

Telomerase Activity Is Decreased in Peripheral Blood Mononuclear Cells of Hemodialysis Patients

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Key Words

Telomeres · Interleukin-6 · Soluble interleukin-6 receptor · Soluble gp130 · C-reactive protein · Peripheral blood mononuclear cells · Serum albumin

Abstract

Background: Telomerase preserves telomere length and structure, preventing cellular senescence, which is associated with alteration of the chromosomal ends. We hypothesized that telomerase activity is altered in peripheral blood mononuclear cells (PBMCs) of hemodialysis (HD) patients. To investigate this hypothesis as well as the relationship between telomerase and inflammation, we measured the activity of this reverse transcriptase as well as the level of several inflammatory markers in PBMCs and serum of an end-stage renal failure (ESRF) population and a non-renal-failure group of subjects. **Methods:** In PBMCs isolated from 42 HD and 39 non-renal-failure subjects of the same age (51.0 ± 12.4 and 51.4 ± 12.1 years, respectively) telomerase activity was measured using PCR-ELISA; the method was based on the telomeric repeat amplification protocol. **Results:** Telomerase activity in PBMCs was detected in

18 (42.9%) HD and 28 (71.8%) non-renal-failure subjects ($p = 0.013$). Among positive subjects, percent telomerase activity in PBMCs was significantly higher in non-renal-failure ($117 \pm 112\%$) than in HD ($47.6 \pm 57.1\%$) subjects ($p = 0.008$). Detectable telomerase activity was lower in long-term than in short-term HD patients (13.3 ± 8.9 vs. $75.0 \pm 64.8\%$, respectively, $p = 0.015$). Although higher in HD group, inflammatory indexes (C-reactive protein, interleukin-6, IL-6, soluble IL-6 and soluble gp130) were not correlated to telomerase activity in PBMCs. **Conclusion:** Telomerase activity in PBMCs is reduced in HD patients. It seems that, at least in this type of cell in this population, defense from senescence, as assessed by telomerase activity, is altered and associated with the chronicity of uremia/HD procedure.

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Introduction

Cellular senescence is an important aspect of cell behavior and seems to occur either after an extended period of cell divisions ('replicative senescence') or more rapidly in response to various physiologic stresses ('stress-in-

Table 1. Characteristics and serum inflammatory markers of the subjects included in the study

	Non-renal-failure subjects (n = 39)	Hemodialysis patients (n = 42)	p
Age, years	51.4 ± 12.1	51.0 ± 12.4	0.891
Sex, F/M	23/16	20/22	0.212
BMI, kg/m ²	25.1 ± 3.9	26.1 ± 4.8	0.288
Smoking	13	9	0.170
Diabetes	1	3	0.336
Hypertension	9	16	0.111
Atherosclerosis ^a	3	9	0.076
Angiotensin-converting enzyme inhibitors	3	8	0.121
Angiotensin receptor antagonists	3	5	0.339
Ca channels blockers	4	9	0.143
Aspirin, 100 mg/day	3	8	0.121
C-reactive protein, mg/l	2.5 ± 2.3	7.6 ± 8.2	<0.001
Interleukin-6, IL-6, pg/ml	2.6 ± 0.7	8.2 ± 10.1	0.001
Soluble IL-6 receptor, sIL-6R, ng/ml	251 ± 106	396 ± 111	<0.001
Soluble gp130, sgp130, ng/ml	365 ± 61	406 ± 94	0.023
(IL-6 × sIL-6R)/sgp130	1.9 ± 1.2	9.0 ± 11.9	<0.001
Serum albumin, g/l	4.8 ± 0.3	4.4 ± 0.3	<0.001

^a Atherosclerotic cardiovascular disease (CVD) profile in each subject was evaluated using the CVD portion of the Index of Co-Existing Disease [7].

duced senescence’) [1]. Telomeres, the nucleoprotein structures protecting chromosome ends, are related to the cellular senescence; erosion to a critical length or change in structure of telomeres is crucial for senescence induction [1, 2]. Telomerase, a specialized reverse transcriptase, maintains telomere length and structure, extending the life span of human cells [1, 3].

Uremic milieu is a stress-inducing condition. Inflammation and oxidative stress are common findings in end-stage renal failure (ESRF) patients [4]. Peripheral blood mononuclear cells (PBMCs) are repeatedly activated during the inflammatory process. The ability of these cells to efficiently maintain telomere structure and length depends on telomerase activity [5]. We postulated that the activity of this specialized enzyme is altered in PBMCs of hemodialysis (HD) patients and is potentially associated with inflammation, which characterizes this population.

To investigate our hypothesis, telomerase activity was measured in PBMCs isolated from a group of HD and a group of non-renal-failure subjects of the same age. C-reactive protein (CRP) as well as interleukin-6 (IL-6), soluble IL-6 receptor (sIL-6R) and soluble gp130 (sgp130), the active circulating components of a main pro-inflammatory cytokine, were also determined in the serum of all the subjects enrolled in the study, for inflammation assessment.

Characteristics of Hemodialysis and Non-Renal-Failure Subjects

Forty-two stable HD patients and 39 non-renal-failure subjects were enrolled in the study. The former were ESRF patients, dialyzed in the same renal unit (Blue Cross Hospital) for, mean ± SD, 60.8 ± 61.0 (min, max, 4–200) months, while the later were hospital (Department of Nephrology, General Hospital of Athens) staff volunteers and their relatives with an estimated glomerular filtration rate (Cockcroft-Gault formula) ≥ 80 ml/min. The two groups were matched for age and other characteristics as shown in table 1. The age range (25th and 75th percentiles) was 21–81 years (42 and 62 years) and 23–73 (41 and 61.2 years) in non-renal-failure subjects and in HD patients, respectively. Both HD patients and non-renal-failure subjects had no clinically evident infection, malignancy, collagen tissue disease, trauma or any major CVD event at the time of blood sample collection (November–December 2004). All participants gave their informed consent for inclusion in the study.

The cause of ESRF in the HD group was chronic glomerulonephritis in 12 patients, polycystic disease in 10 patients, hypertensive glomerulosclerosis in 8 patients, chronic interstitial nephritis in 5 patients, and diabetic nephropathy in 3 patients, while the cause was undetermined in 4 patients. All patients were on conventional HD

and were being dialyzed with bicarbonate dialysate. The type of dialyzer was modified cellulose membrane for all the patients (polysynthane or modified di- or tri-acetate cellulose, Baxter). Water processing (central reverse osmosis water treatment system) and the type of concentrate were also common for the entire group of patients. Of the patients, 17 were being treated with sevelamer hydrochloride, 9 with intravenous (i.v.) vitamin D, 27 with erythropoietin and 7 patients with i.v. iron (maintenance treatment 100 mg once per week); iron administration was discontinued 1 week before blood sampling.

Methods

Blood Samples Collection

In both groups a heparinized and a non-heparinized blood sample of 10 and 5 ml, respectively, were collected, for obtaining PBMCs and serum samples. In dialyzed patients, blood samples were drawn just before the onset of the midweek dialysis session.

Preparation of PBMCs

PBMCs were obtained by a Ficoll-Hypaque (Histopaque 1077, Sigma Aldrich, St Louis, Mo., USA) gradient density centrifugation (400 g for 30 min). The mononuclear layer was then collected and washed 3 times in PBS, and finally an aliquot containing 2×10^5 cells was transferred into an Eppendorf tube and centrifuged at 3,000 g for 5 min at 8°C. The purity of the cell population was tested through microscopic examination of May-Grünwald-Giemsa-stained smears. In all samples, patients and controls, over 90% of the cells were lymphocytes, while the rest consisted of a small number of monocytes and polymorphonuclear neutrophils. The pelleted cells were stored at -80°C until use.

Detection of Telomerase Activity

Telomerase activity was measured using a commercial telomerase PCR – ELISA (Roche Diagnostics Corporation, Indianapolis, USA), based on the telomeric repeat amplification protocol (TRAP) method, described by Kim et al. [6]. Telomerase activity was expressed in %, as described in the instructions provided in the kit, by comparing the signal of the sample to the signal of the high positive control template containing 0.1 amol/ μ l DNA.

CRP, Serum Albumin and Cytokines Assays

The quantitative determination of CRP and serum albumin (S-albumin) were assayed by particle-enhanced immunonephelometry on Behring Nephelometer 2. The high-sensitivity CRP assay was designed to measure CRP concentrations within an overall range of approximately 0.175–1.100 mg/l.

IL-6 and sIL-6R serum levels were quantified with a sandwich enzyme immunoassay technique based on a monoclonal-polyclonal antibody pair (R&D systems, Minneapolis, Minn., USA). For IL-6, the intra-assay precision was 4.2% CV and the inter-assay precision was 6.4% CV at a serum concentration of 16.8 pg/ml, while for sIL-6R the intra-assay precision was 8.6% CV and the interassay precision was 6.4% CV at a serum level of 134 ng/ml. For the determination of sgp130 serum levels, a sandwich enzyme immunoassay

technique based on a monoclonal-biotinylated monoclonal antibody pair was used (Diaclone Research, Besancon, France). In this case, the intra-assay CV value was 3.27% at a serum level of 264 ng/ml while the inter-assay precision was 4.85%. All assays were carried out in the same run and in duplicate.

Statistical Analysis

All values with skewed distribution were transformed to their natural logarithms. Statistical analysis was performed using Student's t test. Correlations were performed using Pearson coefficient in log-transformed values. Differences of distribution of groups 2×2 contingency tables were measured using the χ^2 test. Multiple linear regression analysis was applied as described in the 'Results' section. Unless otherwise reported, results are expressed as mean \pm SD of the natural numbers. In all cases, significant results were declared to be those with $p < 0.05$. Analyses were performed using the SPSS version 11.0 (SPSS Inc., Chicago, Ill., USA).

Results

Telomerase Activity in PBMCs

Telomerase activity in PBMCs was detected in 18 (42.9%) HD and 28 (71.8%) non-renal-failure subjects ($p = 0.013$). Among positive subjects, percent telomerase activity in PBMCs was significantly higher in non-renal-failure ($117 \pm 112\%$) than in HD ($47.6 \pm 57.1\%$) subjects ($p = 0.008$) (fig. 1). Age was not correlated to telomerase activity, neither in the HD patients nor in the control group.

No other correlations between non-renal-failure or HD subjects' characteristics or medications or HD conditions and telomerase activity were noticed, except that in the control group more non-smokers (22 of 28) than smokers (6 of 13) had detectable telomerase activity in PBMCs ($p = 0.022$).

In stepwise multiple linear regression analysis, using % telomerase activity as the dependent variable and all the factors listed in table 1 as well as renal failure presence or not, hemoglobin, white blood cells, % neutrophils, % lymphocytes, serum calcium, phosphorus, cholesterol, low-density lipoproteins, high-density lipoproteins, triglycerides, ferritin, ferrum and treatment (or not) with sevelamer hydrochloride, vitamin D, erythropoietin and i.v. iron, with entry factors at $p < 0.05$, and removing those factors no longer contributing at $p > 0.10$, only the factor 'renal failure presence' was a significant predictor for the % telomerase activity in PBMCs ($\beta = 0.367$, $t = 3.503$, $p = 0.001$).

Duration of HD and Telomerase Activity in PBMCs

When patients were divided into 2 groups according to HD duration (median time on HD, 38.5 months),

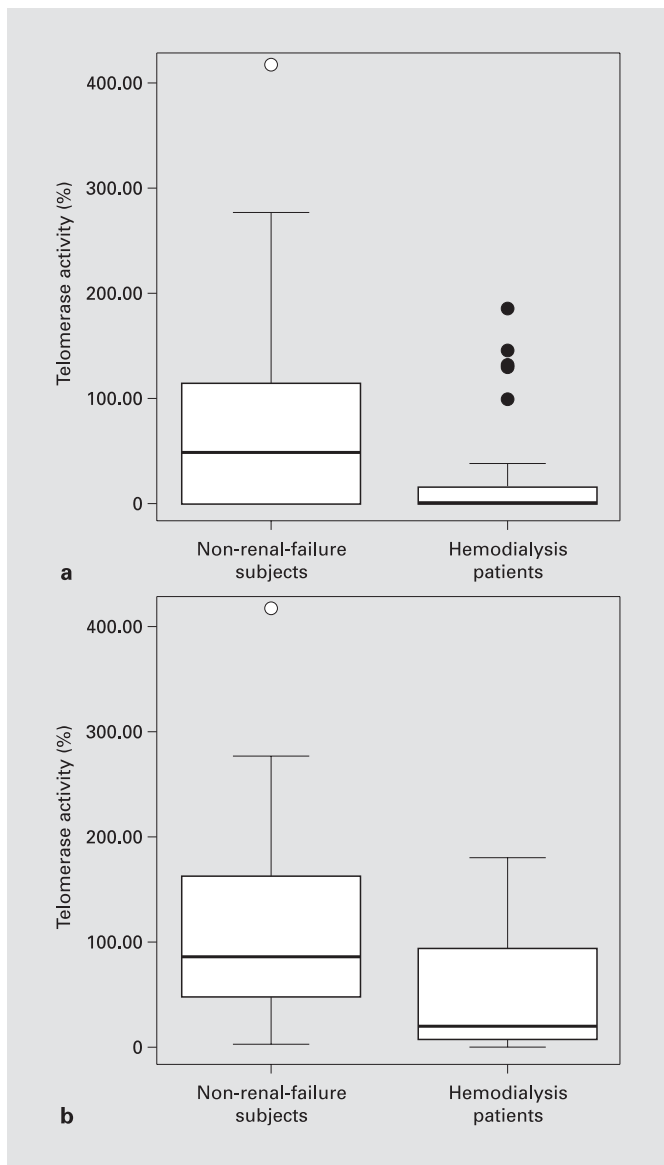


Fig. 1. Percent telomerase activity in PBMCs. **a** Eighty-one subjects (control and hemodialysis, HD, group) with or without detectable telomerase activity included in the study. **b** Forty-six subjects with detectable telomerase activity (28 non-renal-failure and 18 HD subjects) included in the study. Boxplots show the median, quartiles and extreme values of telomerase activity.

telomerase activity in PBMCs was detected in 8 of 21 patients of the long-term HD group (median time on HD 100 months, range 39–200 months) and in 10 of 21 patients of the short-term HD group (median time on HD 23 months, range 4–38 months).

Among positive patients, the 10 short-term HD patients (mean \pm SD, 14.2 \pm 10.7 months on HD) had

significantly higher telomerase activity in PBMCs than the 8 long-term HD patients (104 \pm 51.1 months on HD): 75.0 \pm 64.8 and 13.3 \pm 8.9%, respectively, $p = 0.015$ (fig. 2).

In the renal-failure population only, we performed stepwise multiple linear regression analysis using % telomerase activity as the dependent variable and duration of hemodialysis (months) as well as all the factors listed in table 1 plus hemoglobin, white blood cells, % neutrophils, % lymphocytes, serum calcium, phosphorus, cholesterol, low density lipoproteins, high density lipoproteins, triglycerides, ferritin, ferrum and treatment (or not) with sevelamer hydrochloride, vitamin D, erythropoietin and i.v. iron, with entry factors at $p < 0.05$, and removing those factors no longer contributing at $p > 0.10$. Only the factor 'duration of hemodialysis' was a significant predictor for the % telomerase activity in PBMCs ($\beta = 0.441$, $t = 2.119$, $p = 0.030$).

Inflammation Indexes – Correlation to Telomerase Activity in PBMCs

C-reactive protein, IL-6, sIL-6R and sgp130 were significantly higher and S-albumin significantly lower in serum of HD patients compared to non-renal-failure subjects (table 1). Moreover, the ratio of the two circulating agonistic molecules of IL-6 complex (IL-6 \times sIL-6R), which induce its action, to their natural antagonist (sgp130), that inhibits their activity, was also found to be higher in HD patients (table 1). The above-mentioned ratio was positively and significantly correlated to CRP ($r = 0.396$, $p = 0.009$) and negatively but insignificantly correlated to S-albumin (-0.229 , $p = 0.129$) in HD patients.

No statistically significant correlations between inflammatory markers and telomerase activity in PBMCs were noticed in HD patients or in non-renal-failure subjects.

It is worth noting that, although inflammation was not correlated to telomerase activity in ESRF patients, the long-term, low telomerase, HD patient group had higher serum IL-6 (11.9 \pm 13.3 vs. 4.5 \pm 2.3 pg/ml in short-term, high telomerase HD patients, $p = 0.006$) as well as higher IL-6 \times sIL-6R/sgp130 (13.7 \pm 15.1 vs. 4.4 \pm 3.4, respectively, $p = 0.002$).

Discussion

The main findings of the present study are that telomerase activity in PBMCs is detected in a smaller percentage of HD than non-renal-failure subjects and that among

positive individuals its activity is higher in the latter. Long-term HD patients had lower telomerase activity in this type of cell. Although inflammation is increased in renal-failure patients, no correlation was found between selected inflammatory markers and the activity of this specialized reverse transcriptase in PBMCs.

To the best of our knowledge, telomerase activity in somatic, non-malignant cells of ESRF patients is measured for the first time in the present study. In a recent publication, accelerated telomere shortening was found in PBMCs of HD patients [8]. Taking into consideration the findings of both studies, it seems that not only premature senescence, reflected by telomere shortening, but also the potential of preserving telomere length and structure by telomerase activity is altered, at least in PBMCs, in this population. This last finding should be of importance, especially because recent studies showed that disruption of telomerase activity in normal human cells slows cell proliferation, restricts lifespan and alters the characteristic telomere structure ('telomere uncapping'), without changing the rate of overall telomere shortening [9–11]. Consequently, the smaller percentage of HD patients with detectable telomerase activity in PBMCs as well as the lower activity of this enzyme in those patients (fig. 1) might suggest that this type of cell, in this population, is prone to enter a senescence phase, possibly independently of the natural telomere shortening that follows repetitive cell divisions. On the other hand, repeated activation of PBMCs also occurs in these patients; frequent infections and bioincompatibility of dialysis material are common causes. This fact underlines the importance of telomerase activity in maintaining telomere length and structure in HD patients; accelerated 'replicative senescence' as well as 'stress-induced senescence' – linked more to structure alteration of telomeres [10] – may be inter-related to this reverse transcriptase deficiency, particularly in this population.

Telomerase activity in PBMCs was found to be inversely correlated to HD duration in ESRF patients included in the study. Repetitive activation of PBMCs as well as chronic exposure to oxidative [12] or other uremia-induced stress of long-term HD patients might explain the observed telomerase activity reduction. It is worth noting that although 'dialysis age' was significantly different between the groups of short-term and long-term HD patients (see 'Results'), their mean age was almost identical (mean \pm SD, 50.6 \pm 12.9 and 51.5 \pm 12.2 years, $p = 0.807$). Thus, reduced telomerase activity was age-independent in these patients and possibly linked to the chronicity of stress – chronic oxidative stress or even

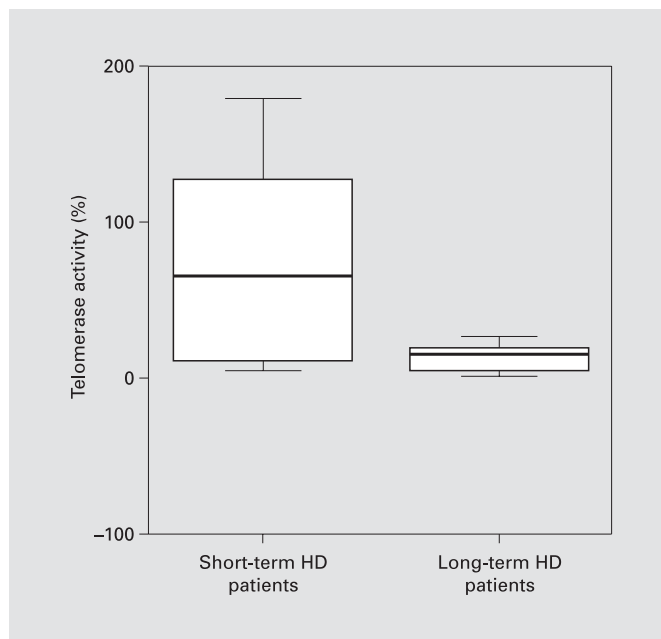


Fig. 2. Percent telomerase activity in PBMCs in positive (n = 18) short-term and long-term HD patients. Boxplots show the median and the quartiles of telomerase activity.

psychological stress, as a recent study showed [13] – that characterizes this population.

Inflammation estimated by serum CRP, the three active soluble components of the IL-6 complex and partially by S-albumin, although increased, was not found to be associated with telomerase activity in HD patients. This may be due to the relatively small number of renal-failure patients included in the study. According to existing data, the telomerase-cytokine relationship seems to be bi-directional, cytokines modulating telomerase activity and vice versa; nuclear factor κ -B may be the link [14, 15]. Furthermore, senescent cells – possibly telomerase-depleted – show spontaneous cytokine production [16]. It is of interest that long-term, low telomerase activity HD patients in our study had higher ratio of agonistic to antagonistic IL-6 circulating components than short-term, high telomerase activity patients (see 'Results').

The number of the subjects included in the present study was relatively small, a limitation which has to be taken into consideration. Moreover, variability in time of the inflammatory indexes cannot be excluded in HD patients [17, 18].

In conclusion, the ability of a main immunity-related type of cell, namely of the PBMC, to prevent senescence, as assessed by telomerase activity, is reduced in HD pa-

tients. Duration of exposure to uremic milieu or to HD-related factors are associated to this age-independent tendency to cellular senescence. Although a cause and effect relationship between telomerase activity and more specific ESRF-related factors cannot be established in the present study, in the case these data are confirmed in future studies, another uremia consequence, namely premature cellular senescence induction, at least in peripheral mononuclear cells of HD patients, could be proposed.

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