additional materials are uploaded at the page of this paper.

1. Figure S1: Amplification curve *Hbg* with dilutions.
2. Figure S2: Amplification Plot *Hbg* with dilutions.
3. Figure S3: Amplification curve TEL with dilutions.
4. Figure S4: Amplification Plot *Hbg* with dilutions.
5. Figure S5: Amplification Plot TEL with dilutions.
6. Figure S6: Amplification Plot TEL and *Hbg*.
7. Figure S7: *Hbg* Standard curves were constructed using a five-point serial dilution series (10^3 to 10^{-1}) of the initial concentration using linear regression. Legend: Reactions exhibited linearity ($R^2 = 0.98$).
8. Figure S8: TEL Standard curves were constructed using a five-point serial dilution series (10^3 to 10^{-1}) of the initial concentration using linear regression. Legend: Reactions exhibited linearity ($R^2 = 0.99$).

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