Circulating prostate-specific antigen and telomere length in a nationally representative sample of men without history of prostate cancer

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ABSTRACT

Background: We investigated the association of prostate-specific antigen (PSA) with leukocyte telomere length, which may be altered in preclinical prostate malignancies.

Methods: This study was based on the 2001-2002 U.S. National Health and Nutrition Examination Survey (NHANES). A subsample of 1,127 men aged 40-85 years without prior history of prostate cancer who provided informed consent and blood samples were selected. Leukocyte telomere length (LTL) relative to standard DNA reference (T/S ratio) was quantified by polymerase chain reaction (PCR). Survey-weighted multivariable linear regression was performed to examine T/S ratio across quintiles of total and free PSA and free-to-total PSA ratio (%fPSA). A sensitivity analysis was performed by excluding men dying from prostate cancer during follow-up through to 31 December 2006. Stratification analyses were carried out to assess any effect modification by age group, race, body mass index (BMI) and levels of C-reactive protein (CRP), a marker of inflammation.

Results: Higher total PSA levels were associated to longer LTL, with approximately 8% increase in log-transformed T/S ratio (95% confidence interval [CI]: 2-13%) among men in the highest quintile of total PSA compared to the lowest in the fully adjusted model (P_{trend}=0.01). No significant association was found for free PSA or %fPSA, although nonlinearity between all PSA measures and T/S ratio was indicated. Similar results were found after excluding men who died from prostate cancer during follow-up. We also found the associations between total PSA and T/S ratio to be strongest among non-Hispanic blacks, non-obese men (BMI <30 kg/m^2), and those with low CRP. However, a significant interaction was only found between total PSA and race/ethnicity ($P_{interaction}$=0.01).

Conclusion: Total PSA levels were strongly associated to leukocyte telomere length, particularly among non-Hispanic blacks. Our findings support a potential link between PSA and specific mechanisms contributing to prostate cancer development.

Background

Telomere shortening is known as a marker for biological ageing [1]. However, there is indication that changes in telomere length may be associated with carcinogenesis [2]. For prostate cancer, prior studies have shown alterations of telomerase activity in prostate cancer compared to normal prostate tissue [3]. Furthermore, pre-diagnostic telomere length has been associated to risk of prostate cancer [4]. In the context of benign prostate disease, telomerase activity has been reported to be increased in benign prostatic hyperplasia (BPH), but the extent differed by cell biology [5]. These findings point towards associations between altered telomere length and prostate carcinogenesis which may correspond to specific biological mechanisms.

Since prostate cancer is the most common cancer and a leading cause of cancer-related deaths among men [6], identification of early diseases and population at high risk is crucial. Prostate-specific antigen (PSA) is a useful tumor marker for prostate cancer and has been widely used as a screening tool for the disease. Lower prostate cancer mortality has been reported among men diagnosed through PSA-based screening [7]. However, the risk of over-diagnosis hampers the benefits of screening programmes for prostate cancer [8]. Knowledge of how PSA is associated with specific mechanisms contributing to prostate carcinogenesis, such as alteration of telomere length, may allow refinement of future screening approaches. To date, there is still a lack of evidence regarding the association between PSA and telomere length in the general population. Therefore, we sought to investigate the association of circulating prostate-specific antigen (PSA) with leukocyte telomere length (LTL) among a nationally representative population of men without prior diagnosis of prostate cancer. In addition to the non-specific total PSA (tPSA), we also assessed free PSA and the ratio of

free-to-total PSA [9], and took into account prostate cancer death which shortly followed index measurements.

Methods

Study population

The National Health and Nutrition Examination Survey (NHANES) is a cross-sectional health survey conducted by the National Center for Health Statistics (NCHS) in representative samples of the non-institutionalized U.S. population [10]. Participants were selected through multi-stage stratified, clustered probability sampling. The survey included an interview conducted at home and an extensive physical examination, which included a blood sample taken in a mobile examination center (MEC). This study was based on the continuous NHANES 2001–2002, which included over 11,000 participants. Among this population, serum PSA was measured in 1,380 men aged 40 years and above who consented and who did not have any history of cancer diagnosis, recent biopsy, rectal examination, cystoscopy, or current infection or inflammation of the prostate. We further selected those with available information on telomere length (N=1,275) and excluded those without complete data on other covariates: poverty-to-income ratio, education level, height, weight, smoking status and levels of C-reactive protein (N=1,127). Linkage with mortality follow-up through to 31 December 2006 provided information on those dying from prostate cancer. Identification of prostate cancer mortality was performed based on the International Classification of Diseases, 10^{th} revision (ICD-10 code: C61) [11].

Telomere length assay

The telomere length assay was performed in the laboratory of Dr. Elizabeth Blackburn at the University of California, San Francisco, using the quantitative polymerase chain reaction

(qPCR) , as described in detail elsewhere [12, 13]. Briefly, for each DNA sample, the factor by which the sample differed from a reference DNA sample in its ratio of telomere repeat copy number to single gene copy number was calculated. This ratio (T/S ratio) should be proportional to the average telomere length [13]. Each sample was assayed three times on three different days. The samples were assayed on duplicate wells, resulting in six data points. Sample plates were assayed in groups of three plates, and no two plates were grouped together more than once. Assay runs with eight or more invalid control wells were excluded from further analysis (<1% of runs) [10, 14]. Control DNA values were used to normalize between-run variability. Runs with more than four control DNA values falling outside 2.5 standard deviations from the mean for all assay runs were excluded from further analysis (<6% of runs). For each sample, any potential outliers were identified and excluded from the calculations (<2% of samples). The mean and standard deviation of the T/S ratio were then calculated normally. The interassay coefficient of variation was 6.5%. The amplification efficiency for reference gene and telomere were 0.968 ± 0.027 and 0.904 ± 0.026, respectively. The Centers for Disease Control and Prevention (CDC) conducted a quality control review before linking the telomere data to the NHANES public data files. The quality control protocol is available at http://www.cdc.gov/nchs/data/nhanes/genetics/Quality_Control_Public.pdf.

PSA assay

Serum PSA concentration (ng/mL) was measured using the Beckman Access Immunoassay System with the Hybritech Total PSA Assay (Beckman Coulter, Fullerton, CA) [15]. Free PSA levels (ng/mL) were measured by a two-site immunoenzymatic "sandwich" assay with the Access Hybritech (Beckman Coulter, Fullerton, CA). We categorised men based on quintiles of total and free PSA, and additionally dichotomised them based on the following clinical cut-off points of total PSA: 2.5, 3.0, and 4.0 ng/mL [15]. A ratio of free to total PSA

(%fPSA) was calculated by dividing the free PSA by total PSA. We categorised %fPSA into five groups based on rounded up values of its quintiles.

Other covariates

Age at measurements was grouped into 40–50, 50–60, 60–70 and 70 years and older. Race/ethnicity was categorised into non-Hispanic white, non-Hispanic black, Mexican-American, and other. We classified educational attainment as less than high school, high school equivalent, and higher than high school. Socioeconomic status (SES) was estimated with poverty-to-income ratio (PIR), a ratio of total family income to the official poverty threshold at the family level. A PIR less than 1 indicated that income was less than the level of poverty. We categorised PIR into <1, 1–2, and ≥2, indicating lowest to highest SES, as previously described [16]. To assess smoking status, participants were asked whether they had smoked at least 100 cigarettes in their entire life, and those who responded positively were asked whether they now smoke cigarettes every day, some days, or not at all. We defined current smokers as those who had smoked at least 100 cigarettes during their lifetime and, at the time of the interview, reported smoking either every day or some days. Former smokers were those who reported smoking at least 100 cigarettes during their lifetime but currently did not smoke. Never smokers were those who had not smoked 100 cigarettes during their lifetime. Body mass index (BMI) was calculated from weight and height. Weight was measured with an electronic weight scale in pounds and automatically converted to kilograms. Participants only wore underwear, disposable paper gowns and foam rubber slippers. Standing height was measured with a fixed stadiometer to the nearest 1 mm. We used BMI to classify participants as non-obese (<30 kg/m^2) and obese (≥30 kg/m^2) [17]. C-reactive protein, an indicator of systemic inflammation, was assessed by latex-enhanced nephelometry and categorised into <10 and ≥10 mg/L, the latter indicating clinical

inflammation [18]. Testosterone levels were available in 181 participants. Levels of testosterone was measured by electrochemiluminescence immunoassays on the Elecsys 2010 autoanalyzer (Roche Diagnostics, Indian-apolis, IN).

Statistical analysis

We estimated proportions of participant characteristics and corresponding mean LTL with the NHANES 2001–2002 sampling weights for genetic data subsample. LTL (T/S ratio) had a skewed distribution; therefore, it was logarithmically transformed in the analysis. To investigate the association between PSA and telomere length, we first used linear regression models adjusted for age as a continuous variable and race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, and other) to estimate mean LTL across quintiles of total and free PSA and categories of %fPSA. Quintiles of PSA variables were assigned as an ordinal variable to obtain a P-value for trend. Analyses were repeated while adjusting for participant PIR (<1, 1–2, ≥2) and education attainment (less than high school, high school equivalent, and higher than high school). Several lifestyle factors such as cigarette smoking and obesity and inflammation have been suggested to alter both PSA levels and telomere length [12, 19–22]. Therefore, we further adjusted our multivariable model for smoking status (current, former, never smokers) BMI categories (<30 kg/m^2, ≥30 kg/m^2) and levels of CRP (<10 mg/L, ≥10 mg/L). Similar fully adjusted regression analyses were performed using clinical categories of PSA (low, high) based on the cut-off points 2.5, 3.0, and 4.0 ng/mL, with the lower levels being the referent categories. Additionally, we performed multivariable logistic regression analyses assessing telomere length as a dichotomous outcome using its 25th percentile, 0.84, as the cut-off point. Odds ratio (OR) and their 95% confidence intervals for LTL below the 25th percentile were computed for PSA categories using higher LTL values as the referent category. In a sensitivity analysis, we excluded men who died of

prostate cancer during follow-up through to 2006 to account for fatal prostate cancers which may exhibit distinct profiles of PSA and telomerase activity [4, 23]. Due to the suggested association between testosterone and PSA [15], a subgroup analysis only including men with available testosterone measurement was conducted with further adjustment for testosterone levels.

To further investigate the association between PSA and telomere length, we used restricted cubic splines to estimate and visualise mean LTL changes with levels of total and free PSA and %fPSA. Relationships between PSA variables as predictors and LTL were plotted using four knots arbitrarily located at the 0.05, 0.35, 0.65, and 0.95 percentiles of each predictor. The models were adjusted for age (continuous), race/ethnicity, PIR categories, education level, smoking status, BMI and CRP clinical categories.

Finally, to assess any effect modification by participant characteristics, the association between quintiles of total PSA and LTL was stratified by age categories, race/ethnicity, PIR categories, education level, smoking status, BMI and CRP clinical categories, adjusting for all covariates except when they were used as strata. All analyses were conducted with SAS release 9.3 (SAS Institute, Cary, NC) and R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria). Restricted cubic splines were performed using the RCS_Reg SAS Macro created by Desquilbet and Mariotti [24].

Results

Mean age of participants was 53 years. The weighted mean values and standard error of total and free PSA and %PSA were 1.46 ± 0.06 ng/mL, 0.35 ± 0.01 ng/mL and 29.68 ± 0.63%, respectively. Among men with available testosterone levels, weighted mean testosterone was

4.53 ± 0.19. As shown in Table 1, the SES of the majority of men was at least double the national poverty level and most men attended higher education. From the mortality follow-up through to 2006, we identified 5 (0.4%) men who died from prostate cancer during a mean follow-up time of 4.8 ± 0.8 years.

Telomere length decreased with age and increased with education level, and was shortest among Mexican-Americans and those with middle levels of income. No difference was found between smokers and non-smokers (former smokers and those who had never smoked, combined) or between men who were obese (BMI 30 kg/m^2 and over) and non-obese men.

When assessing log-transformed LTL by quintiles of total and free PSA and %fPSA, we found that higher levels of total PSA were associated with longer telomere length, with an 8% increase in log-transformed T/S ratio (2–13%) among men with total PSA 2.3 ng/mL or higher, compared to those having PSA <0.5 ng/mL in the age- and race-adjusted model (P_{trend}=0.01). Further adjustments with PIR, education level, BMI, smoking status and level of CRP did not alter these results (Table 2). No statistically significant trend was observed between free PSA and %fPSA. Nevertheless, men in the third quintile of PSA (0.34–0.54 ng.mL) had marginally longer telomeres compared to those in the lowest quintile (<0.15 ng/mL) in the age- and race- adjusted model, with a 4% higher log-transformed T/S ratio (0.8–7%). This association became weaker with adjustments for SES, education, obesity and smoking status (P=0.06). Patterns of association were similar when we used LTL lower than its 25^{th} percentile as the outcome of interest. For instance, there was a 57% reduced chance of having LTL below its 25^{th} percentile (95% CI: 0.24–0.75) with the highest compared to the lowest quintiles of total PSA (Table 2). No association was observed between total PSA and LTL when we used dichotomous values of total PSA based on clinical cut-offs (2.5, 3.0, and

4.0 ng/mL; results not shown). Findings remained similar in a sensitivity analysis excluding men who died from prostate cancer during follow-up (results not shown). Among 181 men with testosterone measurement, associations were similar but weaker, reflecting a lack of statistical power. For instance, there was a 4% decrease in LTL (-0.2– 0.08) with increasing log total PSA despite a significant trend with total PSA quartiles (P=0.03). Adjustment by testosterone levels did not alter associations between PSA measures and LTL (results not shown).

To confirm the association between PSA variables and LTL, we plotted estimates of mean difference in LTL given levels of total and free PSA and %fPSA as depicted in Figure 1. Similar to results from regression analysis with PSA quintiles, a non-linear association was suggested particularly with free PSA and %fPSA. For total PSA, longer telomere length corresponded to higher total PSA, until reaching a plateau when total PSA approached its 95^{th} percentile indicated by the fourth knot. Parabolic and J-shape associations were suggested for free PSA and PSA. However, 95% confidence intervals indicated that the difference in LTL across their levels was not statistically significant.

We further characterised the association between circulating total PSA by observing any effect modification by age, race/ethnicity, PIR, education level, obesity, smoking status, and CRP levels. When stratifying by age, we only observed a borderline positive association between PSA and telomere length among those aged 50 to 60 years (P_{trend}=0.05). Race/ethnicity stratification revealed a strong positive association between total PSA and telomere length among non-Hispanic blacks, with a 15% increase in log-transformed T/S ratio (2–27%) among men in the highest compared to the lowest total PSA quintile. Similar positive trends between telomere length and total PSA were also observed among men in the

lowest category of PIR (P_{trend}=0.02), those with education levels less or higher than high school (P_{trend}=0.007 and 0.03, respectively), non-obese men (P_{trend}=0.02), and never smokers (P_{trend}=0.03), with non-statistically significant findings among other categories. For CRP levels, both men in low and clinically high (10mg/L or higher) categories of CRP had longer telomere with increasing total PSA quintiles (P_{trend}<0.05). Nevertheless, tests for interaction only showed statistically significant interaction between total PSA quintiles and race/ethnicity ($P_{interaction}$=0.01).

Discussion

We found a positive association between total PSA levels and telomere length, measured as log-transformed and categorised T/S ratio, among a nationally representative population of men without any history of prostate cancer. No association was identified between free PSA or %fPSA and LTL, or when using clinical cut-offs of total PSA. Further explaining this lack of trends, non-linear associations were suggested from spline analysis, in particular with free PSA and %fPSA. In stratification analysis, race/ethnicity was identified as an important effect modifier, with strong inverse associations between quintiles of total PSA and LTL among non-Hispanic blacks and other race/ethnicity, a borderline trend among Mexican American, and a lack of association among non-Hispanic white men.

There are several plausible biological explanations that may explain how circulating PSA may be associated with telomere length. PSA is a kallikrein-like, serine protease secreted exclusively by the epithelial cells of prostatic tissue [25]. PSA secretion increased in presence of dihydrotestosterone (DHT) [26], and its gene expression is upregulated by the androgen receptor (AR) [27], a key player in the majority of prostate malignancies [28]. The role of telomeres in prostate cancer has also been shown, with longer telomeres suggested to prevent

genomic instability and short, dysfunctional telomeres found in prostate cancers [29, 30]. In addition to prostate cancer, BPH and high-grade prostatic intraepithelial neoplasia (PIN) also showed a high level of telomere gene fusions, indicating that the role of telomere dysfunction is not limited to malignant disease [31]. Furthermore, telomerase reactivation may follow telomere dysfunction, contributing to cell immortalisation and malignant progression [32]. Like PSA, telomerase activity has been linked to androgens, with increasing telomere length corresponding to higher DHT levels in men [33]. Correspondingly, experimental studies showed that prostate cancer cells treated with AR-antagonist exhibit signs of telomere dysfunction, which was reversed following treatment washout [34]. Finally, inhibition of prostate cancer cell proliferation by silibinin, a flavonoid agent, was shown to result in decreased telomerase activity, PSA mRNA expression and PSA secretion *in vitro* [35]. Despite the plausible link with regards to telomere length in prostate tissue, it is unclear how circulating LTL may be linked to PSA. Indirect associations, driven by circulating androgens, may have occurred given a recent report of LTL increase in response to treatment with synthetic sex hormone, Danazol [36], However, our findings were robust to adjustment for testosterone levels, indicating a more complex association between PSA metabolism and telomere length.

We found evidence for non-linear associations between PSA measures and telomere length, which was most prominent for %fPSA. The differential role of PSA measures may explain our findings. Total PSA levels represent the quantity of PSA secretion, whilst %fPSA has been suggested to be a superior biomarker in detecting prostate cancer compared to both total and free PSA individually [9]. Therefore, the observed strong positive associations between total PSA and telomere length in our study may indicate the common regulatory pathways between PSA and telomerase activity, for instance involving androgens [28, 30]. On the other

hand, the non-linear association between %fPSA and telomere length may represent the complex biological interplay between telomere dysfunction, telomerase reactivation, and clinically detectable prostate cancer. Corroborating the latter, a lack of conclusive findings linking telomere length and risk of prostate cancer has been shown in population studies [4, 37–40]. Our results also support strong effect modification in the association between total PSA and telomere length by race/ethnicity. PSA levels are known to be higher among African American compared to Caucasian men, which may be partly attributed to different rates of PSA metabolism [41]. Our findings further underline the notion that the use of PSA screening requires understanding of how PSA is linked to specific tumour-promoting pathways such as telomere length alteration, which may be able to explain the well-known variation in clinical course and prognosis of the disease by race/ethnicity [23].

Although a consensus exists that PSA screening reduces death from prostate cancer [7, 8, 42], more studies and better refinement of screening methods are necessary to determine the cost-benefit. Several strategies have been proposed to increase the benefit of PSA screening, including refinement of current methods to enable distinction between low-risk and aggressive or fatal cancers. Telomerase activity in prostate tissue has been reported to be positively correlated with Gleason score [43], indicating a link between telomere length and prostate cancer severity. In observational settings, the odds of developing high-grade and fatal prostate cancer were shown to double (OR: 2.04, 95% CI: 1.00–4.17 and 2.37, 95% CI: 1.19–4.72 for high grade and fatal prostate cancer, respectively) with every standard deviation increase in LTL among men with a family history of prostate cancer [4]. Given all this evidence, the relevance of circulating telomere length to subclinical prostate neoplastic processes needs to be explored in refining the predictive value of PSA for prostate cancer.

To our knowledge, this is the first study demonstrating an association between circulating total PSA and telomere length in the population. The NHANES is a representative sample of the US population, and men included in the present study comprised those without prostate cancer or acute prostate disease or procedures which may have affected PSA levels. We were able to take into account potential confounders including lifestyle risk factors and inflammation which have been reported to be implicated in both prostate disease and ageing. A limitation of this study is that PSA and telomere length were measured cross-sectionally. Therefore, our findings only implied association rather than any causality. Telomere length was measured in total leukocytes in NHANES. Given previous indications that telomere length may differ across types of haematological cells [44], such variability may affect the associations observed. Although the selection of older men represented those at higher risk for prostate cancer, this may reduce the generalisability of our results to the general population. Although mortality follow-up was available, there was no information on prostate cancer incidence. We used prostate cancer death as a surrogate for those diagnosed with more aggressive or fatal prostate cancer, however, this may underestimate the actual burden of prostate cancer given the long latency period and survivorship of the disease [45, 46]. Furthermore, mortality data in the NHANES is obtained through probabilistic matching [11], which may have resulted in misclassification and bias towards the null. Although total PSA levels were suggested to be associated with telomere length in this study, there is no current consensus about any threshold of circulating LTL which is clinically meaningful for prostate cancer diagnosis or screening. Investigation into the predictive role of PSA in prostate cancer while taking into account its association with other circulating biomarkers such as telomere length is therefore a potential avenue to explore in future studies.

Conclusion

Total PSA levels were positively associated with LTL in our study, indicating a predictive role of total PSA for telomere length. The observed association and levelling off may indicate the importance of quantifying the dynamic between PSA and specific biological mechanisms with potential implications in prostate cancer.

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Figure Legends

Figure 1. Mean leukocyte telomere length (LTL) as T/S ratio by quintiles of total PSA, free PSA, and free-to-total PSA.

Figure 2. Dose-response association between PSA and leukocyte telomere length (LTL). LTL was measured as log-transformed T/S ratio and coded using an restricted cubic spline function with four knots arbitrarily located at the 0.05, 0.35, 0.65, and 0.95 percentile. Y-axis represents the difference in LTL for any increase in (a) total PSA (tPSA), (b) free PSA (fPSA), or (c) free-to-total PSA (ftPSA). All models were adjusted for age (continuous), race/ethnicity, PIR categories, education level, BMI (continuous), smoking status, and CRP levels.