Persistent herpesvirus infections and telomere attrition over 3 years in the Whitehall II cohort

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Summary: Persistent herpesviruses, including CMV, HSV-1, and HHV-6 were associated with greater telomere attrition over time in a sample of healthy adults aged 53-76.

Abstract:

The determinants of telomere attrition, a potential marker of cellular aging, are not well understood. Persistent herpesvirus infections including cytomegalovirus (CMV) infection may be particularly important for telomere dynamics via mechanisms such as inflammation, oxidative stress, and their impact on peripheral blood lymphocyte composition. This study examined the association of four human herpesviruses (CMV, HSV-1, HHV-6, and EBV) with change in leucocyte telomere length (LTL) over three years in 400 healthy individuals (ages 53-76) from the Whitehall II cohort. CMV, HSV-1, and HHV-6 infection were independently associated with greater 3-year LTL attrition, with no association found for EBV. Their magnitudes were large, e.g. the equivalent of almost 12 years of chronological age for those CMV seropositive. Seropositivity to a higher number of herpesviruses was additively associated with greater LTL attrition (3 herpesviruses vs. none β=-0.07, p-value= 0.02, 4 infections vs none β=-0.14, p-value< 0.001). Higher IgG antibody levels among those seropositive to CMV were also associated with shorter LTL at follow-up. These associations were robust to adjustment for age, sex, employment grade, BMI, and smoking status. These results suggest that exposure to infectious agents should be an important consideration in future studies of telomere dynamics.

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Introduction:

The length of telomeres, the DNA-protein structures that cap and stabilize the physical ends of chromosomes, has been proposed as a marker of cellular aging [1, 2]. In most cells these telomeres shorten with each round of cell division, with critically short telomeres leading to cellular senescence and genomic instability [1, 3]. Shortened leukocyte telomere length (LTL) is associated with all-cause mortality and progression of age-related diseases - in particular cardiovascular disease, cancer, diabetes, and dementia [4, 5]. Shorter telomere length may also play a role in clinically important age-related declines in immune function, as signified by the reduced lymphocyte proliferative capacity and associated impaired response to vaccines and acute infections [6, 7].

While overall telomere shortening with age is well-established, there is large variation in TL among individuals of the same chronological age, with age accounting for only 10% or less of inter-individual telomere length variation [8]. While environmental factors such as diet, obesity, and smoking have been associated with shorter LTL, these findings have been inconsistent across studies [9, 10]. Additional exposures underlying inter-individual variation in LTL are not well characterized but may include infection history, particularly chronic viral infections [11-13]. Herpesviruses such as cytomegalovirus (CMV), Epstein-Barr virus (EBV), herpes simplex virus type 1 (HSV-1), and human herpes virus 6 (HHV-6) are commonly acquired in childhood or early adulthood [14]. In most cases, the host is generally unable to eradicate these infections

the virus remains in a quiescent (i.e., latent) state in the host's cells, with intermittent phases of reactivation. The containment of herpesvirus replication takes up considerable immune resources, with CMV in particular seeming to be immunodominant, becoming the primary target of 10-30% of all circulating CD4 T lymphocytes and up to 50% of all circulating CD8 T lymphocytes in CMV infected elderly individuals [15, 16]. These CMV-specific T cells contribute to large shifts in the cellular composition of peripheral blood, including a large increase in the number of so-called 'effector-memory' T lymphocytes that are characterized by shorter telomeres [12, 17]. Such dramatic expansion of T cells that exhibit short telomeres is not seen for EBV and has not been well investigated for other herpesviruses [18-20]. However, there is some evidence that EBV-CMV co-infection may influence the human T cell repertoire beyond the impact of each individual infection [19, 21]. The potential impact of other herpesviruses on peripheral blood telomere length is less well explored, though it is known that HHV-6 achieves latency through integration with human telomeres and may lead to dysfunction of the enzyme telomerase which is responsible for elongating telomeres, with clinical implications for stem cell transplants [22, 23]. Herpesviruses infections may contribute to telomere attrition through pathways other than repeated proliferation and subset differentiation, such as by stimulating inflammation and oxidative stress processes [24, 25].

To our knowledge, no prospective studies have examined the association between CMV or other herpesviruses and changes in LTL. Previous cross-sectional analyses in the current study population found an association between CMV infection and lower telomerase activity among women [26], but no association between CMV infection and

cross-sectional LTL. This cross sectional association with telomerase activity suggested potential longer-term impacts of CMV on telomere dynamics that could only be explored with data on telomere change over time [26]. Longitudinal studies of telomere change are also important due to the large genetic component of LTL [27], which implies that cross-sectional associations with shorter LTL could reflect predispositions to risk factors and disease rather than causal associations [28]. The current study evaluated the association of seropositivity to four herpesviruses (CMV, HSV-1, HHV-6, and EBV) at baseline and change in telomere length over three years of follow up. The analyses considered the contribution of serological evidence of infections with the individual herpesviruses as well as their combination in relation to LTL change. Associations between LTL and IgG antibody levels to each herpesvirus were also examined, given evidence of positive associations between higher proportions of (short-telomere) peripheral blood lymphocytes[29] and previous cross-sectional associations between higher CMV IgG and lower telomerase activity in this sample [26].

Materials and Measures

Sample:

Participants were from the Heart Scan sub-sample of the Whitehall II epidemiological cohort, recruited during 2006 to 2008, to investigate the psychosocial, demographic and biological risk factors for coronary heart disease [30]. Participants were aged 53-76 at baseline and were screened to ensure that they had no history or objective signs of coronary heart disease, and no previous diagnosis or treatment for hypertension, diabetes, inflammatory diseases or allergies. Volunteers were of white European origin and 56.5%

were in full-time employment. Socioeconomic status was defined by current (or most recent) grade of employment within the British civil service, and selection into the study was stratified to include representation of higher, intermediate and lower grade employment groups. Participants were invited for reassessment after 3 years (mean 1087 days interval). Measurement of LTL did not commence at the beginning of the study; therefore, only 434 participants have LTL data at baseline, with a total of 400 respondents having complete data on LTL and at both time points and infection data at baseline. Ethical approval was obtained from the University College London Hospital Committee on the Ethics of Human Research, and all participants gave signed informed consent.

Isolation of Peripheral Blood Mononuclear Cells

For the assessment of LTL at baseline and at follow-up, we used an adaptation of the method first described by Cawthon[31]. Genomic DNA was extracted from PBMCs in a QIAcube workstation (baseline) or manually (follow-up) with the QIAamp DNA blood mini kit (Qiagen, Crawley, United Kingdom) according to instructions of the manufacturer and stored in 10 mmol/L Tris-hydrochloride, 0.5 mmol/L ethylenediamine tetraacetate, pH 9.0 at -20°C (baseline) or -80^{o}C (follow-up). Relative mean TL was measured by a monochrome multiplex quantitative real-time polymerase chain reaction (PCR) assay with a Bio-Rad CFX96 Real-Time PCR Detection System (Bio-Rad, Hemel Hempstead, United Kingdom) for samples obtained at baseline, and with a Roche Lightcycler 480 real-time PCR machine (Roche Diagnostics Corporation, Indianapolis, IN) on follow-up. Reactions containing serial dilutions of a reference DNA standard were

included in each polymerase chain reaction plate to generate the telomere (T) and β-globin gene (S) standard curves required for quantitation, and relative mean TL, expressed as a T/S ratio, was derived. The coefficient of variation of these assays was 2.3%. Absolute measures of telomere length can vary across laboratories, but rankings of relative length are highly correlated [32]. We therefore computed standardized telomere length scores Z-scores for the baseline and follow-up samples as a robustness check, and statistical findings were identical to those for absolute values, so the latter are presented. CMV, HSV-1, EBV (EBV-EBNA), and HHV-6 IgG antibody titers were measured from thawed serum samples using a solid-phase enzyme immunoassay system as described previously [33]. Briefly, diluted aliquots of serum were reacted with the specific antigen bound to a solid-phase surface. Following the addition of enzyme-linked anti-human IgG and enzyme substrate, quantification of IgG antibody titers was determined by the amount of color generated from the reaction of antigen-bound enzyme and soluble substrate and measured as optical densities read by a spectrophotometer. In each assay run a standard sample was employed and the results were expressed as a ratio between the amount of color generated by the test sample and the amount generated by the standard. A sample was categorized as seropositive if the optical density ratio was >1.2.

Additional covariates

We adjusted for several potential confounders; factors potentially associated with both infections and LTL. Employment grade was used as an indicator of socioeconomic status (SES); participants were classified according to their current or most recent civil service grade at baseline into lower (administrative assistant, administrative officer and executive

officer), intermediate (higher executive officer and senior executive officer) and higher (grades 7 to 1) SES. Smoking status at baseline was assessed by questionnaire and categorized as current, former, or never. Body Mass Index (BMI (kg/m^2) at baseline based on measured height and weight was categorized as normal weight (18.5-24.9), overweight (25.0-29.9), and obese (>=30).

Statistical Analysis

Associations between serostatus for each individual infection at baseline as well as the total number of infections (0-4) and telomere length measured prospectively were analyzed using separate multivariable regression models. Telomere length at follow-up was the dependent variable, with age, gender, grade of employment, smoking status, BMI and TL at baseline included as controls. Additional models using change in LTL between periods (Time 2 minus Time 1) were also run with very similar results (available upon request).

Results

[Table 1 about here]

The mean T/S ratio averaged 0.992 ± 0.07 at baseline, and 0.897 ± 0.15 at follow-up. This indicates a significant decrease in T/S ratio among the study population over the 3-year follow up period ($p < 0.001$). Telomere length at follow-up was inversely associated with age ($p < 0.001$), and was greater in women than men ($p < 0.001$). 47.3% of the sample was CMV+ at baseline, 21% HSV1+, 59% HHV-6+, and 63.3% EBV+ .

[Table 2 about here]

Serological evidence of CMV infection was associated with shorter LTL at follow-up (β = -0.061, SE = 0.014, $p < 0.001$), which was also true for HSV-1 (β = -0.049, SE = 0.016, $p = 0.002$), and HHV-6 (β = -0.033, SE = 0.015, $p = 0.024$) adjusting for baseline age, sex, smoking, employment grade, BMI, and LTL (Table 2, Model 1). As associations for individual infections may be confounded by exposure to other herpesviruses, Model 2 mutually adjusted for all infections. The coefficient estimates were virtually unchanged, but the association with HHV-6 lost statistical significance at conventional levels ($p = 0.065$). The coefficient for EBV infection was close to zero in both models. The other independent predictors of shorter telomeres at follow-up were older age, male sex, and shorter LTL at baseline. Compared to these factors, the magnitude of the coefficient for CMV seropositivity was substantial, roughly equivalent to the coefficient for females vs. males (β = -0.062, SE = 0.014, $p <0.001$), or the equivalent of 11.8 years of additional age (βCMV/ βAGE). Smoking status, BMI, and employment grade were not significant predictors of LTL at follow-up in any model.

[Table 3 about here]

Serological evidence of exposure to multiple herpesviruses was also significantly associated with shorter telomere length at follow-up (Table 3). Being seropositive for three herpesviruses vs. none was associated with a decrease in telomere length (β = -0.069, SE = 0.029, p=0.019), with a doubling of this effect size for four infections compared to none (β = -0.139, SE = 0.037, p=<0.001). Figure 1 illustrates the pattern of mean change in telomere length across the two time periods by the total number of infections for which an individual was seropositive.

To explore whether one particular herpesvirus, such as CMV, was primarily driving these results, we explored all combinations of infection pairs and their association with telomere length, illustrated in Table 3 and Figure 2. The Benjamini-Hochberg procedure was used to adjust for multiple comparisons/false discovery rate, with adjusted p-values shown in Table 3 [34]. Overall, there was evidence of an important role for CMV infection and some interaction effects with other herpesviruses. Co-infection with both HSV-1 and CMV showed the strongest association with shorter telomere length, suggesting an interactive effect of being seropositive for both infections compared to only one. Co-infection with HHV-6 and CMV also showed a stronger association than either infection by itself. In both of these cases, exposure to HHV-6 and HSV-1 among those who were CMV seronegative was not independently associated with shorter telomeres, but their presence amplified the association with telomere length among those who are CMV seropositive. This interaction was not seen for CMV and EBV. Table 3 also shows the remainder of co-infection pair-wise combinations, with some evidence for the importance of co-infection HHV-6 for the association of HSV-1 and LTL.

IgG Antibody Response and LTL

Finally, as higher CMV IgG antibody has previously been associated with reduced telomerase activity, we also examined LTL with respect to continuous IgG immune response among the CMV seropositive (N=189). Higher CMV IgG antibody levels (mean 3.01, S.D. 0.77) were significantly associated with greater LTL attrition (β = -0.029, SE = 0.013, p =0.028). Higher EBV IgG antibody levels among those seropositive was also associated with shorter LTL (β = -0.023, SE = 0.009, p =0.019). No

significant associations were found for IgG levels of HSV-1 (β = 0.085, SE = 0.075, *p* =0.261) or HHV-6 IgG (β = -0.019, SE = 0.013, *p* =0.160).

Sensitivity Analysis:

Because of previous findings of associations between CMV and telomerase activity in women only, we tested for interactions between sex and each infection as well as burden of infection. No significant sex-infection interactions were found. Since inflammation is a hypothesized mechanism through which infections may impact telomere shortening, we also tested models adjusting for available markers of inflammation (serum C-reactive protein, IL-6, and fibrinogen, measured at baseline). None of these inflammatory markers were associated with LTL shortening, and thus did not mediate the associations between infections and LTL.

Discussion

Although leukocyte telomere length is correlated with many important aging-related outcomes, its determinants are not well understood. Persistent viral infections may be an important determinant of individual variation in LTL, but have not been extensively studied *in vivo* [11, 12]. This study was the first to test the association of multiple highly prevalent persistent herpesvirus infections with prospective leukocyte TL in humans. We found decreases in LTL associated with infections of a substantively important magnitude-the equivalent of almost half of a standard deviation in LTL for those CMV+ vs CMV-, and a full standard deviation in LTL for those infected with all four

herpesviruses compared to none. These findings suggest an important role for these infections for understanding LTL dynamics.

There are several reasons why CMV, more so than other pathogens, may contribute to telomere shortening. CMV infection is thought to be associated with a continuous low-grade reactivation, causing a highly characteristic differentiation among T cells that yields a robust expansion of T cells that downregulate telomerase and exhibit short telomeres [35]. A study of 159 healthy volunteers aged 20-95 found that CMV seropositivity amplified the association of increased age with shorter telomeres in T-cell populations, particularly in the lymphocytes of those aged 60 and over. The causality of this association was confirmed in the same paper, showing primary CMV infection among 19 renal transplant recipients coincided with a steep drop in lymphocyte TL. This drop in lymphocyte TL was related to the change in the T cell subset distribution towards a differentiated low TL phenotype of T cells in both CD4 T cells and CD8 cells [11]. Thus, the TL decreases associated with CMV infection may in part be driven by a change in the cellular composition of the peripheral blood, the main cellular source of TL determination, and not a direct cellular effect of telomere attrition.

The association between CMV and LTL shortening may also involve endocrine pathways. Recent results from this sample found that greater cortisol responsivity to acute stressors predicted more rapid telomere attrition[36]. CMV can infect and replicate in human adrenocortical cells, thereby triggering steroidogenesis [37], which in turn has been shown to inhibit telomerase activity in CD4 and CD8 T cells [38]. This group has previously reported an altered diurnal cortisol slope in healthy CMV-positive individuals where those that are infected exhibited a flatter decline over the day compared to those

not infected[39]. Such a flattened secretion pattern of cortisol has repeatedly been associated with impaired immunity and enhanced inflammatory activity[40, 41], which, in turn, may further promote elevated viral reactivation. Indeed, studies have suggested that cells exposed to glucocorticoids increased replication of HSV and CMV in vitro [42].

The mechanisms underlying the observed associations of HSV-1 and HHV-6 and telomere shortening are not known and should be replicated and explored in future work. Likewise, the synergistic effects seen for CMV co-infection with HSV-1 and HHV-6 merit additional study. The lack of an association of EBV with shorter LTL compared to the other herpesvirus infections is consistent with fundamental differences in how these viruses modulate the host immune system, with dramatic expansion of T cells with short telomeres not seen in EBV relative to CMV [18-20].

A major strength of this study is that our findings come from a well-characterized longitudinal population cohort, in contrast to the previous small clinical sample that explored CMV and lymphocyte telomere length [11]. The prospective assessment of LTL over 3 years and data on the serostatus to multiple herpesviruses at baseline is also novel. Our findings identify for the first time independent associations between HSV-1 and HHV-6 and telomere length. Previous cross-sectional work showed that CMV seropositivity was associated with lower levels of telomerase in women [26], but this did not appear to generalize to shorter telomere length in that sample. The present study indicated that despite the lack of association of CMV with baseline LTL, both CMV seropositivity and CMV IgG antibody response were associated with prospective change in LTL. Future work should test whether CMV plays a role, at least in part, in the association between LTL and the onset of chronic disease, especially vascular disease.

Results from a small clinical study found that telomere length in CD8+ cytotoxic T cells was shorter in CMV+ compared to CMV- coronary heart disease patients, and this TL shortening was correlated with a decrease in left ventricular function in CHD patients, suggesting a role for CMV in the co-evolution of CHD and immunosenescence [43].

Our findings of an association between higher CMV IgG and shorter telomeres warrants further investigation, particularly given epidemiological evidence that higher CMV IgG is associated with mortality[44], weaker vaccine response[29], and lower levels of psychological well-being[45]. There are also strong and consistent associations observed between stress and increased herpesvirus IgG [46]. Given the evidence that chronic stress contributes to shorter telomeres [47], our results could have implications for understanding the pathways through which psychosocial factors impact cellular aging. The role of stress-related reactivation of herpesviruses as a mediator of stress and telomere dynamics should be further explored.

There are several limitations to our study. Telomere length was measured in PBMCs, and values may differ in lymphocyte subpopulations. Future flow cytometric analysis of cell subpopulations would allow better mechanistic understanding of how infections decrease LTL. Measures were also made with two different PCR machines at baseline and follow-up; while this may affect comparisons of absolute values, it did not impact the relative results shown here, as indicated by identical findings with standardized measures of telomere length. Furthermore, our results are virtually identical utilizing data on LTL only at follow-up, providing further assurance that changes in LTL measurement methods are not driving our results. Since IgG antibodies reflect any past exposure, it is not known when these herpesviruses were acquired, though it is likely that

many were acquired early in life given the concentration of risk factors for exposure to these infections in early life [48]. Furthermore, while shorter LTL has been shown to increase the risk of experimentally induced upper respiratory infections in younger adults [7], the infections examined here were not associated with LTL at baseline, reducing the risk that reverse causality from shorter LTL to increased risk of infection was driving the observed associations.

In conclusion, our results suggest large and significant associations between highly prevalent persistent herpesvirus infections and telomere shortening in humans. The magnitude of the association for CMV seropositivity was equal to almost half of a standard deviation in LTL, or the equivalent of 12 years of chronological age as estimated in this sample. In contrast, previously identified risk factors for cross-sectional LTL including smoking, obesity, SES, and inflammatory markers were not associated with LTL attrition in this sample, suggesting that infection history may be a more robust predictor of LTL than these prior commonly identified risk factors. The strong associations seen for CMV and LTL may be clinically important and are consistent with evidence that CMV is associated with increased risk of mortality in the overall population [49, 50]. We encourage additional research to replicate these findings and continue to advance our understanding of the infectious determinants of cellular aging. Such knowledge could improve primary prevention and potentially reduce telomere related chronic disease and accelerated aging.

Conflict of Interest Statement

J. Lin is co-founder of Telome Health, a diagnostic company measuring telomere biology. The remaining authors have nothing to declare, and all authors declare no competing financial interests.

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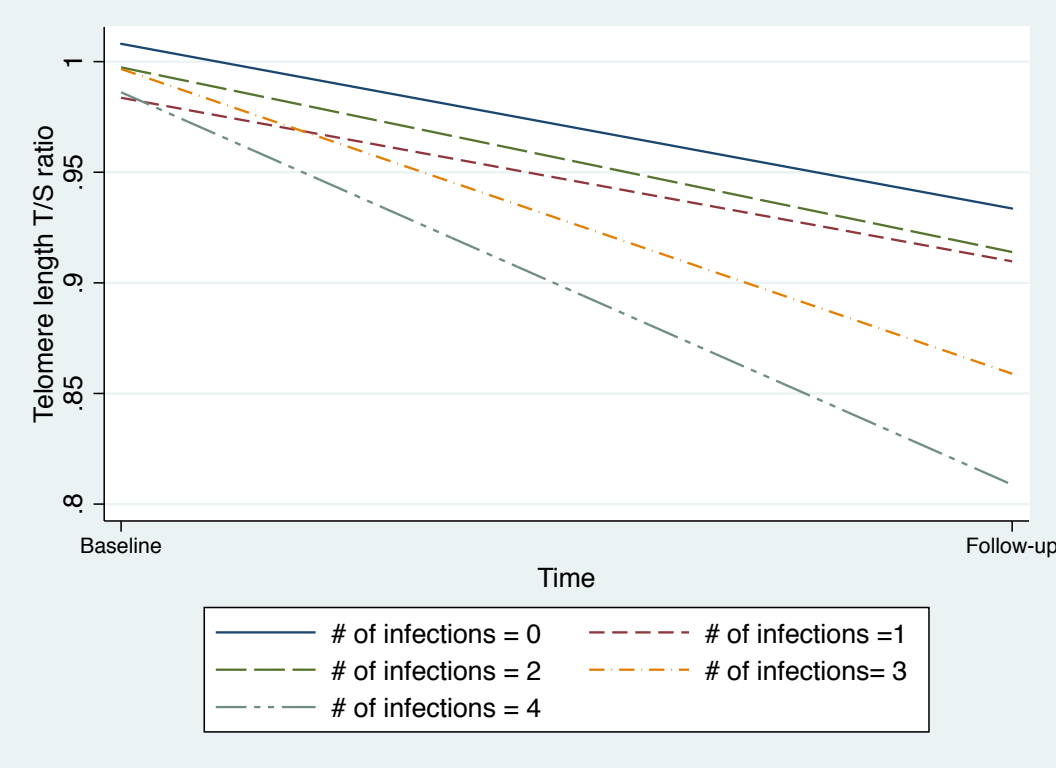
Figure Legends

Figure 1: Number of herpesvirus infections at baseline and mean leukocyte telomere length change (T/S Ratio) over 3 Years

Figure 2: Association of herpesvirus co-infections at baseline and leukocyte telomere length (T/S ratio) at follow-up (β coefficient, linear regression model adjusted for age, sex, and TL at baseline). Error bars indicate 95% confidence intervals.


Telomere length T/S ratio
1
.95
.9
.85
.8
Baseline
Follow-up
Time
# of infections = 0
# of infections =1
# of infections = 2
# of infections= 3
# of infections = 4

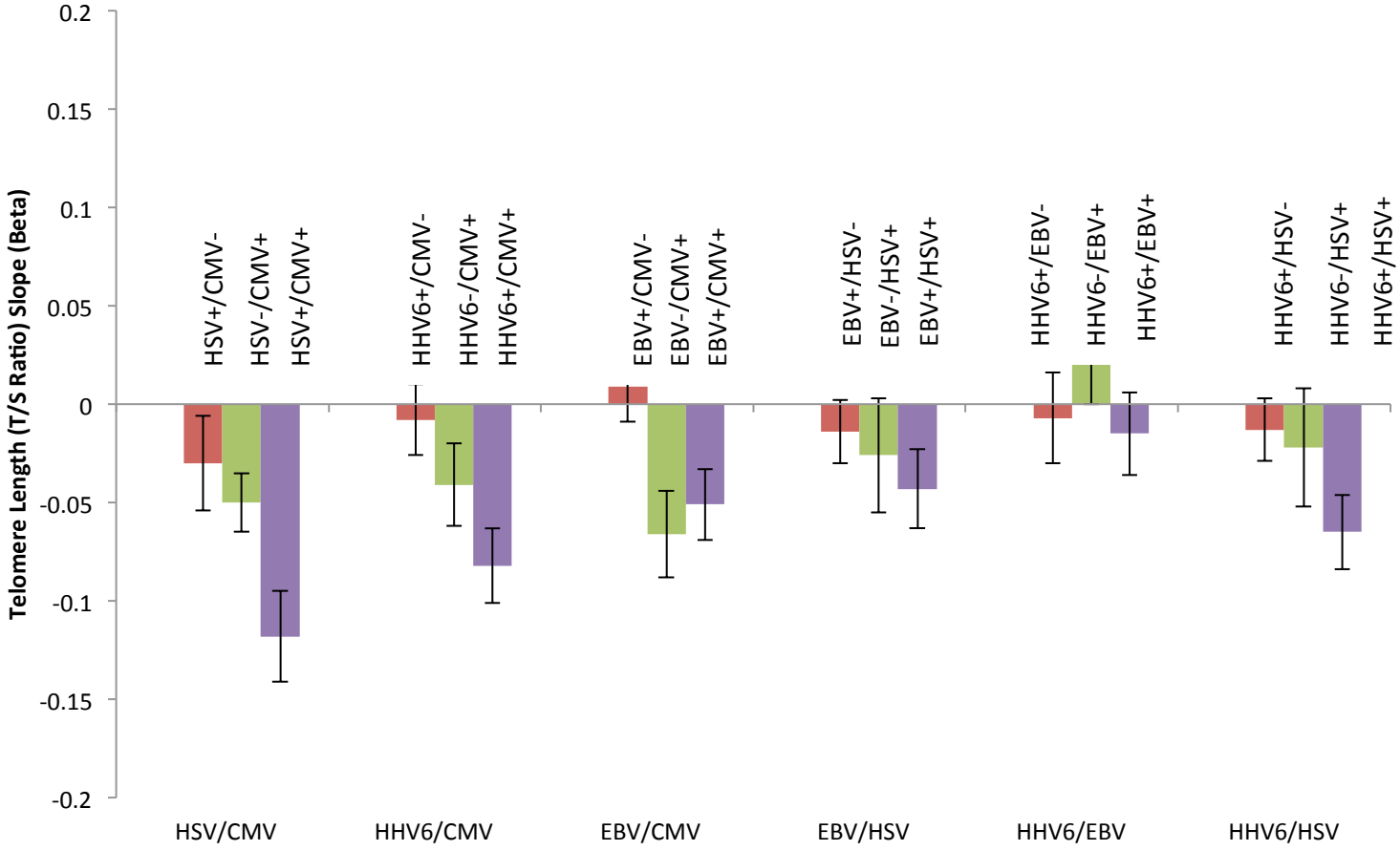

Telomere Length (T/S Ratio) Slope (Beta)
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
HSV+/CMV-
HSV-/CMV+
HSV+/CMV+
HHV6+/CMV-
HHV6-/CMV+
HHV6+/CMV+
EBV+/CMV-
EBV-/CMV+
EBV+/CMV+
EBV+/HSV-
EBV-/HSV+
EBV+/HSV+
HHV6+/EBV-
HHV6-/EBV+
HHV6+/EBV+
HHV6+/HSV-
HHV6-/HSV+
HHV6+/HSV+
HSV/CMV
HHV6/CMV
EBV/CMV
EBV/HSV
HHV6/EBV
HHV6/HSV

Table 1: Descriptive Statistics-Baseline, N=400

Variable	Mean/%	S.D
TL Time 2 (T/S ratio)	0.897	0.15
TL Time 1 (T/S ratio)	0.992	0.07
Age (time 1)	63.413	5.63
Female	53.25%	
CMV +	47.25%	
HSV1 +	21.00%	
HHV6 +	59.00%	
EBV +	63.25%	
Total Pathogen Burden		
0	7.75%	
1	28.25%	
2	36.25%	
3	21.25%	
4	6.50%	

Table 2: Herpesvirus infections and Telomere Length in Whitehall Heartscan Study (N=400)

	TL (T/S ratio (Time 2)			
Whitehall Heart Scan Study	**β (s.e.)**	**P-value**	**β (s.e.)**	**P-value**
	Model 1		**Model 2**	
Pathogens				
CMV+	**-0.061 (0.014)**	**<0.001**	**-0.059 (0.014)**	**<0.001**
HSV-1+	**-0.049 (0.016)**	**0.002**	**-0.047 (0.017)**	**<0.006**
HHV6+	**-0.033 (0.015)**	**0.024**	-0.027 (0.014)	0.065
EBV+	0.001 (0.015)	0.949	0.009 (0.015)	0.521
Covariates				
Age			-0.005 (0.001)	<0.001
Female			0.062 (0.014)	<0.001
TL at Baseline			0.523 (0.093)	<0.001
Current vs. Never Smoking			-0.007 (0.031)	0.831
Obese vs normal weight			-0.021 (0.021)	0.312

All models adjusted for age, sex, smoking, employment grade, categorical BMI & baseline TL
Model 1 shows coefficients for separate regressions with individual infections
Model 2 mutually adjused for the other infections

Table 3: Pathogen burden, Co-infection, and TL

Whitehall Heart Scan Study	TL (T/S ratio (Time 2) β (s.e.)	P-value
Total Pathogen Burden (0-4)		
0 (N=31)	ref	
1 (N=113)	-0.014 (0.028)	0.624
2 (N=145)	-0.025 (0.027)	0.362
3 (N=85)	-0.069 (0.029)	0.019
4 (N=26)	-0.139 (0.037)	<0.001
Pathogen Combinations		
HSV-/CMV- (43.00%)	ref	
HSV+/CMV- (9.75%)	-0.030 (0.024)	0.684
HSV-/CMV+ (36.00%)	-0.050 (0.015)	0.013
HSV+/CMV+ (12.25%)	-0.118 (0.023)	<0.001
HHV6-/CMV- (22.50%)	ref	
HHV6+/CMV- (30.25%)	-0.008 (0.018)	0.684
HHV6-/CMV+ (18.50%)	-0.041 (0.021)	0.583
HHV6+/CMV+ (28.75%)	-0.082 (0.019)	<0.001
EBV-/CMV- (21.50%)	ref	
EBV+/CMV- (31.25%)	0.009 (0.018)	0.684
EBV-/CMV+ (15.25%)	-0.066 (0.022)	0.041
EBV+/CMV+ (32.00%)	-0.051 (0.018)	0.060
EBV-/HSV- (30.00%)	ref	
EBV+/HSV- (49.00%)	0.012 (0.016)	0.684
EBV-/HSV+ (6.75%)	-0.027 (0.029)	0.684
EBV+/HSV+ (14.25%)	-0.048 (0.021)	0.240
HHV6-/EBV- (15.50%)	ref	
HHV6+/EBV- (21.25%)	-0.010 (0.023)	0.684
HHV6-/EBV+ (25.50%)	0.019 (0.022)	0.684
HHV6+/EBV+ (37.75%)	-0.020 (0.021)	0.684
HHV6-/HSV- (35.00%)	ref	
HHV6+/HSV- (44.00%)	-0.015 (0.016)	0.684
HHV6-/HSV+ (6.00%)	-0.026 (0.031)	0.684
HHV6+/HSV+ (15.00%)	-0.069 (0.020)	0.015

All models adjust for age, sex, smoking, employment grade, and TL at Time 1
For pathogen combinations, p-values are adjusted for Benyamini-Hochberg False-Discovery Rate