Association between antinuclear antibody seropositivity and telomere length: a nationwide population-based study

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Received on January 2, 2020; accepted in revised form on February 17, 2020.

Key words: antinuclear antibodies, telomeres, aging

Funding: this work was supported by the National Heart, Lung, and Blood Institute, grant numbers K01HL135459 (CRJ), T32HL007891 (JGN), and K24HL103844 (SMK).

Competing interests; none declared.

ABSTRACT

Objective. *Telomere shortening is a well-established marker of biological aging. Whether telomere erosion coincides with age-related increases in antinuclear antibody (ANA) seropositivity remains unknown. Our study aimed to determine the association between ANA seropositivity and shortened telomeres among 1999-2002 National Health and Nutrition Examination Survey (NHANES) subjects.*

Methods. *We performed a cross-sectional analysis of 2,188 NHANES study participants with available ANA and telomere length data. ANA testing was performed using indirect immunofluorescence. Telomere lengths were measured via quantitative polymerase chain reaction methods. Applying appropriate sample weighting techniques, we used univariate and multivariate logistic regression methods to assess the association between shortened telomeres (i.e. lowest decile of the cohort) and ANA seropositivity.*

Results. *ANAs were positive in 322 out of 2,188 (14.7%, 95% CI 13.3-16.3%) individuals. Subjects with shortened telomeres were more likely to be older (p<0.001), male (p=0.005), and have a cancer history (p<0.001). A higher proportion of non-Hispanic white participants (61.6% vs. 49.3%) and a lower proportion of non-Hispanic black participants (7.8% vs. 17.9%) had shortened telomeres (p<0.001). Shortened telomeres were not independently associated with ANA seropositivity (OR 1.48, 95% CI 0.87-2.52, p=0.14). However, female sex (OR 1.91, 95% CI 1.23-2.96, p=0.006), age ≥80 years (OR 2.06, 95% CI 1.08-3.92, p=0.03), and African American race (OR 1.58, 95% CI 1.00-2.51, p=0.05) were independent risk factors for ANA seropositivity. Neither sex nor race modified the relationship between ANA seropositivity and telomere length.*

Conclusion. *Telomere erosion does not appear to be responsible for age-related increases in the prevalence of ANA seropositivity.*

Introduction

Antinuclear antibodies (ANAs) are associated with autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA). However, ANA seropositivity can exist in the absence of systemic rheumatologic disease. Among healthy adults, the prevalence of ANA seropositivity tends to increase with age (1). In one study, 19% of patients aged 70 years or older were found to have detectable ANAs compared to 13% of patients aged 20 to 39 years (1) Despite this increased expression, elderly patients have not been shown to have a higher prevalence of most autoimmune diseases (2).

Telomeres are specialised structures at the ends of chromosomes that naturally shorten with age (3). They are comprised of repeating AGGGTT nucleotide sequences and protect against genomic instability and eventual apoptosis and immunosenescence (3, 4). Thus, telomere length essentially serves as a marker of biological aging with important functional consequences (3, 5). Telomere length has been linked to a number of autoimmune diseases, including SLE and RA (6-10). Much of this research to date suggests that autoimmune disease likely predates measurably significant telomere shortening, indicating that telomere erosion may be consequential as opposed to causative.

Whether an increase in the prevalence of ANA seropositivity among older healthy adults coincides with markers of biological aging such as telomere length remains largely unknown. A recent small longitudinal study demonstrated an association between baseline telomere length and incident ANA seropositivity. The authors concluded that early autoimmunity may reflect or result from advanced cellular aging (11). This argument counters findings from an earlier study in which shortened telomeres were not associated with increased risk for incident autoimmune disease (6). The purpose of our study was to determine whether telomere length was independently associated with ANA seropositivity among subjects enrolled in the National Health and Nutrition Examination Survey (NHANES) between the years 1999 and 2002.

Methods

Study design and patient population

We performed a cross-sectional study of results generated from NHANES, which comprises surveys representative of the non-institutionalised U.S. population. Survey data are collected in two-year cycles utilising a multistage probability sampling design (12). Measurement of leukocyte telomere length (LTL) was performed during years 1999–2002. Throughout this timeframe, NHANES oversampled a number of specific subgroups to produce more precise estimates, including individuals ages 60 and older, African Americans, Mexican Americans, and low-income individuals. Subjects were considered eligible for our analysis if they were at least 20 years old and had both telomere length and ANA data available.

Antinuclear antibody testing

Standard immunofluorescence ANA testing was performed using commercial HEp-2 ANA slides with 1:80 dilutions of sera, followed by staining with DyLight 488-conjugated donkey anti-human IgG (γ-chain specific) antibodies (13). Cellular patterns of staining and staining intensities were characterised using previously described classification methods (14). Immunofluorescence staining intensities were graded on a 0–4 scale using a standard reference gallery. Samples with intensities of 3 or 4+ were considered positive as previously described (15) and underwent subsequent determination of autoantibody titres from 1:80 to 1:1280 using serial dilution. Specific details on laboratory quality assurance and monitoring are reported elsewhere (15).

Telomere length testing

As noted previously, LTL measurements were obtained for a subset of patients aged 20 years or older. Among 10,291 eligible subjects, a total of 7,827 adults (76%) provided a useable DNA sample. Telomere length measurements were performed at the University of California, San Francisco using the quantitative polymerase chain reaction method, which measures telomere length relative to standard reference DNA (T/S ratio), as described in detail elsewhere (3, 16).

Table I. Baseline demographic and clinical characteristics of NHANES study participants, stratified by measured telomere length in the lowest decile.

	Combined n=2,188	Upper 90th percentile n=1,969	Lowest 10th percentile n=219	*p-value
Age (%)				
<40	822 (37.6)	809 (41.1)	13 (5.9)	<0.001
40-49	347 (15.9)	328 (16.7)	19 (8.7)	
50-59	303 (13.8)	270 (13.7)	33 (15.1)	
60-69	328 (15.0)	279 (14.2)	49 (22.4)	
70-79	225 (10.3)	172 (8.7)	53 (24.2)	
80+	163 (7.4)	111 (5.6)	52 (23.7)	
Sex (%)				
Male	1,013 (46.3)	892 (45.3)	121 (55.3)	0.005
Female	1,175 (53.7)	1,077 (54.7)	98 (44.7)	
Race/ethnicity (%)				
Non-Hispanic white	1,105 (50.5)	970 (49.3)	135 (61.6)	<0.001
Non-Hispanic black	370 (16.9)	353 (17.9)	17 (7.8)	
Mexican American	517 (23.6)	464 (23.6)	53 (24.2)	
Other Hispanic	119 (5.4)	108 (5.5)	11 (5.0)	
Other race (including multi-racial)	77 (3.5)	74 (3.8)	3 (1.4)	
BMI category (%)				
<25	788 (37.1)	715 (37.4)	73 (34.8)	0.04
25-29.9	746 (35.1)	656 (34.3)	90 (42.9)	
30+	589 (27.7)	542 (28.3)	47 (22.4)	
Education (%)				
Less than high school	749 (34.3)	658 (33.5)	91 (41.6)	0.01
High school diploma or equivalent	488 (22.3)	435 (22.1)	53 (24.2)	
More than high school	948 (43.4)	873 (44.4)	75 (34.2)	
Smoking history (%)				
Never	1,115 (51.0)	1,011 (51.4)	104 (47.5)	0.001
Former	597 (27.3)	516 (26.2)	81 (37.0)	
Current	474 (21.7)	440 (22.4)	34 (15.5)	
Cancer history (%)	192 (8.8)	151 (7.7)	41 (18.7)	<0.001
Hypertension (%)	663 (30.6)	582 (29.9)	81 (37.2)	0.03
Diabetes (%)	202 (9.2)	176 (8.9)	26 (11.9)	0.16
Thyroid disease (%)	58 (2.7)	50 (2.5)	8 (3.7)	0.33
Positive ANA (%)	322 (14.7)	281 (14.3)	41 (18.7)	0.08
ANA titre (%)				
Negative	1,866 (85.3)	1,688 (85.7)	178 (81.3)	
1:80	1 (<0.1)	1 (0.1)	0 (0.0)	0.45
1:160	5 (0.2)	5 (0.3)	0 (0.0)	
1:320	82 (3.7)	71 (3.6)	11 (5.0)	
1:640	90 (4.1)	80 (4.1)	10 (4.6)	
1:1,280	144 (6.6)	124 (6.3)	20 (9.1)	

ANA: antinuclear antibody; BMI: body mass index.
*p-values reflect the significance of individual chi-squared analyses for each covariate presented, comparing subjects with LTL in the lowest 10th percentile to subjects with LTL in the upper 90th percentile.

Assessment of sociodemographic and biobehavioural measures

Sociodemographic data including race, ethnicity, and education level, medical comorbidities such as hypertension, diabetes mellitus, and cancer, and smoking history were based on self-report. Body mass index (BMI) was calculated from patient-reported height and weight and subsequently divided using standard cut-points (normal weight <25 kg/m^2, overweight 25–29.9 kg/m^2, and obese 30+ kg/m^2).

Statistical analysis

The patients were categorised based on whether or not their measured LTL fell below the 10th percentile within the total cohort. Differences in patient characteristics, stratified by LTL below or above the 10th percentile, were described using a series of chi-squared tests. To account for the sampling methods, we applied appropriate weighting techniques as recommended by the NHANES investigators using ANA subsample weights. We performed univariate lo-

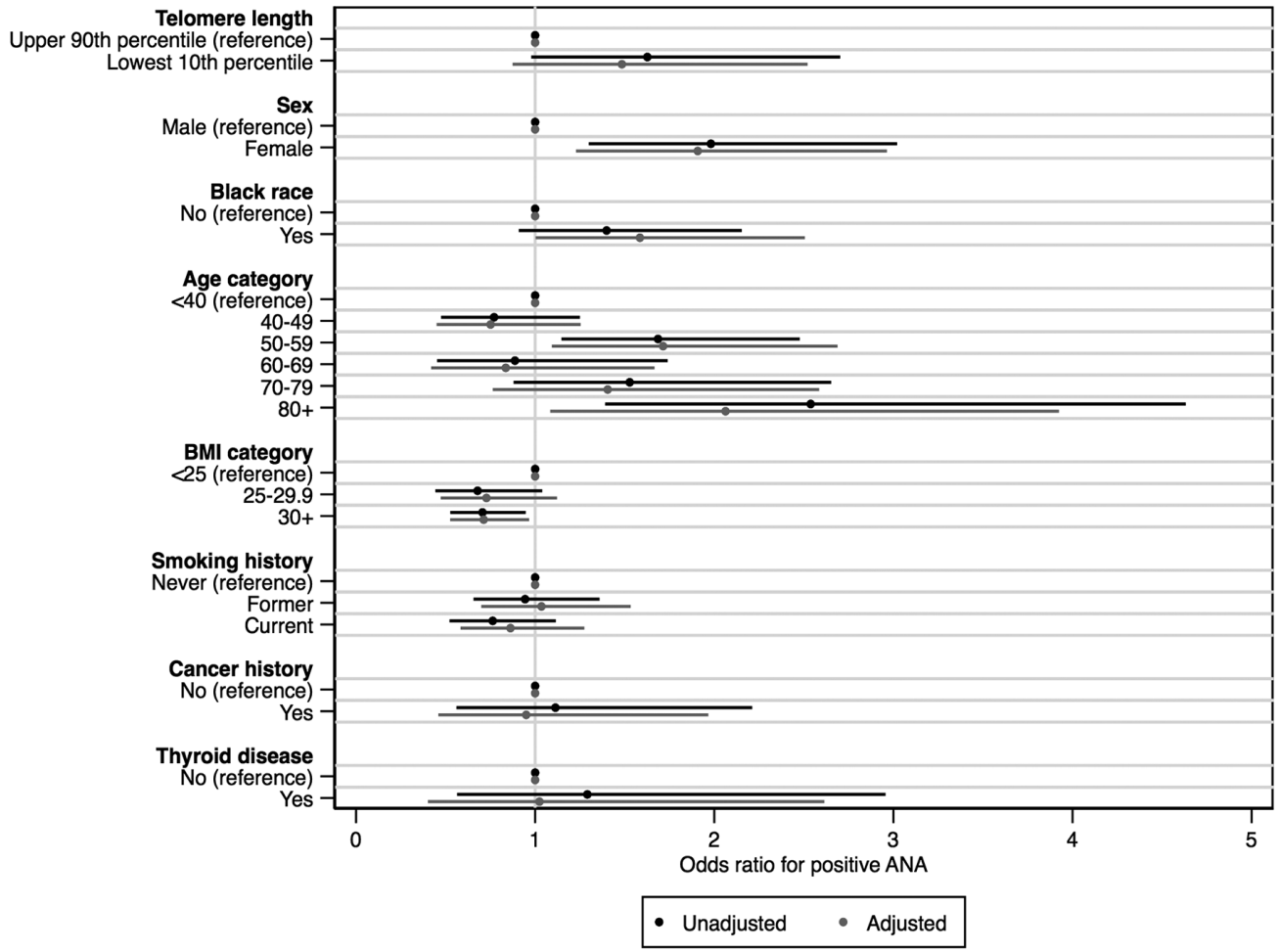

Fig. 1. Unadjusted and adjusted associations between clinical covariates, including telomere length in the lowest decile, and ANA seropositivity (multivariate model includes all of the covariates listed in the figure above).

gistic regression analyses with ANA seropositivity as the dependent variable for the following clinical covariates, all of which were selected *a priori*: LTL below the 10th percentile, age, sex, race (African American *vs*. non-African American), BMI, tobacco use, cancer history, and active thyroid disease. We then performed a multivariate logistic regression analysis incorporating all of the aforementioned covariates. We also tested the relationship between LTL in the lowest decile and ANA seropositivity for the presence of effect modification by either sex or race by incorporating interaction terms into two separate multivariable models. Statistical significance was defined as $p<0.05$. All analyses were performed using Stata/IC, v. 15.1 (College Station, TX).

Results

During the years 1999–2002, there were 21,004 NHANES study participants. We excluded 18,816 subjects on the basis of absent ANA or telomere length data, leaving 2,188 eligible subjects in our study population. Among those, there were 322 (14.7%, 95% CI 13.3–16.3%) participants with positive ANAs (all but 6 with titres 1:320). Baseline demographic and clinical characteristics of NHANES study participants, stratified by LTL in the lowest decile, are shown in Table I. Relative to subjects under age 40, there was an age decade-dependent increase in the prevalence of measured LTL in the lowest decile ($p<0.001$). In addition, study participants with LTL in the lowest decile were more likely to be male (55.3% *vs*. 45.3%, $p=0.005$) and have a history of cancer (18.7% *vs*. 7.7%, $p<0.001$). There were also statistically significant differences between patients with and without LTLs in the lowest decile with regard to racial composition, education level, BMI, tobacco use, and history of hypertension, as summarised in Table I.

Results of univariate and multivariate logistic regression analyses are summarised in Figure 1 and in online Supplementary Table S2. Telomere length in the lowest decile was not associated with ANA seropositivity in either the univariate (OR 1.63, 95% CI 0.98–2.70, $p=0.06$) or multivariate (OR 1.48, 95% CI 0.87–2.52, $p=0.14$) logistic regression analyses. Female sex and black race were both independently associated with ANA seropositivity (OR 1.91, 95% 1.23–2.96, $p=0.006$ and OR 1.59, 95% CI 1.00–2.51, $p=0.05$, respectively). Age categories 50-59 years and 80+ years were both independently associated with ANA seropositivity (OR 1.71, 95% 1.09–2.69, $p=0.02$ and OR 2.06, 95% CI 1.08–3.92, $p=0.03$,

respectively), whereas other age categories had no statistical relationship with ANA seropositivity. Lastly, a BMI of 30+ was protective against having a positive ANA (OR 0.71, 95% CI 0.53–0.97, p=0.03). Similar findings were noted on a sensitivity analysis that included a subsample of the cohort with measured height and weight data. Neither smoking nor a history of cancer were significantly associated with ANA seropositivity on both univariate and multivariate analyses. Finally, female sex (p=0.58 for interaction term) and race (p=0.62 for interaction term) did not appear to modify the relationship between LTL in the lowest decile and ANA seropositivity.

Discussion

To our knowledge, this is the first study using a nationally representative cohort to explore whether shortened telomeres are a risk factor for having detectable ANAs. Despite the age-related increase in ANA expression previously described in the literature (1), we found that telomere length in the lowest decile was not independently associated with ANA seropositivity. Thus, ANA production among healthy adults without clinically evident autoimmune disease is likely not driven by cellular aging. Although immune dysregulation and inflammation associated with aging are established risk factors for a variety of age-related diseases, immune function decline appears to occur independent of telomere erosion. Our data do suggest, however, that ANA seropositivity is associated with both black race and older age, as demonstrated previously (1).

Our results are partially concordant with a recently published study of disease-free individuals that did not demonstrate a statistically significant cross-sectional association between telomere length and ANA seropositivity (11). In this study, however, telomere length at baseline was associated with incident ANA positivity at follow-up averaging 13 years (11). This finding contrasts earlier research suggesting telomere shortening consequentially occurs after the onset of autoimmune disease (6, 7, 9, 10). Ultimately, the authors conclude that early autoimmunity may reflect or result from advanced cellular aging; however, these conclusions are limited by lack of specificity regarding the racial composition of the cohort, as well as a small sample size.

Our study has several limitations. Because of its cross-sectional nature, we do not know whether short telomeres are an independent risk factor for the subsequent development of detectable ANAs. Furthermore, our study may have been inadequately powered to detect differences in ANA seropositivity among certain subgroups. Also, RA was the only autoimmune disease inquired about in NHANES during the years 1999–2002. While there were no differences in ANA seropositivity between patients with and without RA, we were not able to evaluate the potential impact of other autoimmune diseases on our observations. Lastly, subjects with lower-intensity immunofluorescence (1 and 2+) were categorised as having a negative ANA, which explains why so few study participants had low-titre positivity (*e.g.* 1:80 or 1:160). Thus, the distribution of titres reported may not reflect that of the general population.

Our findings suggest that short telomeres are not associated with ANA seropositivity. Additional studies are needed to better understand why greater ANA prevalence with age does not coincide with biological markers of aging such as telomere length.

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