

Original Article

Telomere shortening in patients on long-term hemodialysis

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Abstract

Background: Leukocyte telomere length shortening is a characteristic of premature senescence, a process that can be accelerated by oxidative stress. In general, patients with end-stage renal disease undergoing regular hemodialysis (HD) are repeatedly exposed to oxidative stress. Patients undergoing HD tend to have cardiovascular diseases associated with oxidative stress and inflammation. Therefore, we assumed that telomere length is associated with HD vintage and the degree of vascular calcification.

Methods: A total of 144 patients undergoing regular HD before kidney transplantation and 62 patients on hemodialysis, but not undergoing kidney transplantation, were enrolled. We measured common laboratory values, such as calcium, phosphate, and hemoglobin levels, and assessed the degree of vascular calcification in the patients. The leukocyte telomere length was measured using reverse transcription polymerase chain reaction, and Spearman correlation was used for correlation analysis.

Results: The leukocyte telomere length was negatively associated with age ($\rho = -0.306$, $P < 0.01$); it was shorter in middle-aged patients than in young patients (13.48 ± 4.80 vs. 15.86 ± 4.51 , $P < 0.01$). The telomere length was significantly different among patients aged 52–74 years in groups with different HD vintages. Additionally, the telomere length was positively associated with serum hemoglobin (Hb) levels in all patients ($\rho = 0.290$, $P < 0.01$). There was a significant difference among patients divided into three groups according to the degree of anemia (17.09 ± 5.64 vs. 14.40 ± 4.07 vs. 13.99 ± 3.95 , $P < 0.01$). Further, a significant difference was observed in the telomere length among patients with different degrees of vascular calcification

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(16.79 ± 4.91 vs. 13.61 ± 2.82 vs. 14.62 ± 3.63 vs. 10.71 ± 3.74 , $P < 0.01$). The telomere length was shorter in the patients on hemodialysis who did not receive a kidney transplant than in the surgical patients (8.12 ± 1.83 vs. 14.33 ± 4.63 , $P < 0.01$).

Conclusion: This study demonstrated that the telomere length was significantly correlated with HD vintage in patients of a certain age group. The telomere length was shorter in patients on hemodialysis who matched for age and dialysis vintage with kidney transplant patients. It was also associated with vascular calcification and serum Hb levels in all patients undergoing HD.

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Keywords: Telomere length; Hemodialysis; Chronic kidney disease; Cardiovascular disease; Hemoglobin

Introduction

Patients with end-stage renal disease (ESRD) usually require renal replacement therapy, such as hemodialysis (HD), peritoneal dialysis, or kidney transplantation.¹ As the number of patients on HD with chronic kidney disease (CKD) continues to increase worldwide² and the annual mortality rate of patients undergoing dialysis is as high as 10%–20%,¹ some indices should be monitored for better patient management and prognosis. Recent studies have suggested that telomere shortening is associated with mortality in patients with CKD and that it is a more stable and convenient detection index to determine the risk of complications in patients with CKD.^{3,4}

Telomeres are nucleoprotein structures found at the end of each chromosome arm to maintain genome stability.⁵ When telomeres are critically short, cell senescence mechanisms are activated, which in turn permanently arrest the cell cycle.⁶ With increasing chronological age, telomere shortening has been associated with various disorders, such as cardiovascular disease (CVD), osteoporosis,⁷ renal transplant dysfunction,⁸ and neurological disorders.^{7,9} It has been hypothesized that oxidative stress can induce telomere shortening, a characteristic feature of premature senescence, resulting in organ dysfunction.^{10,11}

Patients with ESRD usually undergo HD at regular intervals, which can lead to the repeated activation of mononuclear cells.¹² Hence, repeated stimulation during HD sessions induces a replicative senescence process in mononuclear cells,¹³ and patients undergoing long-term HD have shorter telomeres due to increased cumulative exposure to oxidative stress.¹⁴

Furthermore, patients undergoing HD have a substantially increased risk of cardiovascular mortality,¹⁵ predominantly caused by atherosclerosis.¹⁶ Oxidative stress and inflammation associated with shorter telomeres play a key role in atherosclerotic plaque progression.¹⁷ Moreover, atherosclerosis is an age-related change in the structural and functional properties of

elastic arteries,¹⁸ and cellular senescence can also be influenced by age.¹⁹ Therefore, patients with CKD who develop CVD may have various telomere lengths depending on the severity of CVD.

In this study, we hypothesized that telomere length is associated with the duration of HD and aimed to explore the relationship between telomere length and some variables affected by oxidative stress in patients undergoing HD to determine whether telomere length shortening was a prognostic indicator. We also evaluated the degree of vascular calcification in these patients to determine whether telomere shortening was associated with atherosclerosis.

Methods

Ethical approval

The study was approved by the Research Ethics Board of our Institute. All patients who participated in this study provided written informed consent. The study protocol was performed in accordance with relevant guidelines.

Study population

A total of 144 patients with ESRD who underwent kidney transplantation at the Kidney Disease Center of First Affiliated Hospital of Zhejiang University Hospital from November 2016 to June 2018 were selected. We also matched another group, including 62 hemodialysis patients who did not receive kidney transplantation, with our 144 patients for sex, age, and dialysis vintage in the same period. The selection criteria were as follows: (1) age >18 years, regardless of sex and ethnicity; (2) patients with ESRD undergoing treatment for ≥ 3 months; and (3) voluntary participation and provision of informed consent. The exclusion criteria were as follows: (1) acute kidney injury; (2) active inflammatory diseases; (3) parathyroidectomy; (4) other concomitant diseases (such as

malignant tumors) that affect calcium levels and soft tissue calcification in the body; (4) pregnant and lactating women; and (5) patients who could not be tested for arterial or valve calcification or whose results were unreliable (e.g., those who had undergone amputations and those with severe peripheral vascular disease).

Patients aged >18 years, irrespective of sex, were enrolled in the study. Among the 144 patients, 101 patients aged 18–44 years (including 44 years old) were classified into the young age group (Group A), and the remaining patients aged 44–74 years were classified into the middle-aged group (Group B) according to the age classification criteria from the World Health Organization (WHO). Each group was further divided into two groups according to the median age: (1) 18–33 years, (2) 34–44 years, (3) 45–52 years, and (4) 53–74 years. All patients underwent HD three times a week (4–5 h per session); the median HD time (vintage) was 48 (range, 6–168) months.

Information regarding general demographic characteristics, including age (years) and sex (male or female), were collected at the commencement of HD. Hospitalization records were reviewed to determine the duration of dialysis, primary cause of ESRD, and presence or absence of hypertension or coronary artery disease. Medication orders were provided for the administration of P-binders and calcitriol.

Specimen processing and collection

Blood samples of the 144 surgical patients were collected preoperatively, whereas those of the 62 patients on hemodialysis, but not undergoing transplantation were collected at the time of inclusion, centrifuged, and serum samples were stored at -80°C until use. The following levels of the following parameters were measured using routine serum samples at the laboratory of our hospital: calcium (Ca), phosphate (P), hemoglobin (Hb), triglycerides (TG), total cholesterol (TC), alkaline phosphatase (ALP), albumin (Alb), low-density lipoprotein (LDL), high-density lipoprotein (HDL), blood urea nitrogen (BUN), serum creatinine (Cr), and estimated glomerular filtration rate (eGFR).

During the kidney transplantation procedure, the proximal portion of the inferior epigastric artery was ligated, and a sample of vessels measuring approximately 1–2 cm was removed. Each vessel was cut with a single-edge blade into 3–4 transverse specimens measuring approximately 3–5 mm in thickness and processed for paraffin embedding. Sections were

stained with hematoxylin and eosin and the von Kossa method to evaluate calcification, and von Kossa positivity in the tunica media was semiquantitatively scored. The classification criteria were as follows: 0, no calcification; 1, focal calcification spots; 2, partial calcification covering 20%–80% of the arterial circumference; and 3, circumferential calcification.²⁰

Telomere measurements by RT-PCR

Reverse transcription (RT)-PCR was used to determine the telomere length in human neutrophils. First, genomic DNA (Axygen, USA) was extracted from PBMCs, and the telomere length of a set of standard cell lines was assessed using Southern blotting. RT-PCR was performed as previously described.²¹ Briefly, telomere (T) PCR and single-copy gene human β -globin (S) PCR were conducted. Reactions were performed using Prism7500 (Applied Biosystems, USA), and data were collected and analyzed using ABI Prism 7500 SDS v.1.7.

Statistical analysis

Statistical analyses were performed using the SPSS 23.0 (IBM Corp., USA). Normal measurement data are expressed as mean \pm standard deviation (SD), skewed data are expressed as median (interquartile range), and categorical variables are expressed as frequencies. Univariate analysis of variance was used to compare normal distribution data. A nonparametric test was used for data that did not meet the normal distribution. Spearman correlation was used for correlation analysis, and categorical variables were compared between the groups. Statistical significance was set at $P < 0.05$.

Results

General characteristics and the association between age and telomere length

We included 144 patients of both sexes. The general characteristics of the study population are described in [Table 1](#). The patients were grouped by age, given that leukocyte telomere length was negatively associated with age ([Fig. 1A](#); $\rho = -0.306$, $P < 0.01$).

As shown in [Table 1](#), the leukocyte telomere length was shorter in middle-aged patients than in young patients ([Fig. 1B](#); 13.48 ± 4.80 vs. 15.86 ± 4.51 , $P < 0.01$), whereas HD vintage was significantly longer in middle-aged patients than in young patients (48 vs. 24, $P < 0.01$). There were significant

Table 1
General characteristics of patients undergoing HD.

Characteristics	All patients (n = 144)	Young (n = 101)	Middle-old-aged (n = 43)
Age (years)	39.26 ± 10.85	33.48 ± 6.24	52.84 ± 6.47 ^b
Dialysis vintage (months)	48 (34.72)	24 (5.60)	48 (26.72) ^b
Cause of CKD, n (%)			
Glomerulonephritis	120 (83)	88 (87)	32 (74)
Lupus nephritis	3 (2)	2 (2)	1 (2)
Polycystic kidney disease	2 (1)	1 (1)	1 (2)
Hypertension	4 (3)	2 (2)	2 (5)
Others	2 (1)	1 (1)	1 (2)
Not confirmed	13 (9)	7 (7)	6 (14)
Hypertension, n (%)	124 (86)	85 (84)	39 (91)
History of CVD, n (%)	11 (8)	2 (2)	9 (21)
Prescription, n (%)			
P-binder use	28 (19)	18 (18)	10 (23)
Calcitriol	6 (4)	0 (0)	6 (14)
Laboratory values			
Ca ²⁺ (mmol/L)	2.16 ± 0.20	2.18 ± 0.19	2.09 ± 0.19 ^a
P (mmol/L)	1.72 ± 0.57	1.76 ± 0.60	1.60 ± 0.44
Alb (g/L)	41.79 ± 5.02	42.89 ± 5.23	39.21 ± 3.40 ^b
ALP (U/L)	70(57.93)	70(58.91)	75(56.5.99)
Hb (g/L)	108.25 ± 13.59	108.11 ± 12.63	108.55 ± 15.90
HDL (mmol/L)	1.09 ± 0.30	1.08 ± 0.31	1.08 ± 0.27
LDL (mmol/L)	2.15 ± 0.68	2.12 ± 0.67	2.21 ± 0.72
TG (mmol/L)	1.23 ± 0.85	1.32 ± 0.92	0.99 ± 0.59 ^a
TC (mmol/L)	3.82 ± 0.86	3.80 ± 0.87	3.87 ± 0.85
BUN (mmol/L)	19.15 ± 6.48	18.82 ± 6.72	19.91 ± 5.92
Cr (mmol/L)	785.5 (616.0,956.5)	815.0 (648.0,999.0)	716.0 (569.0,889.0) ^b
eGFR (mL/min/1.73m ²)	7.05 ± 3.25	6.80 ± 3.43	7.62 ± 2.79
Telomere length (kb)	15.16 ± 4.70	15.86 ± 4.51	13.48 ± 4.8 ^b

HD: hemodialysis; CKD: chronic kidney disease; CVD: Cardiovascular disease; Alb: albumin; ALP: alkaline phosphatase; Hb: hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglyceride; TC: total cholesterol; BUN: blood urea nitrogen; Cr: creatinine; eGFR: estimate glomerular filtration rate.

Data are presented as n(%), mean ± SD or Median (quarter, three quarters).

^a*P* < 0.05, vs. Young; ^b*P* < 0.01, vs. Young.

differences in serum calcium, albumin, and triglyceride levels between the two groups (2.18 ± 0.19 vs. 2.09 ± 0.19, 42.89 ± 5.23 vs 39.21 ± 3.40, 1.32 ± 0.92 vs 0.99 ± 0.59, *P* < 0.05).

However, there was no statistically significant difference between men and women regarding telomere length or age. Furthermore, higher HDL and total cholesterol levels were observed in women than in men.

Significant difference in telomere length in different groups

When patients were further divided into four groups by age, patients who underwent long-term HD had shorter telomere lengths than patients with dialysis vintage <24 months in the group aged 52–74 years (Fig. 2A; 15.58 ± 3.89 vs. 10.54 ± 3.30, *P* < 0.05); no such differences were observed in the other groups.

Moreover, we graded the degree of vascular calcification in each patient and classified the patients into four groups. A significant difference was observed in the telomere length among patients with different degrees of vascular calcification (Fig. 2B; 16.79 ± 4.91 vs. 13.61 ± 2.82 vs. 14.62 ± 3.63 vs. 10.71 ± 3.74, *P* < 0.01).

Further, another group of patients on hemodialysis who did not receive kidney transplantation was matched with our 144 patients for sex, age, and dialysis vintage. Finally, 62 patients were included from each group. The basic information is shown in Table 2. There was no significant difference in age or dialysis vintage between the two groups. The telomere length was shorter in the patients on hemodialysis who did not receive a kidney transplant (Fig. 3; 8.12 ± 1.83 vs. 14.33 ± 4.63, *P* < 0.01). In addition, the hemodialysis group had higher serum lipid and serum calcium, and phosphorus levels than the transplantation group.

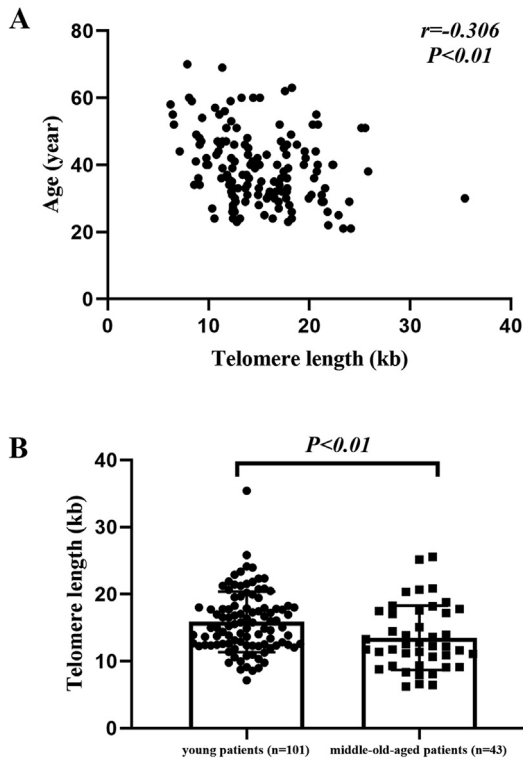


Fig. 1. The leukocyte telomere length was negatively associated with age in 144 patients ($\rho = -0.306$, $P < 0.01$) (A). In young patients, the leukocyte telomere lengths were longer than those in middle-old-aged patients (13.48 ± 4.80 vs. 15.86 ± 4.51 , $P < 0.01$) (B).

Telomere length was correlated with serum hb

Telomere length was positively associated with serum Hb levels in all patients (Fig. 4A; $\rho = 0.290$, $P < 0.01$). There was a significant difference among patients divided into three groups according to the degree of anemia (Fig. 4B; 17.09 ± 5.64 vs. 14.40 ± 4.07 vs. 13.99 ± 3.95 , $P < 0.01$).

We also analyzed these associations separately in young and middle-aged patients (Table 3). Telomere length was strongly and negatively correlated with age and Hb levels in young patients, but no association was found in middle-aged patients.

Discussion

Various factors influence telomere shortening. Many previous studies have reported an inverse relationship between telomere length and age.^{22,23} Telomere length has also provided useful information about the aging process before the disease progresses, making it a promising therapeutic target.²⁴ Our results showed that

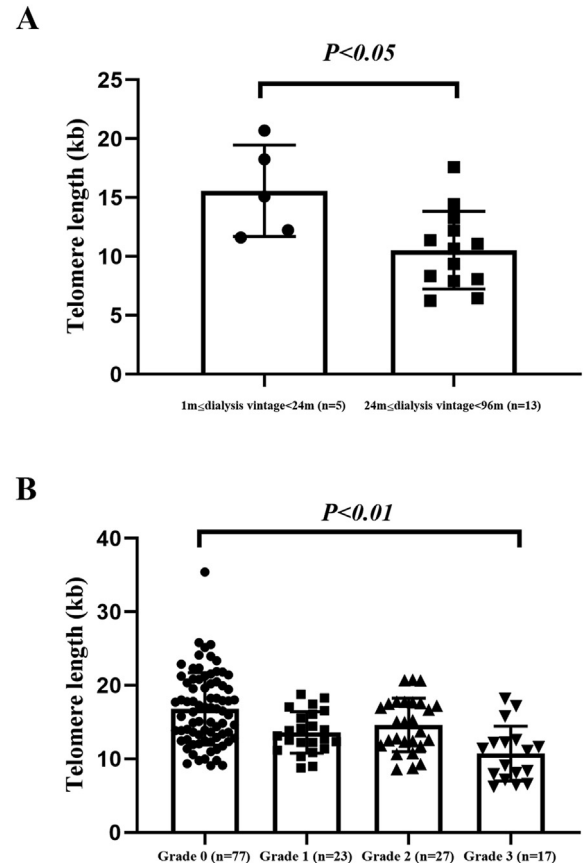


Fig. 2. A total of 18 patients aged 52–74 years who underwent a longer period of HD had shorter telomere lengths than those with hemodialysis vintage < 24 months (15.58 ± 3.89 vs. 10.54 ± 3.30 , $P < 0.05$) (A). A total of 144 patients were classified into four groups according to the degree of their vascular calcification; there was a significant difference in telomere length among the groups (16.79 ± 4.91 vs. 13.61 ± 2.82 vs. 14.62 ± 3.63 vs. 10.71 ± 3.74 , $P < 0.01$) (B). The classification criteria were as follows: 0, no calcification; 1, focal calcification spots; 2, partial calcification covering 20%–80% of the arterial circumference; and 3, circumferential calcification. m: months.

telomere length shortened with age in patients with CKD, according to its characteristic as a biomarker of chronological aging.^{25–27}

Differences in the telomere length between men and women showed different results under different conditions. Most studies have found that women have longer telomeres than men among patients on dialysis as well as in the general population;^{3,28–30} this is consistent with the theory that estrogen may exert a protective effect on telomere length, directly or indirectly, due to its anti-inflammatory and antioxidant properties. Carrero et al.³ also proposed that the state of inflammation and CVD may differ between male and

Table 2
General characteristics of HD patients underwent transplantation surgery or not.

Characteristics	All patients (n = 124)	Transplantation group (n = 62)	Hemodialysis group (n = 62)
Age (years)	45.53 ± 9.98	46.08 ± 10.43	44.98 ± 9.64
Dialysis vintage (months)	48 (24.84)	48 (24.72)	47 (21,108)
Cause of CKD, n (%)			
Glomerulonephritis	80 (65)	47 (76)	33 (53)
Lupus nephritis	4 (3)	2 (3)	2 (3)
Polycystic kidney disease	7 (6)	1 (2)	6 (10)
Hypertension	6 (5)	4 (6)	2 (3)
Diabetes	13 (10)	0 (0)	13 (21)
Others	4 (3)	1 (2)	3 (5)
Not confirmed	10 (8)	7 (11)	3 (5)
Laboratory values			
Ca ²⁺ (mmol/L)	2.19 ± 0.18	2.16 ± 0.19	2.22 ± 0.17 ^a
P (mmol/L)	1.77 ± 0.58	1.60 ± 0.51	1.95 ± 0.61 ^b
Alb (g/L)	39.29 ± 4.03	39.87 ± 4.00	38.71 ± 4.03
Hb (g/L)	104.31 ± 15.76	106.84 ± 14.49	101.79 ± 16.79
HDL (mmol/L)	1.07 ± 0.35	1.09 ± 0.33	1.06 ± 0.37
LDL (mmol/L)	2.52 ± 0.86	2.13 ± 0.65	2.91 ± 0.87 ^b
TG (mmol/L)	1.43 ± 0.81	1.04 ± 0.60	1.81 ± 0.81 ^b
TC (mmol/L)	4.15 ± 1.09	3.79 ± 0.81	4.50 ± 1.23 ^b
Telomere length (kb)	11.23 ± 4.68	14.33 ± 4.63	8.12 ± 1.83 ^b

HD: hemodialysis; CKD: chronic kidney disease; Alb: albumin; Hb: hemoglobin; HDL: high-density lipoprotein; LDL: low-density lipoprotein; TG: triglyceride; TC: total cholesterol.

Data are presented as n(%), mean ± SD or Median (quarter, three quarters).

^a*P* < 0.05, vs. Transplantation group; ^b*P* < 0.01, vs. Transplantation group.

female patients undergoing hemodialysis. However, sex differences did not affect the telomere length in our study, possibly because of the relatively small number of cases and the study included patients undergoing HD and not healthy subjects.

The incidence of ESRD continues to increase worldwide and has a major effect on HD needs.³¹ Although we could not find an association between

telomere length and HD vintage in all patients, we found that patients who underwent long-term HD had shorter telomere lengths than those with HD vintage <24 months in the group of patients aged 52–74 years. This finding suggests that patients with relatively longer HD vintage, that is, those who are repeatedly exposed to oxidative stress, have relatively shorter telomere lengths. CKD has been hypothesized as a clinical model of premature aging.³² The development of chronic diseases and the process of dialysis can accelerate telomere attrition, and patients with longer telomere lengths may indicate less proliferation to replace lost leukocytes.³³ Contradictory findings regarding the relationship between telomere length and HD vintage have been reported in some studies. Carrero et al³ reported that leukocyte telomere length was not related to HD vintage. In contrast, Boxall et al³⁴ and Mueillo-Ortiz et al⁴ reported that shortened dialysis time was independently associated with increased telomere shortening in patients undergoing HD, consistent with our study results. In non-dialysis-dependent patients with CKD, Raschenberger et al³⁵ showed a U-shaped association between telomere length and CKD duration, indicating the gradual activation of telomerase in patients with long-term diseases to counteract the shortening of telomere length caused by oxidative stress and inflammation could lead

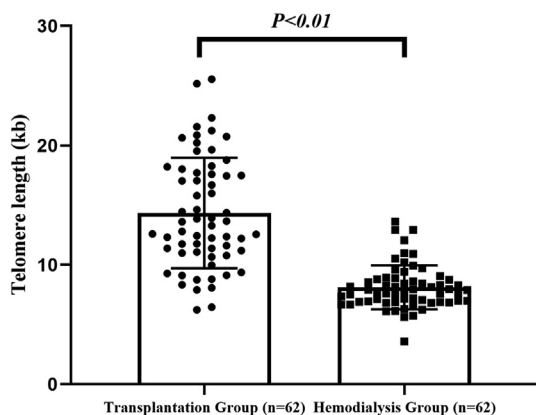


Fig. 3. The telomere length was shorter in the patients on hemodialysis who did not receive a kidney transplant when matched for sex, age, and dialysis vintage with our 144 patients on hemodialysis and who received kidney transplant (8.12 ± 1.83 vs. 14.33 ± 4.63 , $P < 0.01$).

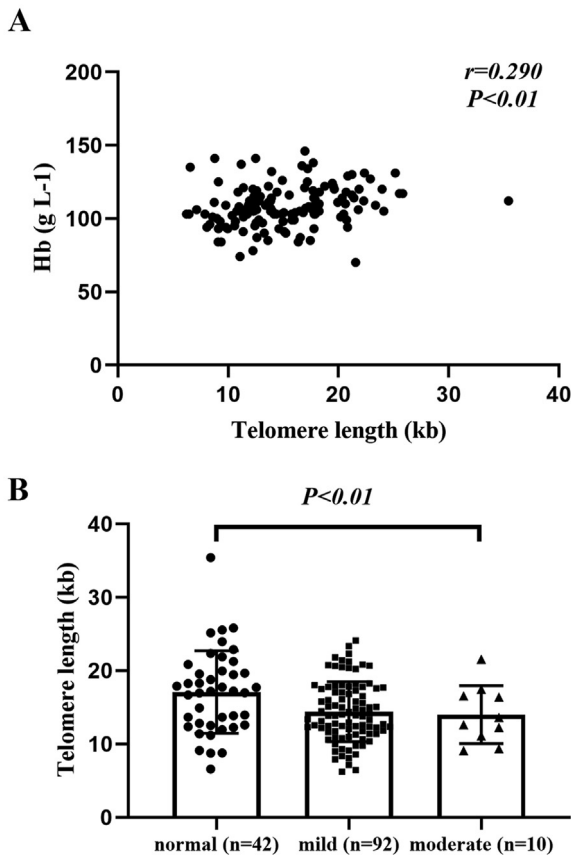


Fig. 4. The telomere length was positively associated with serum hemoglobin levels in all patients ($\rho = 0.290$, $P < 0.01$) (A). A significant difference was observed among patients divided into three groups according to the degree of anemia (17.09 ± 5.64 vs. 14.40 ± 4.07 vs. 13.99 ± 3.95 , $P < 0.01$) (B).

to longer telomere length. With the progression of CKD to ESRD, patients are increasingly exposed to inflammation, oxidative stress, and uremic toxicity³⁶ and it is uncertain whether the dialysis procedure can aggravate or delay cellular senescence. There may be a dynamic change in the telomere length in patients with CKD undergoing dialysis. Further studies are needed to compare the leukocyte telomere length between patients who are non-dialysis-dependent and dialysis-dependent.

Table 3

Spearman rank correlation between leukocyte telomere length and selected parameters in 144 patients.

Variables	Young (n = 101)		Middle-old-aged (n = 43)	
	Rho	P-Value	Rho	P-Value
Age	-0.208	0.037	-0.159	NS
Hb	0.268	0.007	0.279	NS

We also compared the kidney transplantation group to a group of patients on hemodialysis, but did not undergo transplantation, who were matched for age and dialysis vintage. Although there was no significant difference in age or dialysis vintage between the two groups, the telomere length was shorter in the patients on hemodialysis who did not receive a kidney transplant. For patients with ESRD, the decision to undergo a kidney transplant depends on several factors, including health status, comorbid conditions, the availability of a suitable match, psychosocial assessment, etc.³⁷ Telomere length shortening may be an indicator of evaluating the candidate's profile to access the surgery.

Our study showed a significant difference in the telomere length among patients with different degrees of vascular calcification, and patients with greater severity of vascular calcification tended to have shorter telomeres. It is necessary to find a sensitive and effective marker to monitor the early progression of CVD, which is slow and inconspicuous, to reduce its high morbidity and mortality. In particular, the risk of calcification of blood vessels is greatly increased in patients on dialysis.³⁸ Several related studies have demonstrated that telomere length can be identified as a novel risk factor for CVD.^{39,40} The clustering of CVD risk factors has been significantly correlated with arterial stiffness.⁴¹ Jeanclous et al⁴² also suggested that telomere shortening was associated with hypertension, endothelial dysfunction, atherosclerosis, and cardiovascular mortality. In patients with CKD, due to oxidative stress and inflammation, vascular calcification can be aggravated, which increases the risk of CVD, a phenomenon that can also be reflected by the shortened telomere length. Telomere length measurements may help assess the risk of cardiovascular complications in patients undergoing cardiological procedures and evaluate the effectiveness of some drugs.⁴³ CVD is also a major cause of death in patients undergoing long-term hemodialysis. The existing diseases in patients with end-stage kidney disease may also accelerate cellular aging and lead to disease, including age-related CVDs. Because cells with shorter telomeres tend to senesce earlier and upregulate proinflammatory cytokines when they are in growth arrest, shorter telomeres may directly contribute to atherosclerosis, thereby gradually aggravating the progression of CVD. Therefore, our findings also suggest that telomere length can be regarded as an early indicator for evaluating and monitoring the occurrence and development of cardiovascular disease in patients.

We also demonstrated a significant association between reduced telomere length and serum Hb levels. Erythrocytes may be injured when exposed to oxidative stress,⁴⁴ leading to decreased Hb levels. Therefore, CKD patients with anemia may have shorter telomere lengths due to oxidative stress and inflammation. Although the mechanism is unknown, the type and concentration of Hb seem to play roles in the early detection of acute kidney injury (AKI).⁴⁵ A series of studies have shown a relationship between decreased Hb levels and the incidence of AKI.^{46,47} Deterioration of renal function in patients with anemia can also lead to shortened telomere lengths. In contrast, we also found that older patients with shorter telomeres had significantly lower albumin levels than middle-aged patients. Albumin can also reflect the nutritional status of patients. Hypoalbuminemia in patients on hemodialysis may also result from chronic inflammation and increased albumin loss, and not just represent a sign of dystrophy. Therefore, both, patients with anemia and hypoproteinemia have shorter telomere length due to the chronic inflammatory process of the disease itself or its complications, although the exact mechanism for this remains unclear.

Hb levels have been thought to be associated with an elevated risk of cardiovascular disease,⁴⁸ and related studies on the direct relationship between Hb levels and pulse wave velocity (PWV) have also suggested a significant association between high Hb levels and an increased risk of cardiovascular events and mortality, represented by increased arterial stiffness.⁴⁹ However, in this study, the telomere length in patients with anemia or severe vascular calcification was relatively short. Hence, we can assume that telomere length and other factors are interrelated; they do not influence each other.

In our study, the telomere length was strongly and negatively correlated with age and Hb levels in young patients, but no such association was found in middle-aged patients. Thus, we can deduce that the telomere length of young patients is mainly associated with age and Hb levels, but with increased age, the effect of HD vintage becomes apparent. It can also be observed that oxidative stress and inflammatory processes have a long-term impact on cell aging.

In addition, an increase in white cell turnover, possibly due to inflammation or the dialysis membrane used in patients undergoing HD, also influences the telomere length, considering that the length decreases with each cell division.³⁹ However, with cumulative exposure to oxidative stress, cells can also be activated

when dialyzed with cellulosic membranes.¹³ Therefore, this study showed that identifying independent factors affecting telomere length is difficult. Additional studies should be performed to explore more variations in the telomere length and determine their relationships.

This study has some limitations. First, the number of cases was relatively small, and the selection was biased as we chose only surgery patients, making our analyses less reliable. The results of this study were based on a single random blood sample, which may be unstable information. Further studies are needed to clarify whether telomere shortening can increase the risk of mortality in patients undergoing HD.

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Conflict of interest

None.

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