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Peripheral blood leukocyte telomere length and endometriosis

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Abstract

Endometriosis is a common gynecologic disease defined by the presence of endometrial-like tissue outside the uterine cavity. While its etiology is largely unknown, accumulating evidence suggests inflammation plays a major role. Our objective was to investigate the association between peripheral blood leukocyte telomere length (LTL) and endometriosis using data from two large population-based studies, the New England Case-Control Study (NEC; n=877) and the National Health and Nutrition Examination Survey (NHANES; n=2,268). NEC control participants were

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Authors' contributions: NS was responsible for formulation of the study hypotheses and study design, statistical analyses, results interpretation, manuscript writing, revision, and finalization. JY was responsible for study design, statistical analyses, manuscript revision and finalization. AFV was responsible for data collection, development, and implementation of NEC, study analysis methods, statistical analyses, results interpretation, and manuscript revision and finalization. DWC was responsible for data collection, development, and implementation of NEC, manuscript revision and finalization. LJT was responsible for data collection, development, and implementation of NEC, and manuscript revision and finalization. IDV was responsible for data generation in NEC data and manuscript finalization. SAM was responsible for analysis methods, results interpretation, manuscript revision and finalization. KLT was responsible for study design and analysis methods, results interpretation, manuscript revision and finalization, and was responsible for the overall conceptualization of study.

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Ethics approval: For the New England Case Control Study, the Human Subjects Review Committees at Brigham and Women's Hospital and Dartmouth Medical School approved this study, and each participant provided informed consent. For National Health and Nutrition Examination Survey, the NCHS Research Ethics Review Board approved the study.

Availability of data and material: The NHANES datasets are available online [<https://www.cdc.gov/nchs/nhanes/index.htm>]. The NEC data that support the findings of this study are available upon request and review by study leadership. The data are not publicly available due to them containing information that could compromise research participants' privacy and consent.

Code availability: All statistical analyses were conducted using SAS; programs are available from the corresponding author upon request.

identified through a combination of random digit dialing, drivers' license lists, and town resident lists. In NHANES, selection algorithms were used to identify a nationally representative sample. Blood samples and demographic, reproductive, and health-related information were available from both data sources. Endometriosis was defined as self-report of physician-diagnosed endometriosis. LTL was measured using quantitative polymerase chain reaction. Multivariable logistic regression was used to calculate odds ratios (OR) and 95% confidence intervals (CI) for the association between LTL and endometriosis. Shorter LTL was associated with greater odds of history of endometriosis. In NEC, women with the shortest LTL tertile compared to the longest had a 2.5-fold greater odds of endometriosis ($OR_{T3/T1}=2.56$, 95%CI=1.16–5.63; p-value, test for linear trend=0.02). The association was stronger among women who usually experienced moderate or severe menstrual pain ($OR_{T3/T1}=3.50$, 95%CI=1.12–10.97). In NHANES, the data suggested a similar but attenuated association ($OR_{T3/T1}=1.29$, 95%CI=0.85–1.96). The observed associations in NEC suggest shorter LTL may be associated with greater odds of endometriosis. A better understanding of how LTL influences endometriosis risk could elucidate novel disease pathophysiology.

Keywords

Endometriosis; leukocyte telomere length; Menstrual Pain; Infertility

Introduction

Telomeres are the repeated DNA sequences (TTAGGG_n) that protect chromosomal ends and shorten with each cell division [1], and studies have shown that the shortening of telomere length is accelerated in the presence of inflammation [2–4]. Therefore, peripheral blood leukocyte telomere length (LTL) reflects, among other influences, the lifelong replication of white blood cells while also capturing the cumulative burden of inflammation on the systemic immune system [5].

Endometriosis is a gynecologic disorder defined by the presence of endometrial-like tissue outside the uterine cavity affecting approximately ten percent of reproductive-aged women and is often associated with severe pain and infertility [6–8]. While the etiology of endometriosis is largely unknown, women with endometriosis have increased circulating inflammatory markers [8–11]. Given the observed associations between peripheral blood LTL and inflammation, LTL shortening could play a role in the endometriosis pathogenesis as well.

Two studies have previously examined the association between peripheral blood LTL and endometriosis, resulting with conflicting findings [12, 13]. One study reported longer LTL among women with endometriosis compared to those without while another study reported no association between peripheral blood LTL and endometriosis. However, these studies were both small in sample size and used surgical control groups (i.e. women who underwent surgery for other gynecologic indications) to examine the associations, which could have erroneously attenuated the observed associations [14]. Here, we examined the association

between peripheral blood LTL and self-reported endometriosis using data from two population-based studies.

Materials and Methods

Study Population

The New England Case Control Study (NEC) is a population-based case-control study of ovarian cancer, enrolling 2,076 epithelial ovarian cancer cases and 2,100 population-based controls from eastern Massachusetts and New Hampshire over three phases (1992–1997, 1998–2002, 2003–2008). Details regarding the study design have been described previously [15]. Population-based controls were recruited through random digit dialing, drivers' license lists, and town resident lists. All participants were interviewed at the time of enrollment about exposures that occurred at least one year before enrollment and provided blood specimens.

The National Health and Nutrition Examination Survey (NHANES) is an ongoing series of cross-sectional population-based survey study (1999–present), examining a nationally representative sample of about 5,000 participants each year [16]. Details regarding the study design are described elsewhere [17]. In brief, participants were recruited using a complex, multistage probability sampling scheme to identify a representative sample of the non-institutionalized US population. Participants were interviewed about their demographic, lifestyle, and reproductive characteristics as well as health related information, and provided physical examination and biospecimens including blood samples.

Endometriosis definition

Endometriosis was defined as any self-reported diagnosis of endometriosis, regardless of location, severity, or laparoscopic confirmation. In NEC, in-person interviews were conducted to all participants at enrollment, which included questions on endometriosis history. Specifically, data on endometriosis diagnosis were abstracted from questions assessing past medical conditions of endometriosis, reasons for infertility, and reasons for hysterectomy. In NHANES, prior endometriosis diagnosis was assessed in female participants between 20 to 54 years old using a reproductive health questionnaire that specifically asked “Has a doctor or other health professional ever told you that you had endometriosis?”.

Covariates

Demographic, lifestyle, and reproductive characteristics were assessed by questionnaires in NEC and NHANES including age at blood draw (years), body mass index (kg/m^2) calculated from reported weight (kg) per height squared (m^2), cigarette smoking status (never, current, former), self-reported race/ethnicity (White, Black, Hispanic, other for NEC; Non-Hispanic White, Non-Hispanic Black, Hispanic (combining other Hispanics and Mexican Americans), other for NHANES), education background (less than college, college graduate or above), age at menarche (<12 years old, 12 years old), usual menstrual pain during their twenties and thirties (no/mild, moderate/severe; NEC only), oral contraceptive (OC) use (ever, never; ever use defined as having used OCs for more than 3 months), age at

endometriosis diagnosis, history of infertility (never, ever; NEC only asking “Did you ever try for more than 1 year to get pregnant without success, or see a doctor about having difficulty in getting pregnant or carrying a pregnancy to term”), parity (nulliparous, parous), and hysterectomy (never, ever). Regarding menopausal status categorization (premenopausal, postmenopausal), women were classified as premenopausal if they reported having regular periods in the past year or if they reported not having regular periods because they had been pregnant, breastfeeding, or having irregular cycles. Women were classified as postmenopausal if they had reported no periods in the past year due to menopause or had a bilateral salpingo-oophorectomy (BSO). Women who reported no periods in the past year because of hysterectomy without BSO or because of other conditions were classified as premenopausal if they were younger than age 50 and postmenopausal if they were age 50 years or older.

Telomere length assessment

Genomic DNA from peripheral blood leukocytes was used to measure leukocyte telomere length (LTL) in both NEC and NHANES. In NEC, telomere length was measured in participants enrolled from 1992–2002. Details were described previously [18]. In brief, genomic DNA was extracted, standardized, and concentration measured using PicoGreen quantification by 96-well spectrophotometer (Molecular Devices, Sunnyvale, CA). Quantitative polymerase chain reaction (PCR) was used to determine the LTL. The assay estimated the ratio (T/S) of telomeric DNA to that of a single gene (36B4) copy number derived from peripheral blood leukocytes. LTL is the ratio (T/S) of telomeric (T) DNA to that of a single (S) copy gene (36B4) and is proportional to the average telomere length of a sample [19]. Each plate contained eight quality control samples, and the intraplate CV for the T/S ratio of QC samples was 10%. The LTL values were adjusted for 384-well plate using batch adjustment methods described previously [20], and outliers identified using the extreme studentized deviate many-outlier procedure [21] were excluded from further analyses (n=29).

In NHANES, telomere length was measured in 1999–2000 and 2001–2002. Details are described previously and available online https://wwwn.cdc.gov/Nchs/Nhanes/1999-2000/TELO_A.htm [22, 23]. Briefly, quantitative PCR was used to measure telomere length relative to standard reference DNA (T/S ratio) as previously described [22, 23]. Each assay plate contained eight quality control samples, representing 5% of the complete set which were blinded to the investigators. The interassay coefficient of variation was 6.5%. Outliers identified using the extreme studentized deviate many-outlier procedure were excluded from further analyses (n=1).

Statistical Analysis

In NEC, of the 1,243 population-based controls enrolled in 1992–2002, we excluded control participants with missing blood, had insufficient DNA quantity or quality, or outlying telomere length measurement (n=296) and those who self-reported any prior cancer diagnoses other than non-melanoma skin cancer (n=70), resulting in a total of 877 population-based controls included in the analysis. In NHANES, of the 21,004 participants in cycle 1999–2002, we excluded those who were male (n=10,214), missing blood samples

or outlying telomere length measurement (n=6,734), missing reproductive questionnaire data (n=242), missing information on history of endometriosis diagnosis since endometriosis was only assessed in women aged 20 to 54 years old (n=1,472), or who reported prior cancer diagnosis other than non-melanoma skin cancer (n=74), resulting in a total of 2,268 women included in the analysis.

Demographic, lifestyle, and reproductive characteristics of the study participants were described with means, standard deviations, and proportions. Tertiles of relative LTL values were determined based on the LTL distribution in each study, with a higher tertile indicating shorter LTL. Logistic regression was used to estimate the odds ratios (OR) and 95% confidence intervals (CI) comparing the odds of endometriosis across tertiles of LTL. Multivariable models were adjusted for age (continuous), BMI (continuous), smoking status (never, current, former), OC use (never, ever), parity (nulliparous, parous), and age at menarche (< 12, 12–17 years). In NHANES, the multivariable model additionally adjusted for race/ethnicity since this was a more heterogeneous population compared to NEC. These variables were selected *a priori* as possible confounders due to their known association with telomere length and endometriosis [24]. In the age-adjusted and multivariate model, we excluded those missing BMI (n=1) and age at menarche (n=5) in NEC. In NHANES, we excluded those missing smoking status (n=2), OC use (n=1), and BMI (n=20); missing indicators were used for those missing age at menarche (n=68) and parity (n=82). Two-sided Wald p-values to test for linear trends were calculated by modeling the median of each tertile as a continuous term. Effect modification was assessed for menstrual pain levels, history of infertility, and menopausal status at time of blood draw using a likelihood ratio test comparing the multivariate model with the cross-product term between the exposure variable and potential effect modifier with the model with main effects only. All analyses using NHANES data used SAS survey procedures with the DOMAIN option for analyzing the subpopulations and used sampling weights according to the NHANES analytic guidelines [25]. Statistical analyses were performed using SAS Version 9.4 (SAS Institute Inc, Cary, NC).

Results

A total of 877 women (53 with self-reported endometriosis and 824 without) in NEC and 2,268 women (151 with self-reported endometriosis and 2,117 without) in NHANES were included in the analysis. The average age at interview and blood draw of women with and without endometriosis was 49 and 50 years, respectively, in NEC and 40 and 37 years in NHANES (Table 1). About half of the women in NEC and 30% in NHANES were normal weight. The majority were White in both NEC (>95%) and NHANES (>60%); within NHANES, women with endometriosis were more likely to be White compared to the other race/ethnicity groups. In both NEC and NHANES, women with endometriosis were more likely to have ever smoked, ever used OCs, be nulliparous, and to have had a hysterectomy. In NEC, women with endometriosis were more likely to report moderate or severe menstrual pain and to have had infertility, which may also have been their indication for diagnosis. The average age at endometriosis diagnosis for cases was 30 years in NEC and 29 years in NHANES.

We observed an inverse association between LTL assessed in tertiles and odds of endometriosis (Table 2). In NEC, women with the shortest LTL, compared to the longest, had an approximate 2.5-fold higher odds of endometriosis ($OR_{T3/T1}=2.56$, 95%CI=1.16–5.63) with a significant linear trend ($p\text{-trend}=0.02$). In NHANES, the data suggested a modest association between shortest LTL and odds of endometriosis ($OR_{T3/T1}=1.29$, 95%CI=0.85–1.96), but confidence intervals were wide. We also did not observe significant associations when stratified by age.

We further examined whether the association between telomere length and endometriosis may differ by menstrual pain status or history of infertility in NEC (Table 3). Among women with moderate or severe menstrual pain, those with the shortest LTL, compared to the longest, had a 3.5-fold greater odds of endometriosis ($OR_{T3/T1}=3.50$, 95%CI=1.12–10.97). An elevated odds of endometriosis was suggested in women with no or mild menstrual pain ($OR_{T3/T1}=2.00$, 95%CI=0.65–6.10), but was not statistically significant with very wide confidence intervals. Among those who have had infertility, shorter LTL was suggestively associated with a more than 3-fold increase in odds of endometriosis ($OR_{T3/T1}=3.40$, 95%CI=0.93–12.50). Among those without a history of infertility, shorter LTL was suggestively associated with elevated odds of endometriosis ($OR_{T3/T1}=1.90$, 95%CI=0.66–5.46) but the association was not significant. While there was no statistically significant heterogeneity by menstrual pain status or infertility status, the association between LTL and endometriosis was stronger among women with moderate or severe menstrual pain or infertility. When we further examined the association between LTL and endometriosis among those who had both moderate to severe menstrual pain and infertility, we observed a non-significant positive association with shorter LTL and odds of endometriosis. However, there were only 12 women with endometriosis and 47 women without who reported both menstrual pain and infertility, hence we were limited in sample size.

Since endometriosis is an estrogen-dependent disease with symptoms usually minimizing or vanishing altogether after menopause [8, 26, 27], we further stratified by menopausal status at time of blood draw (Table 4). In NEC, both a shorter and a medium LTL were associated with significantly greater odds of endometriosis among premenopausal women, and these associations were substantially attenuated among postmenopausal women. In NHANES, shorter LTL was suggestively associated with greater odds of endometriosis among both pre- and postmenopausal women although not statistically significant.

Discussion

Using two population-based studies, we observed that shorter LTL was associated with greater odds of history of endometriosis, and the association was stronger among women who had experienced moderate or severe menstrual pain. Interestingly, this association was observed only among women who were premenopausal at time of blood draw in NEC.

To our knowledge, only two studies have previously examined peripheral blood LTL in relation to endometriosis with inconsistent results [12, 13]. One study reported that women with endometriosis had a significantly longer LTL compared to controls at time of surgery [12]. However, this study was based on 86 endometriosis cases and 21 controls who had

received surgery for other indications (i.e. myomectomy, tubal ligation), which may have biased the results toward the null if the surgical indications were also associated with telomere length [14]. Another study measured TL in endometrial tissue and peripheral blood among endometriosis cases (n = 29) and in controls (n = 27) who were receiving laparoscopic surgery for tubal ligation [13]. While the investigators did not observe a statistically significant association between LTL and endometriosis, the small sample size limited their ability to observe any significant results. In addition, since parity has been reported to be associated with shorter telomere length [28], using multiparous controls who had tubal ligation would likely bias the results toward the null given that a high proportion of the women with endometriosis are likely to be nulliparous, [14].

Our observations are consistent with what is known of the underlying biology of endometriosis. Inflammation is observed in endometriosis patients [9, 10, 29]. Inflammatory factors, including interleukin-1 β (IL-1 β), IL-6, tumor necrosis factor- α (TNF- α) and high-sensitivity C-reactive protein (hs-CRP), are elevated in the peripheral blood of women with endometriosis compared to controls [8–10]. Furthermore, chronic, systemic inflammation increases white blood cell turnover, which increases telomere attrition rate [30]. Proinflammatory cytokine TNF- α may lead to telomere shortening by downregulating telomerase, an enzyme that helps maintain telomere length [31]. Another study reported that higher levels of IL-6 and TNF- α were associated with shorter LTL [4]. Therefore, it is plausible that this combination of increased inflammation as well as increased leukocyte proliferation contribute to telomere shortening in women with endometriosis.

A recent Mendelian randomization study of chronic diseases broadly reported that longer telomeres were associated with a greater risk of cancer while shorter telomeres were associated with greater risk of cardiovascular and autoimmune disease [32]. Genetically-predicted telomere length was not associated with endometriosis in this Mendelian randomization analysis, possibly because endometriosis was based on GWAS SNPs, which may reflect only a subset of those with endometriosis - particularly those with advanced disease with endometrioma specifically or act as a marker of fibrosis and scarring [33]. Given our finding that the association between LTL and endometriosis may differ by pain presenting or infertility presenting phenotype, use of these SNPs for endometriosis may have attenuated the association. Importantly, while the endometriosis SNPs used in the mendelian randomization analysis was based on a modest sample size endometriosis GWAS conducted in 2012 among 4,604 endometriosis cases and 9,393 controls [33], endometriosis GWAS from larger studies including more than 750,000 women are now available for future MR consideration [34].

We observed a suggestive differential association of LTL and endometriosis by menstrual pain status and infertility status. Female infertility factors such as recurrent miscarriage, ovarian insufficiency, and oocyte maturation have been reported to be associated with shorter LTL [35], which is in line with our observation of endometriosis patients with infertility being associated with shorter LTL. In terms of pain, three studies examined the association between LTL and pain (i.e. fibromyalgia, osteoarthritis, chronic pain) observing no associations [36–38]. No studies have investigated the association between LTL and menstrual pain. While our observed results may be due to small sample size, this is the first

study to report the association between telomere length and endometriosis-associated infertility and menstrual pain. Further investigations are warranted on whether pain severity and infertility status modify the association between telomere length and endometriosis. Furthermore, we observed the significant association between LTL and endometriosis only among women who were premenopausal at time of blood draw and not among postmenopausal women in NEC, suggesting that the systemic hormonal milieu may be involved in the interaction between LTL and endometriosis pathophysiology.

Peripheral blood LTL was measured potentially years after endometriosis diagnosis in both NEC and NHANES, and therefore may not reflect active endometriosis status but instead may reflect primarily the milieu given many years of chronic exposure. Given that telomere shortening is accelerated in the presence of inflammation, shorter LTL being significantly associated with endometriosis in NEC may be in part due to chronic exposures to pain and inflammation in women with endometriosis since these women had their blood drawn at about 20 years after their endometriosis diagnosis. Endometriosis has also been reported to be associated with increased risk of chronic diseases such as cardiovascular diseases, cancer, and autoimmune diseases, and it is possible that accelerated shortening of LTL may mediate these associations. However, prospective studies are needed to elucidate these associations more in-depth.

Our study is the first to examine the association between circulating LTL and endometriosis by symptom presentation using population-based data. We were able to adjust for potential confounding factors including age, BMI, OC use, and parity. However, both studies collected information about endometriosis retrospectively. Prospectively measured endometriosis diagnosis and telomere length are needed to establish temporality. Peripheral blood LTL was measured potentially years after endometriosis diagnosis in both NEC and NHANES, and therefore may not reflect active endometriosis status or may reflect primarily the milieu given many years of chronic exposure. Since all endometriosis cases were self-reported and not restricted to those who were surgically confirmed, there may be some cases who may have misclassified themselves as having endometriosis. Also, as with all studies of endometriosis, there are likely undiagnosed cases among the women who reported never having been diagnosed with endometriosis. However, both of these misclassifications would most likely result in bias towards the null. Although our study is large, the number of endometriosis cases limited our ability to assess interactions and stratified analyses. Also, we were not able to assess differential associations by infertility or menstrual pain symptoms in NHANES since these data were not available. Despite our best efforts, residual or unmeasured confounding may still exist. However, we anticipate this to be minimal since the age adjusted and multivariable adjusted effect estimates were similar, and thus the known potential confounders for which we could adjust did not confer a confounding effect. Results from this study have limited generalizability given that the majority of the participants were White race, although we could expect the biological relationship would be similar across different racial groups. Thus, the association between LTL and endometriosis requires further study, ideally using prospective designs in diverse populations.

CONCLUSIONS

In summary, we observed that shorter LTL was associated with greater odds of history of endometriosis even after adjusting for potential confounders in a population-based study. Further research is warranted to replicate our findings, assess temporality, and elucidate the interrelationship between leukocyte telomere length and the inflammatory and hormonal milieu among endometriosis patients to further our understanding of endometriosis pathophysiology.

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Table 1.

Demographic and reproductive characteristics of two population-based studies.

	New England Case Control Study (NEC) participants without ovarian cancer, 1992– 2002 ^a		National Health and Nutrition Examination Survey (NHANES), 1999–2002 ^b	
	Endometriosis		Endometriosis	
Characteristics	Yes	No	Yes	No
	(n = 53)	(n = 824)	(n = 151)	(n = 2117)
Age at blood draw, years				
Mean (95% CI)	49 (46.1–51.9)	50 (49.4–51.2)	40 (38.8–41.0)	37 (36.4–37.6)
Body mass index (BMI), kg/m ² , n (%) ^c				
<20	1 (2)	70 (9)	10 (9)	129 (8)
20–24	29 (55)	372 (45)	41 (29)	586 (33)
25–29	17 (32)	242 (29)	44 (27)	615 (27)
30+	6 (11)	139 (17)	55 (35)	766 (32)
Smoking, n (%) ^c				
Never	15 (28)	398 (48)	82 (53)	1329 (58)
Former	30 (57)	280 (34)	22 (14)	332 (18)
Current	8 (15)	146 (18)	47 (32)	453 (25)
Race/Ethnicity, n (%) ^c				
White	51 (96)	798 (97)	110 (87)	924 (67)
Black/African American	0	8 (1)	20 (6)	381 (11)
Hispanic	1 (2)	13 (2)	15 (4)	727 (17)
Other	1 (2)	5 (0.5)	6 (3)	85 (5)
Education Level, n (%) ^c				
< College	29 (55)	482 (59)	112 (76)	1683 (74)
College	24 (45)	342 (42)	39 (24)	431 (26)
Age at menarche, years				
Mean (95% CI)	12 (12.0–12.9)	13 (12.5–12.7)	13 (12.2–12.8)	13 (12.6–12.7)
Oral contraceptive use, n (%) ^c	37 (70)	500 (61)	117 (81)	1415 (73)
Nulliparous, n (%)	13 (25)	158 (19)	34 (23)	385 (24)
Menstrual pain, n (%) ^c				
None or Mild	27 (51)	558 (68)	-	-
Moderate or Severe	26 (49)	261 (32)	-	-
Infertility, n (%) ^c	24 (45)	142 (17)	-	-
Hysterectomy, n (%) ^c	11 (21)	64 (8)	62 (55)	157 (9)
Menopausal status at blood draw, n (%) ^c				
Premenopausal	28 (53)	425 (52)	97 (60)	1883 (87)

	New England Case Control Study (NEC) participants without ovarian cancer, 1992– 2002 ^a		National Health and Nutrition Examination Survey (NHANES), 1999–2002 ^b	
	Endometriosis		Endometriosis	
Postmenopausal	25 (47)	399 (48)	54 (40)	234 (13)
Age at endometriosis diagnosis, years				
Mean (95% CI)	30 (27.6–32.0)	-	29 (27.0–30.4)	-

^aMissings in NEC: body mass index at enrollment (n=1), body mass index at age 18 (n=1), age at menarche (n=5), menstrual pain (n=5)

^bMissings in NHANES: smoking status (n=2), OC use(n=1), BMI (n=20), age at menarche (n=68), parity (n=82)

^cUnweighted number and weighted percentage in NHANES

Table2.

Associations between circulating leukocyte telomere length (LTL) and endometriosis.

New England Case Control Study (NEC), 1992–2002				
	Endometriosis		Model 1 ^{a,c}	Model 2 ^{b,c}
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI)	Odds Ratio (95%CI)
Tertile				
T1 (longest)	10 (19)	295 (36)	ref	ref
T2	21 (40)	261 (32)	2.47 (1.14–5.35)	2.37 (1.08–5.19)
T3 (shortest)	22 (42)	268 (33)	2.61 (1.20–5.70)	2.56 (1.16–5.63)
p-trend ^d			0.01	0.02
National Health and Nutrition Examination Survey (NHANES), 1999–2002				
	Endometriosis		Model 1 ^{a,f}	Model 2 ^{b,f}
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI)	Odds Ratio (95%CI)
Tertile				
T1 (longest)	39 (27)	709 (34)	ref	ref
T2	51 (32)	691 (32)	1.19 (0.69–2.05)	1.19 (0.70–2.05)
T3 (shortest)	60 (41)	691 (34)	1.32 (0.83–2.09)	1.29 (0.85–1.96)
p-trend ^d			0.20	0.22

^a Adjusted for age (years)^b Adjusted for age (continuous), smoking status (never, current, former), body mass index (BMI) (continuous), oral contraceptive use (never, ever), parity (nulliparous, parous), age at menarche (< 12, 12–year)^c Exclude missing BMI (n=1) and age at menarche (n=5)^d p-trend was calculated by modeling the median of each category as continuous term.^e Additionally adjusted for race/ethnicity (white, black, hispanic, other)^f Exclude missing smoking status (n=2), OC use(n=1), bmi (n=20), and used missing indicator for those missing age at menarche (n=68) and parity (n=82)

Table 3.

Associations between circulating leukocyte telomere length (LTL) and endometriosis stratified by menstrual pain and infertility status in the New England Case-Control Study (NEC), 1992–2002

	Mild or no menstrual pain			Moderate or severe menstrual pain			
	Endometriosis			Endometriosis			
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI) ^a	Yes	No	Odds Ratio (95%CI) ^a	p-het ^b
Tertile							
T1 (longest)	5 (19)	193 (35)	ref	5 (19)	100 (38)	ref	0.68
T2	12 (44)	177 (32)	2.37 (0.80–7.01)	9 (35)	83 (32)	2.34 (0.73–7.51)	
T3 (shortest)	10 (37)	188 (34)	2.00 (0.65–6.10)	12 (46)	78 (30)	3.50 (1.12–10.97)	
p-trend ^c			0.21			0.03	
	No infertility			Infertility			
	Endometriosis			Endometriosis			
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI) ^a	Yes	No	Odds Ratio (95%CI) ^a	p-het ^b
Tertile							
T1 (longest)	6 (21)	251 (37)	ref	4 (17)	44 (31)	ref	0.69
T2	13 (45)	216 (32)	2.50 (0.92–6.80)	8 (33)	45 (32)	2.00 (0.53–7.52)	
T3 (shortest)	10 (34)	215 (32)	1.90 (0.66–5.46)	12 (50)	53 (37)	3.40 (0.93–12.50)	
p-trend ^c			0.2			0.06	

^a Adjusted for age (continuous), smoking status (never, current, former), body mass index (BMI) (continuous), oral contraceptive use (never, ever), parity (nulliparous, parous), age at menarche (< 12, 12 year)

^b Effect modification was assessed with a likelihood ratio test that compared the model with the cross-product term between the exposure variable and stratifying variable with the model with main effects only.

^c p-trend was calculated by modeling the median of each category as continuous term.

Table 4.

Associations between circulating leukocyte telomere length (LTL) and endometriosis stratified by menopausal status at time of blood draw.

New England Case Control Study (NEC), 1992–2002							
	Premenopausal			Postmenopausal			
	Endometriosis			Endometriosis			
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI) ^a	Yes	No	Odds Ratio (95%CI) ^a	p-het ^b
Tertile							
T1 (longest)	4 (14)	188 (44)	ref	6 (24)	107 (27)	ref	0.06
T2	15 (54)	132 (31)	5.20 (1.64–16.52)	6 (24)	129 (32)	0.78 (0.24–2.58)	
T3 (shortest)	9 (25)	105 (25)	3.62 (1.06–12.38)	13 (52)	163 (41)	1.71 (0.60–4.84)	
p-trend ^c			0.02			0.30	
National Health and Nutrition Examination Survey (NHANES), 1999–2002							
	Premenopausal			Postmenopausal			
	Endometriosis			Endometriosis			
Leukocyte Telomere Length	Yes	No	Odds Ratio (95%CI) ^a	Yes	No	Odds Ratio (95%CI) ^a	p-het ^b
Tertile							
T1 (longest)	27 (30)	651 (36)	ref	12 (23)	58 (23)	ref	0.90
T2	35 (35)	626 (32)	1.24 (0.74–2.07)	16 (28)	65 (31)	1.21 (0.43–3.39)	
T3 (shortest)	34 (35)	584 (32)	1.22 (0.80–1.87)	26 (50)	107 (46)	1.53 (0.47–4.97)	
p-trend ^c			0.32			0.46	

^a Adjusted for age (continuous), smoking status (never, current, former), body mass index (BMI) (continuous), oral contraceptive use (never, ever), parity (nulliparous, parous), age at menarche (< 12, 12–14, 15–17, ≥ 18 years)

^b Effect modification was assessed with a likelihood ratio test that compared the model with the cross-product term between the exposure variable and stratifying variable with the model with main effects only.

^c p-trend was calculated by modeling the median of each category as continuous term.