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Leukocyte telomere length predicts subsequent infectious morbidity among Colombian schoolchildren

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ABSTRACT

Objective: Telomere length (TL) attrition is related to chronic disease risk. However, less is known on whether TL predicts infectious outcomes, especially in childhood. We examined whether leukocyte TL (LTL) was associated with subsequent infectious morbidity in schoolchildren.

Methods: We assessed LTL in 717 Colombian children 5-12 years-old at the beginning of a school year and followed them through the year for daily occurrence of common infection symptoms and doctor visits. We estimated adjusted incidence rate ratios (IRR) with 95% confidence intervals (CI) of gastrointestinal and respiratory syndromes for quartiles of standardized LTL Z-score and per unit LTL Z-score.

Results: A longer LTL was associated with increased incidence of all infectious morbidity syndromes considered. Adjusted IRR (95% CI) per unit LTL Z-score were 1.55 (1.20, 2.00) for diarrhea with vomiting, 1.34 (1.13, 1.60) for cough with fever, 1.70 (1.28, 2.28) for ear infection, and 1.66 (1.36, 2.02) for doctor visits with symptoms.

Conclusions: Longer LTL is related to increased incidence of common infectious morbidities in middle childhood.

Keywords: telomere length, infection, diarrhea, respiratory morbidity, children

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INTRODUCTION

There is growing interest in telomere length (TL) attrition as a mechanism that may link early life stressors to the risk of chronic disease in later life (Marasco, Smith, & Angelier, 2022). TL shortens in proportion to the number of cell replications, and shorter TL in adults has been associated with chronic disease and all-cause mortality (Mons et al., 2017). Less is understood about the potential role of TL on infectious outcomes, particularly in childhood, since most studies of TL have been conducted in adults. This question is relevant because the burden of pediatric infectious morbidity is substantial, especially in low- and middle-income countries, and understanding the role of TL at this age could shed light into immunocompetence mechanisms. Shorter leukocyte TL (LTL) has been associated with an increased risk of infection in adults in both observational (Helby, Nordestgaard, Benfield, & Bojesen, 2017) and experimental (Cohen et al., 2013) studies. In a randomized trial of medically-induced upper respiratory infection with a common cold virus among healthy adults, shorter telomeres were associated with greater odds of infection, independent of pre-challenge virus-specific antibody, demographic characteristics, and body mass index (Cohen et al., 2013). Because leukocytes pertain to the cellular arm of the immune response, a decreased ability to replicate could contribute to an increased infection risk. However, few studies have prospectively described the relation between TL and subsequent infectious morbidity in children.

In this report, we examined whether LTL was associated with the incidence of common gastrointestinal and respiratory infectious morbidity during a school year in Colombian children.

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MATERIALS AND METHODS

Study design and population

We used data from the Bogotá School Children Cohort, a longitudinal investigation of nutrition and health. Details of the study design have been previously reported (Arsenault et al., 2009). In brief, we recruited 3202 children ages 5-12 years through random selection from public primary schools at the start of the 2006 academic year. At enrollment, parents completed a sociodemographic survey and research assistants measured the children's height and weight, and obtained fasting blood samples. All participants provided informed consent prior to enrollment. The study was approved by the Ethics Committee at the National University of Colombia Medical School; the University of Michigan Institutional Review Board approved the use of data and samples.

Whole blood collected in EDTA tubes was separated into its components by centrifugation. DNA was extracted from the buffy coat and cryopreserved. LTL was quantified in the DNA samples of a random subgroup of participants (n=723) as the telomere-to-single-copy-gene (T/S) ratio, using a multiplex qPCR method. LTL measurements had high internal and external validity (Flannagan et al., 2020) (Supporting Information Appendix 1). Morbidity data was collected for the year following enrollment using weekly pictorial diaries maintained by the parents, as detailed elsewhere (Thornton, Mora-Plazas, Marin, & Villamor, 2014). These diaries depicted drawings of children with common infectious morbidity symptoms such as fever, diarrhea, vomiting, and cough, as well as doctor or hospital visits; parents were instructed to checkmark each day the child experienced any of these events. This method has been validated in other settings to prospectively obtain clinical infectious morbidity information (Stanton et al., 1987).

Data analysis

Of 723 children with LTL results available, 717 children (99%) also had at least one week of complete morbidity data and they constituted the analytic sample.

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Exposure. LTL was standardized to the study population and expressed as a Z-score with mean=0 and SD=1; it was considered both in quartiles and as a continuous variable.

Outcomes. Outcomes were the number of days with combinations of symptoms representing the most common infectious morbidities in this age group (Pappas, Hendley, Hayden, & Winther, 2008). These included diarrhea with vomiting, a proxy for gastrointestinal illness; cough with fever, a proxy for respiratory infections; earache or ear discharge with fever, a proxy for otitis media; and the number of doctor visits with symptoms during the surveillance period, to indicate severe illness.

Statistical analysis. We examined the distribution of background characteristics by LTL quartiles. Next, we estimated rates of each outcome as the proportion of days with the corresponding combination of symptoms out of the total number of days observed. Rates were compared by LTL quartiles with use of incidence rate ratios (IRR) and 95% confidence intervals (CI) from log-binomial regression models for repeated measures within child, with the presence of symptoms on each day as the binary outcome and robust variance estimators to account for non-independence of observations. Modelling each day of observation accounts for the overdispersed nature of morbidity data, since a majority of children would have 0 days with a given combination of symptoms during the surveillance period. We also estimated rate ratios per unit LTL Z-score, assuming linearity of the associations. We identified potential confounders per prior knowledge on independent predictors of the morbidity outcomes (Thornton et al., 2014) that could also be related to LTL (Flannagan et al., 2020) without being its consequence. These included child's sex, age, height- and BMI-for-age Z-scores, and household socioeconomic status per the city government's classification of neighborhoods. We also considered white blood cells count and the proportion of granulocytes in the blood sample where LTL was assessed because LTL can vary with leukocyte count (Codd et al., 2022). Because 25 children (3.5%) had missing values on adjustment covariates, we re-estimated the unadjusted IRR excluding them. A similarity of unadjusted IRR estimates between the entire analytic sample (*n*=717) and the subsample with complete

data (*n*=692) would argue against selection bias. Because the length of follow-up was variable and some children had few days of observation, we conducted sensitivity analysis by restricting the sample to only children who reported morbidity data for at least 12 weeks. Analyses were carried out with use of Statistical Analysis Software version 9.4 (SAS Institute, Cary, NC).

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RESULTS

Mean ± SD age at enrollment was 8.6 ± 1.7 years; 54.7% of participants were girls. Male sex, age, and % granulocytes were inversely related to LTL Z-score (Supporting Information Table S1).

Median follow-up was 140 days (IQR: 91, 182). Diarrhea with vomiting, cough with fever, earache or ear discharge with fever, and symptomatic doctor visits occurred, respectively, on 1.05, 3.01, 0.61, and 0.60 days per child-year.

LTL was positively associated with each of the morbidity outcomes considered (Table 1). Rates of diarrhea with vomiting increased with LTL quartiles in a dose-response manner; every unit LTL Z-score was related to a 1.55-times (95% CI: 1.20, 2.00; *P*=0.0009) higher rate, after adjustment for potential confounding (Supporting Information Tables S2, S3). Adjusted IRR per unit LTL Z-score for cough with fever, earache or ear discharge with fever, and symptomatic doctor visits were, respectively, 1.34 (95% CI: 1.13, 1.60; *P*=0.001), 1.70 (95% CI: 1.28, 2.28; *P*=0.0003), and 1.66 (95% CI: 1.36, 2.02; *P*<0.0001). Unadjusted estimates in the subset without missing covariate values were similar to those in the entire sample. Analyses restricted to children with ≥12 weeks of morbidity data yielded similar results (Supporting Information Table S4).

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DISCUSSION

In this longitudinal investigation of Colombian school-age children, LTL was associated with increased incidence of common gastrointestinal and respiratory infectious morbidities during a school year.

These findings are unexpected since previous studies had reported inverse rather than positive associations between TL and incidence of infections (Cohen et al., 2013; Helby et al., 2017). Several reasons could explain this discrepancy. First, while previous studies focused on adults, our investigation pertained to children and both TL dynamics and infectious morbidity patterns may differ between the two age groups. Second, differences in length of follow-up and outcome definitions between studies also limit comparability of results. While we examined the cumulative number of days with infectious morbidity symptoms over one academic year, an experiment in adults focused on the incidence of a single acute infection over the course of a few days (Cohen et al., 2013) and a population-based observational study followed participants for over 20 years for the incidence of specific infection episodes (Helby et al., 2017). Third, there are also differences in the definition of exposure across investigations. The intervention study (Cohen et al., 2013) ascertained TL in specific immune cell types, whereas we quantified TL in all leukocytes.

Our study has several strengths. Morbidity was ascertained using a valid method to capture episodes of infection. The prospective nature of morbidity data collection precluded differential misclassification of outcome. Also, the detailed characterization of the cohort enabled us to control for important potential confounders such as the nutritional status, white blood cell counts, and socioeconomic conditions. Some limitations are also worth noting. First, variability in follow-up duration could have resulted in outcome misclassification since fewer reporting days may not represent the annual morbidity pattern. Nevertheless, results did not change in sensitivity analyses excluding children with the shortest follow-up. Second, we lacked etiological definitions of the infection episodes, and the

role of TL on infection risk could vary according to the causative microorganism. Third, the study design captured symptomatic illnesses but not asymptomatic infections. Fourth, there could be residual confounding by genetics or race/ethnicity, which we did not assess. Finally, we cannot rule out reverse causation if the infection patterns we measured prospectively were correlated with those unobserved prior to TL assessment. If that were the case, longer TL could be a consequence, rather than a cause, of increased infectious morbidity; nonetheless, such a positive association would also run counter to most available evidence which suggests infections shorten rather than lengthen TL (Asghar et al., 2018; Giraudeau, Heidinger, Bonneaud, & Sepp, 2019).

In conclusion, LTL in middle childhood is positively associated with infectious morbidity symptoms. Further longitudinal investigations are warranted to elucidate the potentially complex role of TL as a marker of immunocompetence.

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AUTHOR CONTRIBUTIONS

Gwenyth O. Lee and Eduardo Villamor conceived the study and analyzed the data. Gwenyth O. Lee wrote the first manuscript draft. Mercedes Mora-Plazas and Constanza Marín collected the data and contributed to the interpretation of results in the local context. All authors reviewed and approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study can be made available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

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Table 1. Leukocyte telomere length and infectious morbidity in school-age children from Bogotá, Colombia

	LTL Z-score quartiles				Per 1 SD
	1	2	3	4	
Median LTL Z-score	-0.92	-0.35	0.07	0.89	
Number of children	179	179	180	179	
Child-days of observation	22,937	23,515	24,027	23,524	

Diarrhea with vomiting								
Days with symptoms reported[a]	47		55		74		93	
Rate per child-year[b]	0.75		0.85		1.12		1.44	
Unadjusted rate ratio (95% CI)[c]	1.00	1.14	(0.53, 2.44)	1.50	(0.71, 3.17)	1.93	(0.95, 3.93)	1.46 (1.10, 1.94)
Unadjusted rate ratio (95% CI)[d]	1.00	1.09	(0.51, 2.33)	1.41	(0.67, 2.99)	1.87	(0.92, 3.80)	1.47 (1.10, 1.96)
Adjusted rate ratio (95% CI)[e]	1.00	1.13	(0.51, 2.52)	1.54	(0.77, 3.09)	1.89	(0.88, 4.05)	1.55 (1.20, 2.00)
Cough with fever								
Days with symptoms reported[a]	156		185		151		283	
Rate per child-year[b]	2.48		2.87		2.30		4.39	
Unadjusted rate ratio (95% CI)[c]	1.00	1.16	(0.62, 2.17)	0.92	(0.54, 1.58)	1.77	(1.06, 2.94)	1.29 (1.08, 1.54)
Unadjusted rate ratio (95% CI)[d]	1.00	0.89	(0.50, 1.56)	0.88	(0.52, 1.50)	1.70	(1.02, 2.83)	1.32 (1.11, 1.58)
Adjusted rate ratio (95% CI)[e]	1.00	0.86	(0.49, 1.53)	0.88	(0.52, 1.51)	1.66	(0.99, 2.80)	1.34 (1.13, 1.60)
Earache or ear discharge with fever								
Days with symptoms reported[a]	21		38		34		65	
Rate per child-year[b]	0.33		0.59		0.52		1.01	
Unadjusted rate ratio (95% CI)[c]	1.00	1.77	(0.69, 4.54)	1.55	(0.68, 3.50)	3.02	(1.17, 7.78)	1.55 (1.09, 2.21)
Unadjusted rate ratio (95% CI)[d]	1.00	1.69	(0.66, 4.34)	1.47	(0.65, 3.33)	2.93	(1.14, 7.53)	1.56 (1.10, 2.22)
Adjusted rate ratio (95% CI)[e]	1.00	1.66	(0.66, 4.17)	1.54	(0.70, 3.39)	2.76	(0.98, 7.77)	1.70 (1.28, 2.28)
Doctor visits on days with symptoms								
Days with visits reported[a]	31		14		37		73	
Rate per child-year[b]	0.49		0.22		0.56		1.13	
Unadjusted rate ratio (95% CI)[c]	1.00	0.44	(0.18, 1.11)	1.14	(0.34, 3.81)	2.30	(0.82, 6.41)	1.58 (1.12, 2.23)
Unadjusted rate ratio (95% CI)[d]	1.00	0.42	(0.17, 1.06)	1.09	(0.33, 3.62)	2.23	(0.80, 6.21)	1.59 (1.13, 2.25)
Adjusted rate ratio (95% CI)[e]	1.00	0.46	(0.19, 1.12)	1.06	(0.37, 3.05)	2.27	(0.81, 6.39)	1.66 (1.36, 2.02)

[a] Total number of days, summed across all child-days of observation, when the symptoms combination was reported.

[b] Proportion of days with symptoms out of all days of observation, multiplied by 365.25 (one year).

[c] From log-binomial regression for repeated measures within child, with each combination of symptoms on the day of observation as the outcome and LTL Z-score (quartiles or the continuous variable for estimates per 1 SD) as predictors. Robust estimates of variance were specified in all models.

[d] Restricted to children without missing values in covariates (height- or body mass index-for-age Z-scores, white blood cell count, granulocytes, or household socioeconomic status; *n*=692).

[e] Adjusted for child sex, age, height- and body mass index-for-age Z-scores, white blood cell count, granulocytes as a percentage of total white blood cells, and household socioeconomic status. Complete case analysis (*n*=692).

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