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Leukocyte telomere length in a population-based case–control study of ovarian cancer: a pilot study

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Abstract

Objectives—Telomeres are structures at chromosome ends that contribute to maintaining genomic integrity. Telomere shortening with repeated cell divisions may lead to genomic instability and carcinogenesis. Studies suggest that shorter telomeres in constitutional DNA are associated with bladder, breast, lung, and renal cancer. Ovarian cancer tissues also have shortened telomeres and increased telomerase activity, suggesting that telomere abnormalities may be related to ovarian cancer.

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Methods—We investigated leukocyte telomere length in 99 women with serous ovarian adenocarcinoma and 100 age-matched cancer-free controls enrolled in a population-based case–control study.

Results—Cases tended to have shorter telomeres than controls ($P_{wilcoxon}$ = 0.002). Compared to subjects with telomere lengths in the longest tertile, those in the middle and shortest tertiles showed respective age-adjusted odds ratios (95% confidence intervals) of 2.69 (1.23–5.88) and 3.39 (1.54–7.46) (P_{trend} = 0.002). Strongest associations were found for subjects with poorly differentiated carcinomas (OR = 4.89, 95% CI 1.93–12.34).

Conclusions—This study shows that short leukocyte telomeres are associated with serous ovarian adenocarcinoma. These findings should be confirmed in large, prospective studies.

Keywords

Ovarian cancer; Telomere length; Case–control study; Epidemiology

Introduction

Telomeres consist of nucleotide repeats $(TTAGGG)_n$ and protein complexes that help maintain genomic structural integrity by protecting chromosome ends from degradation, end-to-end fusion, and atypical recombination [1]. Telomeric repeats progressively shorten with each cell division, and potentially contribute to genetic instability and risk of malignant change [2, 3]. Telomere shortening is a characteristic of aging, and might partly underlie the rising incidence of cancer with aging [4]. There is growing evidence that short telomeres are associated with the initiation and progression of cancer [5–7].

Shorter telomeres, in blood or buccal cell DNA, have been associated with bladder, breast, head and neck, lung, and renal cell cancers [8–12]. Several studies have also demonstrated that tumor cells and their precursor lesions have shorter telomeres than surrounding non-malignant cells [5, 13, 14]. A small case–control study (32 cases, 45 controls) found that telomere length (TL) in plasma-derived free DNA was significantly shorter in ovarian cancer patients than in healthy controls, and was correlated with TL in ovarian tumor tissue [15]. Telomerase activity and shorter telomeres have been found in ovarian cancer tissue, with the highest telomerase activity detected in poorly differentiated tumors [16–18]. Therefore, we hypothesized that shorter TL in DNA of peripheral leukocytes may be associated with ovarian cancer.

Materials and methods

Study population

The Polish Ovarian Cancer Study is a population-based case–control study of incident ovarian cancer among woman age 20–74 years residing in two cities in Poland (Warsaw and Lodz), as described elsewhere [19]. The main study has 341 cases and 1,994 controls; 78 and 69% of eligible cases and controls agreed to participate. All study participants were of Polish Caucasian origin. The study protocol was reviewed and approved by local and NCI Institutional Review Boards; all study participants provided written informed consent. All participants underwent a detailed interview to assess known or suspected ovarian cancer risk factors. The time from diagnosis to interview and sample collection ranged from 5 days to 1 year (median: 2.4 months). Cases were women with newly diagnosed and histologically confirmed ovarian carcinoma or a borderline tumor identified during 2001–2004. Controls were selected from population lists of women without ovarian cancer or bilateral oophorectomy (n = 100), and were frequency matched to cases (n = 99) on age (5-year groups) and study site. To reduce potential etiologic and biological heterogeneity, cases in

this study were restricted to serous adenocarcinoma. Thirty-seven cases had chemotherapy 8 days to 11 months prior to blood collection, with the majority (60%) receiving treatment less than 3 months prior to sample collection.

Telomere length measurement

Genomic DNA was extracted from buffy coat fractions by standard procedures (Gentra Autopure). Relative TL was measured using a multiplexed quantitative polymerase chain reaction (Q-PCR) method previously described [20]. Briefly, the average, relative TL was estimated from the ratio of the telomere (*T*) repeat copy number to a single gene copy number (human β-globin gene; *S*), expressed as the *T*/*S* ratio for each sample using standard curves. All PCR reactions were performed on the Bio-Rad MyiQ Single Color Real-Time PCR detection system. TL measurement by the terminal restriction fragment (TRF) method is the current standard to which other methods are compared and includes the subtelomeric sequence. The comparable TRF TL in base-pairs (bp) for a *T*/*S* ratio of 1.0 is approximately 7.06 kb based on the updated Cawthon method [20]. If the subtelomeric region is excluded, a *T*/*S* of 1 is approximately 3.33 kb [20]. Ten blinded quality control samples were included to assess variability, and each sample was run in triplicate. The coefficient of variation for repeats was 6.40%.

Statistical analysis

Telomere length was analyzed as a continuous and as a categorical variable. The Wilcoxon rank-sum test was used to compare TL among case and controls as a continuous variable. Unconditional logistic regression was used to obtain the odds ratio (OR) and 95% confidence intervals (CI) for the strength of the association between ovarian cancer and TL, adjusting for age. TL tertile and 50th percentile values were determined based on the distribution in control subjects. The relationship of TL to case–control status was also assessed using multivariate models to investigate possible interactions with the following ovarian cancer risk factors: number of pregnancies, family history of ovarian or breast cancer, lifetime ovulatory cycles (LOC; calculated as described [21]), use of hormone replacement therapy (HRT) and oral contraceptives (OC). Spearman rank correlations and general linear models adjusted for age (except the age variable) were used to investigate associations between TL and age, smoking status, and ovarian cancer risk factors (see list above). All statistical tests were calculated using SAS software 9.1 (SAS Institute, Cary, NC).

Results

The characteristics of the cases and matched controls are shown in Table 1. The mean age of both cases and controls was 56 years. TL was inversely correlated with increasing age in the control population, as anticipated, confirming data quality. Prior studies of TL and smoking have been inconsistent; we found no association between TL and smoking status in controls (data not shown). TL was significantly shorter in patients with poor tumor grade compared to those with well or moderate tumor grade. Thirty-seven cases received chemotherapy prior to blood draw, however, TL was not different between ovarian cancer cases who had or had not received chemotherapy (unadjusted median TL for those with no chemotherapy = 0.92 vs. 0.90 for those that had chemotherapy; $P = 0.90$; Table 1). There were no statistically significant associations between individual ovarian cancer risk factor variables (number of pregnancies, family history, LOC, HRT, or OC usage) and ovarian cancer, possibly due to sample size and/or unique features of the Polish study [19]. These variables were not associated with TL (Table 1).

Telomere length was significantly shorter in ovarian cancer patients (age-adjusted *T*/*S* ratio 0.91, 95% CI 0.86–0.95) than in the controls (1.01, 95% CI 0.97–1.06) ($P_{wilcoxon}$ = 0.002), corresponding to a 700 bp deficit in cases. After categorization of subjects into TL tertiles, based on the TL distribution among controls, unconditional logistic regression models adjusting for age found that ovarian cancer risk was highest in individuals with the shortest telomeres. Using the longest tertile as the referent, the OR was 2.69 (95% CI 1.23–5.88) for the middle tertile and 3.39 (95% CI 1.54–7.46) for the shortest tertile (P_{trend} = 0.002; Table 2). The association between short TL and ovarian cancer risk remained significant after adjustment for traditional ovarian cancer risk factors (TL dichotomized at the median: OR 3.00, 95% CI 1.55–5.79). To exclude the potential affect of chemotherapy on TL, we investigated this relationship in only those cases that had not received chemotherapy prior to blood collection, and also observed a similar association (TL dichotomized at the median: OR 3.36, 95% CI 1.34–8.39).

We investigated the effect of shorter leukocyte telomeres on the association with ovarian cancer after stratifying the patients according to tumor grade of differentiation; the increased risk of ovarian cancer was only evident among patients with poorly differentiated tumors (OR adjusted = 4.89, 95% CI 1.93–12.34) (Table 3). A significant interaction was also observed between TL and tumor grade in the multivariate model (P = 0.02). Individuals with stage I/II and III tumors both had shorter telomeres associated with ovarian cancer, but this was statistically significant only in those with stage III tumors. This may be due to the higher percentage of these tumors being poorly differentiated (68 vs. 53%; data not shown).

To explore possible effect modifiers of the association between TL and ovarian cancer, we performed analyses stratified by ovarian cancer risk factors (Table 3). Significant associations between shorter median TL and ovarian cancer were also found among never users of HRT and those with greater LOC. Earlier age at first menarche and later age of menopause were also associated with shorter TL (data not shown). It is possible that these findings are an artifact of the small sample size. Shorter TL was associated with ovarian cancer in both young and older subjects (data not shown). There were no significant interactions between these variables and TL (data not shown).

Discussion

We found that leukocyte TL was significantly shorter among patients with poorly differentiated serous ovarian cancer than among unaffected controls and that the strength of association was inversely related to TL. This association was only observed in those with poorly differentiated tumors and could reflect hypothesized differences in the biology of low- versus high-grade serous carcinomas or differences in the systemic effects of these tumors (reviewed in [22]). This hypothesis suggests that there are two distinct pathogenic mechanisms in ovarian cancer; one mechanism leads to low-grade serous carcinomas and the other leads to high-grade ones [23]. Our findings lend some support to the presence of different molecular pathways of carcinogenesis, although small sample size limits further interpretation of these results. The age-adjusted TL was approximately 700 bp lower in our ovarian cancer cases than in controls. A recent longitudinal study found an average rate of leukocyte TL loss of 41 bp/year in healthy individuals [24]; this potentially demonstrates accelerated telomere erosion in our cases. In conjunction with prior studies showing that telomeres are shorter and telomerase activity is increased in malignant epithelial ovarian tumor tissue [16–18], our data suggest that telomere dysfunction may be associated with high-grade serous ovarian carcinoma risk. Although we were unable to assess TL in ovarian or ovarian cancer tissues, one small study suggested they may be correlated [15].

This is the first population-based study to evaluate associations between leukocyte TL and ovarian cancer but its case–control design is a limitation. There is the potential for reverse causation bias whereby the presence of cancer might impact leukocyte TL. There is some evidence that chemotherapy can alter TL [25, 26], and although we did not observe a TL difference between those that received and had not received chemotherapy, we cannot exclude the possibility of an effect. Small sample size is also a potential limitation. However, we had at least 80% statistical power to detect an OR of 1.65 and 2.72 with TL in the second and first tertiles (assuming a type I error of 0.05), respectively, providing us adequate power to detect our ORs of 2.69 and 3.39, respectively.

In summary, our findings suggest that leukocyte TL is shorter in patients with ovarian cancer, especially in patients with poorly differentiated tumors. Larger, prospective studies which include pre- and post-diagnosis leukocyte TL are required to fully understand the implications of these findings.

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Table 1

Distribution of select study subjects' characteristics and associations with relative leukocyte telomere length

Variables‡	Controls		Ovarian cancer patients	
	No. (%)	Telomere length† (95% CI)	No. (%)	Telomere length† (95% CI)
Overall	100 (50.2)	1.01 (0.97–1.06)	99 (49.8)	0.91 (0.86–0.95)
*P**				0.001
Age				
≤55	50 (50.0)	1.06 (0.99–1.13)	48 (48.5)	0.95 (0.88–1.01)
>55	50 (50.0)	0.97 (0.90–1.04)	51 (51.5)	0.87 (0.81–0.93)
r§ (*P* value)		−0.23 (0.02)		−0.21 (0.03)
Oral contraceptive use				
Never	89 (89.9)	1.03 (0.98–1.08)	89 (91.8)	0.91 (0.87–0.96)
Ever	10 (10.1)	0.86 (0.69–1.03)	8 (8.2)	0.83 (0.68–0.99)
P¶		0.06		0.34
Ever used hormone replacement therapy				
No	78 (79.6)	1.02 (0.97–1.08)	75 (76.5)	0.91 (0.86–0.96)
Yes	20 (20.4)	0.99 (0.88–1.10)	23 (23.5)	0.89 (0.79–0.98)
P		0.64		0.71
Lifetime ovulatory cycles				
<369	30 (32.6)	0.99 (0.90–1.09)	20 (21.1)	0.93 (0.82–1.04)
369–419	31 (33.7)	0.95 (0.86–1.03)	35 (36.8)	0.92 (0.85–0.99)
>419	31 (33.7)	1.10 (1.01–1.19)	40 (42.1)	0.88 (0.80–0.95)
r (*P* value)		0.12 (0.24)		−0.11 (0.28)
Family history of breast and/or ovarian cancer in first-degree relatives				
No	86 (86.0)	1.01 (0.96–1.07)	78 (78.8)	0.91 (0.86–0.96)
Yes	14 (14.0)	1.02 (0.89–1.15)	21 (21.2)	0.89 (0.79–0.99)
P		0.88		0.74
Tumor grade of differentiation				
Well/moderate[a]		–	20 (33.3)	1.03 (0.93–1.12)
Poor		–	40 (66.7)	0.86 (0.79–0.92)
P				0.005
Tumor stage				
I/II[b]		–	18 (26.9)	0.89 (0.79–1.00)
III		–	49 (73.1)	0.91 (0.85–0.98)
P				0.72
Chemotherapy prior to sample collection				
No		–	34 (34.3)	0.91 (0.82–0.99)
Yes		–	37 (37.4)	0.89 (0.81–0.97)
Unknown		–	28 (28.3)	0.92 (0.84–0.99)
P				0.90¥

‡Not all variable information was available for all subjects

† Telomere length refers to *T*/*S* ratio (95% confidence intervals) adjusted for age (except the age variables)

§ Spearman rank correlations for continuous variables adjusted for age (except the age variables)

* *P* value for the TL difference between cases and controls using a general linear model adjusting for age

¶ *P* value for the association with TL for categorical variables using general linear models adjusting for age

¥ *P* value for TL difference between patients with and without chemotherapy treatment

a Since there were only three patients with well differentiated tumors and their TL's did not significantly differ they were combined with those with moderately differentiated tumors

b Since there were only 2 stage II patients and their TL's did not significantly differ they were combined with the stage I's

Table 2

Association of ovarian cancer with relative leukocyte telomere length

Telomere status	Controls *n* (%)	Cases *n* (%)	OR[a] (95% CI)	P_{trend}
Categorized by tertile in controls				
1st (short)	33 (33.0)	45 (45.5)	3.39 (1.54–7.46)	0.002
2nd (medium)	34 (34.0)	40 (40.4)	2.69 (1.23–5.88)	
3rd (long)	33 (33.0)	14 (14.1)	1.00 (ref)	

[a]Odds ratios (95% confidence intervals) adjusted for age; *n* = number of subjects

Table 3

Association of ovarian cancer with relative leukocyte telomere length for selected variables

Telomere status	Controls *n* (%)	Cases *n* (%)	OR[†] (95% CI)	*P* value
Well/moderate tumor grade of differentiation				
Short[a]	50 (50.0)	8 (40.0)	0.82 (0.29–2.28)	0.696
Long	50 (50.0)	12 (60.0)	1.0 (ref)	
Poor tumor grade of differentiation				
Short	50 (50.0)	33 (82.5)	4.89 (1.93–12.34)	<0.001
Long	50 (50.0)	7 (17.5)	1.0 (ref)	
Never used HRT				
Short	39 (50.0)	55 (73.3)	2.70 (1.36–5.38)	0.005
Long	39 (50.0)	20 (26.7)	1.0 (ref)	
Ever used HRT				
Short	10 (50.0)	15 (65.2)	2.25 (0.55–9.15)	0.259
Long	10 (50.0)	8 (34.8)	1.0 (ref)	
LOC[b] ≤ 419				
Short	30 (49.2)	32 (58.2)	1.49 (0.70–3.16)	0.298
Long	31 (50.8)	23 (41.8)	1.0 (ref)	
LOC > 419				
Short	16 (51.6)	31 (77.5)	4.45 (1.42–13.94)	0.010
Long	15 (48.4)	9 (22.5)	1.0 (ref)	

[†] Odds ratios (95% confidence intervals) and corresponding *P* values adjusted for age; *n* = number of subjects

[a] Telomere length dichotomized at the median based on the distribution in the controls

[b] The first and second tertiles of lifetime ovulatory cycles were combined