Original Article

The association between relative leukocyte telomere length of prostate cancer patients at diagnosis with cancer prognostic parameters

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Leukocyte telomere length, prostate cancer, castration resistance, metastasis, survival

Abstract

Objectives: In this study, relative leukocyte telomere length (LTL) was investigated as a prognostic marker to evaluate association of LTL at the time of diagnosis and prostate cancer-specific survival, metastasis-free survival, overall survival, with castrate-resistant prostate cancer (CRPC).

Materials and Methods: In this retrospective cohort study, pertinent data from 81 patients were collected. Patients underwent prostate cancer (PCa) treatment procedures determined by staging and current recommendation. Blood samples from suspected PCa patients were obtained before the initiation of diagnosis and treatment. LTL was determined by the quantitative polymerase chain reaction method. Relative LTL was compared to the main clinical outcome measures. Prostate cancer-specific survival, metastasis-free survival, overall survival and CRPC were calculated retrospectively, for a mean follow-up period of 30 months.

Results: This analysis showed relative LTL was not associated with tumor stage, Gleason score, grade group, metabolic disease, or smoking. However, older age was significantly associated with short LTL ($p < 0.001$). All main outcomes were not associated with LTL. In contrast, a subgroup analysis of patients who underwent primary androgen deprivation therapy (ADT) showed a CRPC association with relatively long LTL ($p = 0.039$). To our knowledge, these results are novel and give further strength to our hypothesis that relative LTL might be used as a prognostic marker in PCa especially in patients who will receive primary ADT.

Conclusion: Aging was significantly associated with relatively short LTL. There was no significant association between LTL in PCa patients at diagnosis and cancer-specific survival, metastasis-free survival, or overall survival. However, patients who underwent ADT treatment alone showed CRPC associated with relatively long LTL.

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Introduction

At present, aging is an important risk factor in relation to disease development. Malignancy, degenerative diseases, metabolic syndrome and cardiovascular disease are associated with advanced age. The length of telomere is a known marker of biological aging[1]. Metabolic diseases, cardiovascular diseases, and heavy smoking are also associated with shortened telomere length[2-5].

Current research has produced varying results regarding relative leukocyte telomere length (LTL). One study showed that telomere length of prostatic cancer cells was shorter than in normal prostatic cells[6]. Nevertheless, other studies reported that LTL between prostate cancer (PCa) patients and non-PCa patients, benign prostatic hypertrophy and prostatitis, were not significantly different[7,19]. A meta-analysis demonstrated that shortened LTL is associated with the risk of cancer[8]. Some studies showed an association between shorter LTL and worse cancer outcomes[9-11], but several prospective studies showed an association between long LTL and the increasing risk of certain cancers[12-15]. Some studies demonstrated a null association between cancer risk and LTL[16,17]. The most probable explanation of differences between the results included genetics, epigenetics, immune components, hormones and stress[18]. Moreover, differences in study design (retrospective vs prospective), type of cancer, and technical variability of LTL measurement by real time polymerase chain reaction could have impact on the results and create variation.

Currently, the prevalence of PCa is increasing and is one of the most common male cancer mortalities, therefore, diagnostic and prognostic factors for PCa are very important in enabling early diagnosis and informing choice of treatment dependent on risk classification. Unfortunately, to date, past and ongoing studies have not furnished significant data on the potential association of relative LTL with prognostic markers of PCa. In one prospective cohort study, longer LTL at baseline was a significant PCa risk factor[15]. Another study found that patients with long LTL (median) had a significantly worse prostate cancer–specific and metastasis-free survival rate compared to patients with short LTL[19]. However, another recent study found shorter LTL was associated with a significantly increased risk of biochemical recurrence in localized PCa patients receiving radical prostatectomy and radiotherapy[20]. This study aimed to elucidate the association of relative LTL as a prognostic marker of various aspects of prostate cancer including cancer-specific survival, overall survival, metastasis-free survival and relation of CRPC.

Materials and Methods

This study was conducted in the Division of Urology, Department of Surgery, Maharaj Nakorn Chiang Mai Hospital, Chiang Mai, Thailand. The study protocol was approved by the Research Ethics Committee, Faculty of Medicine, Chiang Mai University. This was a retrospective study including data from 87 patients diagnosed with PCa between March 2017 and December 2018 in Maharaj Nakorn Chiang Mai Hospital. Data from six cases was excluded due to missing key parameters including TNM stage, Gleason score, PSA at diagnosis, PSA nadir level, month of death, or month of CRPC. The LTL from genomic DNA (20 ng/mL) was evaluated at baseline before initiation of any treatment by the monochromic multiplex-quantitative real-time polymerase chain reaction method (MMQPCR), as previously described in Cawthon RM. and Shen M. et al[21,22].

Briefly, the T/S value was calculated as the ratio of telomere repeat copies (T) to single-copy-gene number and albumin gene (S) to represent the average length of telomere. In each sample, the quantity of telomere repeats, and single-copy-gene copies were determined and compared to the reference samples. All samples were assayed in triplicate to minimize the sample-to-sample variation. Once the PCR was completed, the Applied Biosystems QuantStudio™ 6 Flex Real-Time PCR analysis software was used to determine the T and S values for each experimental sample based on the standard curve method (Applied Biosystems, Foster City, CA, USA). The results were expressed in terms of T/S ratio or LTL.

Clinical data including age, weight, height, waist circumference, underlying diseases and current medication were collected. The laboratory data including serum PSA, fasting blood glucose, LDL, HDL, triglycerides, Gleason score and grade group reported were recorded.

Data from PCa patients included choice of treatment (radical prostatectomy, external beam radiation therapy, androgen deprivation therapy), stage after follow-up, time to metastasis,

time to death, time to castration resistance and cause of death. Details of pathologic stage and pathologic grade group were collected if the patient underwent radical prostatectomy. Decline in velocity, time to PSA nadir and PSA nadir were collected if the patient underwent primary ADT.

Fisher's exact test was used to compare LTL to clinical parameters including age, smoking, metabolic syndrome, Gleason score, T stage, stage of cancer, metastatic cancer and CRPC. All parameters were observed retrospectively, for a mean follow-up period of 30 months. Survival analysis was performed using Kaplan–Meier survival curves with the log-rank test; univariate comparison was investigated to determine if they carry any prognostic information. The patients were subdivided into two groups, with the median LTL (value 5.3) as the cut-off and with PC-specific death, overall death, time to metastasis, and time to castration resistance as the event. Statistical significance was determined as p-value < 0.05.

Results

Complete data from 81 patients were collected. Baseline characteristics of patients are reported in Table 1. The mean age of prostate cancer patients was 69.35 years old. 43.2% were aged over 70 years of age. Only 27.5% patients had smoked more than 15-pack-years. About 39% were diagnosed with metabolic syndrome. Notably, many cases in this study were locally advanced (45.7%) and metastasis had occurred (34.6%).

Median LTL was 5.3 which became a cut-off point to dichotomize the data. As expected, patients over 70 years old were significantly associated with short LTL ($p < 0.001$). In contrast, other factors including smoking, metabolic syndrome, Gleason score, T stage, stage of cancer, metastatic cancer and castrate resistant cancer were not associated with LTL with p values of 0.45,1.0, 0.51, 0.59, 0.41, 0.24 and 0.57 respectively (Table 2). In line with current studies, factors including T stage, stage, Gleason score, CRPC and metastasis were factors which significantly impact overall survival with p-values < 0.001, 0.009, 0.018, 0.008 and < 0.001 respectively. The main outcome of this study was that prostate cancer-specific survival and overall survival were not associated with relative LTL with p values of 0.769 and 0.206 respectively (Figure 1 and 2). Four patients developed metastasis in the median follow-up time, so metastasis-free survival could not be analyzed or interpreted.

Table 1. Demographic data

Parameters	N=81
Age (years)	
Mean (SD)	69.35 (8.09)
Median	68
Age group (years) n (%)	
≤ 70	46 (5679)
> 70	35 (43.21)
Label smoker n (%)	
Yes	22 (27.50)
No	58 (72.50)
Metabolic syndrome n (%)	
Yes	30 (38.96)
No	47 (61.04)
Telomere length mean (SD) median	5.43 (0.72), 5.3
Telomere length	
≤ 5.3	44 (54.32)
> 5.3	37 (45.68)
Stage n (%)	
IIa, IIb and IIc	16 (19.75)
IIIa, IIIb, IIIc, IVa and IVb	65 (80.25)
Gleason score n (%)	
< 7	14 (17.28)
≥ 7	67 (82.72)
Metastasis n (%)	
Yes	28 (34.57)
No	53 (65.43)
Treatment n (%)	
ADT	37 (45.68)
RP	33 (40.74)
EBRT+ADT	7 (8.64)
WW	2 (2.47)
AS	2 (2.47)
CRPC n (%)	
Yes	15 (18.52)
No	66 (81.48)
Survival n (%)	
Survived	67 (82.72)
Death	14 (17.28)

There were 37 patients (45.7%) who underwent primary androgen deprivation therapy (ADT). In the subgroup analysis of this group, CRPC was significantly ($p = 0.039$) associated with long LTL. However, decline velocity (≤ 9 month) and time to PSA nadir (≤ 11ng/mL/month) showed no correlation with LTL (Table 3). However, CRPC was associated with decline velocity (≤ 9 month), time to PSA nadir (≤ 11ng/

Table 2. Association of relative LTL and other parameters.

Parameters	Telomere length		P-value
	≤ 5.3 (n=44)	> 5.3 (n=37)	
Age (years) n (%)			< 0.001
≤ 70	17 (38.64)	29 (78.38)	
> 70	27 (61.36)	8 (21.62)	
Smoker			0.452
Yes (%)	14 (31.82)	8 (22.22)	
No (%)	30 (68.18)	28 (77.78)	
Metabolic syndrome n (%)			1.000
Yes	16 (39.02)	14 (38.89)	
No	25 (60.98)	22 (61.11)	
T stage n (%)			0.586
T1c, T2a, T2b and T2c	17 (38.64)	18 (48.65)	
T3a, T3b	12 (27.27)	10 (27.03)	
T4	15 (34.09)	9 (24.32)	
Stage n (%)			0.407
IIa, IIb and IIc	7 (15.91)	9 (24.32)	
IIIa, IIIb, IIIc, IVa and IVb	37 (84.09)	28 (75.68)	
Gleason score			0.407
≤ 7	18 (40.91)	18 (48.65)	
> 7	26 (59.09)	19 (51.35)	
Metastasis			0.243
Yes	18 (40.91)	10 (27.03)	
No	26 (59.09)	27 (72.97)	
CRPC			0.574
Yes	7 (15.91)	8 (21.62)	
No	37 (84.09)	29 (78.38)	
Survival			0.126
Survived	30 (68.18)	31 (83.78)	
Dead	14 (31.82)	6 (16.22)	

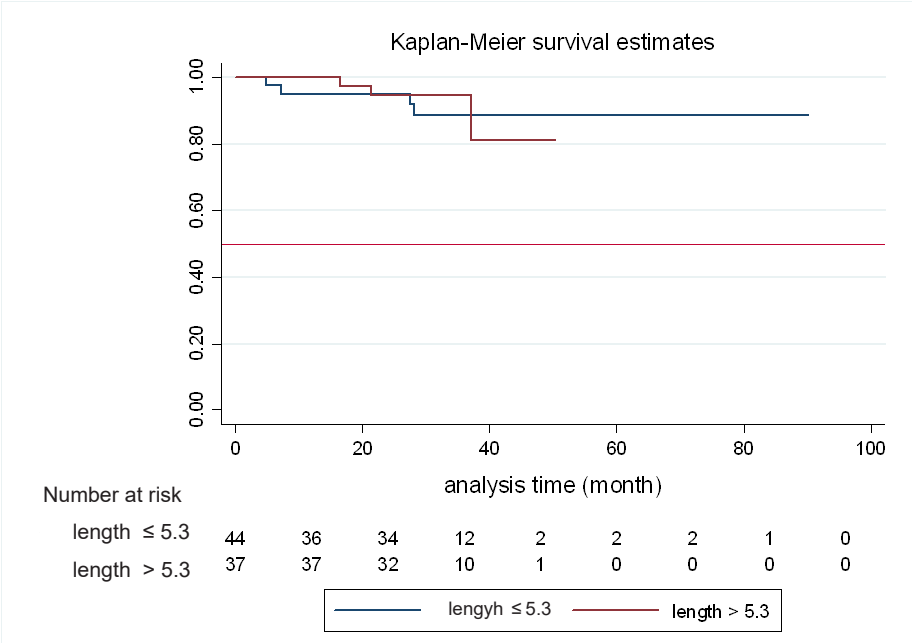

Figure 1. Cancer specific free survival estimates by relative leukocyte telomere length.

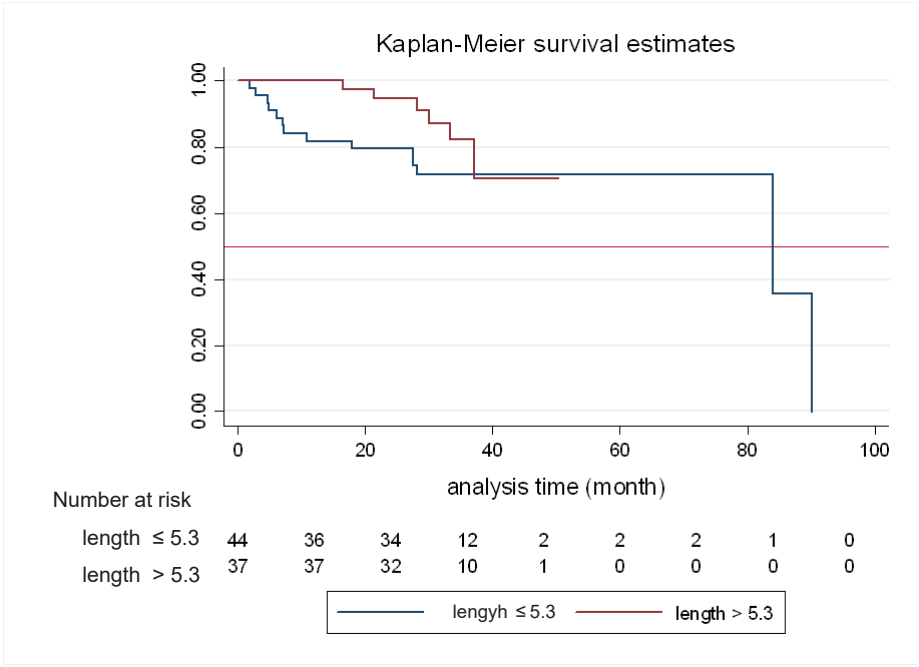

Figure 2. Overall survival estimates by relative leukocyte telomere length.

Table 3. Subgroup analysis of patients who underwent primary ADT compared with relative LTL and other parameters.

Parameters	Telomere length		P-value
	≤ 5.3 (n=24)	> 5.3 (n=13)	
CRPC n (%)			0.039
Yes	6 (25.00)	8 (61.54)	
No	18 (75.00)	5 (38.46)	
Time to PSA nadir n (%)			0.091
≤ 9 months	11 (45.83)	10 (76.92)	
> 9 months	13 (54.17)	3 (23.08)	
Decline velocity n (%)			0.300
≤ 11 ng/ml/month	13 (54.17)	4 (30.77)	
> 11 ng/ml/month	11 (45.83)	9 (69.23)	
PSA nadir			0.512
Mean (SD)	26.82 (72.84)	51.15 (151.00)	
Median	1.05	4.50	

mL/month) and PSA nadir ≥ 0.2 ng/mL with P-values of 0.007, 0.040 and 0.007 respectively (Table 4).

Discussion

In this study, we investigated the association between relative LTL and prognostic markers of various aspects of prostate cancer including cancer-specific survival, overall survival, metastasis-free survival, and castrate-resistant prostate cancer. We found that the only factor which affects the LTL was old age (more than 70 years old).

Patients who undergo ADT inevitably show progression to CRPC[23]. We are reporting a novel finding, specifically that a relatively long LTL measurement in newly diagnosed patients with primary ADT may be correlated with an increased risk of progression to CRPC. In this group, patients with long LTL had significantly more CRPC in 30 months of follow-up. CRPC was correlated with decreased PCa-specific survival, metastasis-free survival, and overall survival. This finding was in line with data from Svenson et al.[19] and Renner et al.[24]

Svenson et al.[19] measured LTL in 272 PCa patients (162 controls and 110 newly diagnosed PCa). This study excluded high-grade prostatic intraepithelial neoplasia (PIN) and other cancers.

Table 4. Subgroup analysis of patients who underwent primary ADT compared with CRPC and other parameters.

Parameters	CRPC		P-value
	Yes (n=14)	No (n=23)	
Telomere length n (%)			0.039
≤ 5.3	6 (42.86)	18 (78.26)	
> 5.3	8 (57.14)	5 (21.74)	
Time to PSA nadir n (%)			0.007
≤ 9 months	12 (85.71)	9 (39.13)	
> 9 months	2 (14.29)	14 (60.87)	
Decline velocity n (%)			0.040
≤ 11 ng/ml/month	3 (21.43)	14 (60.87)	
> 11 ng/ml/month	11 (78.57)	9 (39.13)	
PSA nadir			0.007
< 0.2 ng/ml	0 (0.00)	9 (39.13)	
≥ 0.2 ng/ml	14 (100.00)	14 (60.87)	

LTL was evaluated in relation to PCa diagnosis, risk classification and level of serum PSA. This study is reporting that PCa patients with long LTL(≥ median) had a significantly worse PCa-specific and metastasis-free survival compared to those with short LTL and confirmed the use of LTL as a prognostic marker for PCa. Another study by Renner et al.[24] reported that longer LTL predicts higher overall mortality in patients with PCa on EBRT +/- ADT.

Ji G. et al.[25] studied 185 patients with PCa who had received ADT as the primary therapy. That study reported the presence of distant metastasis before ADT, higher PSA nadir, and a velocity of PSA decline > 11ng/mL per month. They further determined that a time to PSA nadir ≤ 9 months was significantly associated with an increased risk of progression to CRPC. In line with our findings from the subgroup analysis of patients who underwent primary ADT, a higher PSA nadir of more than 0.2 ng/mL, a velocity of PSA decline > 11ng/mL per month, and a time to PSA nadir ≤ 9 months were significantly associated with an increased risk of progression to CRPC (Table 4). However, we were unable to demonstrate a significant correlation of these parameters with LTL but not CRPC which may be associated with long LTL.

Due to a limitation of the study, we could not show a significant main outcome related to PC-specific death, overall death, or time to metastasis. We had a small number of patients compared with prior studies. In addition, the short follow-up (median time only 30 months) may have affected our outcomes, especially the time to metastasis and time to death. Furthermore, only baseline blood samples were taken; thus, no analyses of LTL such as telomere shortening dynamics were available during follow-up. In future studies these limitations would need to be addressed to add weight to the findings.

Conclusion

In this study, aging was significantly associated with relatively short LTL. We found no significant association between telomere length of prostate cancer patients at diagnosis, cancer specific survival, metastasis-free survival, or overall survival. However, the subgroup analysis of patients who underwent ADT treatment alone showed castrate-resistant prostate cancer may associated with relatively long LTL. Future large, prospective-longitudinal studies are needed to confirm these initial findings.

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Conflict of Interest

The authors declared no conflict of interest.

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