

Original Article

Serum oxidized low-density lipoprotein is inversely correlated to telomerase activity in peripheral blood mononuclear cells of haemodialysis patients

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SUMMARY:

Background: Telomerase preserves telomeres' function and structure preventing cellular senescence. Its activity is reduced in peripheral blood mononuclear cells (PBMC) of haemodialysis (HD) patients. The purpose of this study is to investigate the potential correlation between increased oxidative stress/inflammation and telomerase activity in PBMC of HD patients.

Methods: Telomerase activity was measured by PCR-ELISA in PBMC isolated from a group of 42 HD patients and 39 subjects with estimated glomerular filtration rate ≥ 80 mL/min (control group). Serum oxidized low-density lipoprotein (ox-LDL), tumour necrosis factor- α (TNF- α) and interleukin-10 (IL-10) were also measured in both groups by ELISA.

Results: Ox-LDL was negatively correlated to percentage telomerase activity in PBMC ($r = -0.506$, $P = 0.000$ in the whole group of 81 HD and normal subjects and $r = -0.559$, $P < 0.001$ in HD patients). TNF was also inversely associated with percentage telomerase activity in the whole group studied ($r = -0.492$, $P = 0.000$) while IL-10 was not. In stepwise multiple linear regression, taking into consideration the most important characteristics of the HD patients and control group, the only significant predictors for percentage telomerase activity in PBMC were ox-LDL and TNF ($\beta = -0.421$, $t = -4.083$, $P = 0.000$ and $\beta = -0.381$, $t = -3.691$, $P = 0.000$, respectively) while examining separately HD patients, the predictors for the same parameter were ox-LDL and HD duration ($\beta = -0.671$, $t = -4.709$, $P = 0.000$ and $\beta = -0.349$, $t = -2.447$, $P = 0.023$, respectively).

Conclusion: Ox-LDL serum level is inversely correlated to telomerase activity in PBMC of HD patients. Our study proposes a new consequence of increased oxidative stress in HD patients: the premature cellular senescence potentially related to atherosclerosis through LDL oxidation.

KEY WORDS: cytokine, inflammation, oxidative stress, renal failure, telomere, tumour necrosis factor.

Telomerase activity is crucial for preservation of telomeres' function and structure.¹ Telomeres, the ends of eukaryotic chromosomes, are structures strictly related to the phenomenon of cellular senescence.² Cellular senescence occurs

after an extended period of cell divisions, but it can occur earlier in case the cell is exposed to various types of stresses, oxidative stress being one of them.¹ Telomerase activity may also be interrelated to inflammation, especially in cell types like peripheral blood mononuclear cells (PBMC) implicated in this process.³

We found that in haemodialysis (HD) patients telomerase activity is decreased in PBMC.⁴ On the other hand, oxidative stress and inflammation characterize these patients.^{5,6} The aim of the present study is to investigate the potential correlation of the above-mentioned entities to telomerase activity. For this purpose, we used a HD patient and a control group, in which we had previously measured

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PBMC' telomerase activity. In serum – isolated and stored at the time of blood collection for telomerase activity measurement in PBMC – we further determined oxidized low-density lipoprotein (ox-LDL), an index of oxidative stress and a well-known atherogenic factor, as well as the levels of a pro- and an anti-inflammatory cytokine – tumour necrosis factor (TNF) and interleukin-10 (IL-10) – in both groups and correlated them to the activity of telomerase in PBMC as well as with other characteristics of the studied groups.

PATIENTS AND METHODS

Thirty-nine subjects with estimated glomerular filtration rate = 80 mL/min (control group) and 42 HD patients were included in the study. Hemodialysis and control group were matched for age (51.0 ± 12.4 and 51.4 ± 12.1 years, $P = 0.891$), sex, body mass index, smoking, diabetes, hypertension or atherosclerosis presence – evaluated using the cardiovascular disease portion of the index of coexisting disease⁷ – as well as for angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, Ca blockers or aspirin treatment and had no clinically evident inflammation at the time of blood collection.⁴ All participants gave their informed consent for inclusion in the study.

The method for detection of telomerase activity and isolation of PBMC is described elsewhere.⁴ Briefly, telomerase activity in PBMC was measured using a commercial telomerase PCR-ELISA (Roche Diagnostics Corp., Indianapolis, IN, USA), based on the telomeric repeat amplification protocol.⁸

For the determination of ox-LDL – in serum isolated and stored at the time of blood collection for telomerase activity measurement in PBMC – a sandwich enzyme immunoassay technique based on a monoclonal-antibody pair was used (Mercodia, Uppsala, Sweden). The intra-assay precision varies between 5.5% and 6.2% at the corresponding serum concentrations of 8.5 U/L and 32 U/L, while the inter-assay precision varies between 6.2% and 4.0% for the same serum concentrations. IL-10 and TNF serum levels were quantified with ELISA (R&D Systems, Minneapolis, MN, USA).

Serum levels of all biochemical markers (total cholesterol, high-density lipoprotein cholesterol, triglycerides, etc.) were determined utilizing Olympus AU 560 random access analyser (Olympus Corp., Japan). LDL-cholesterol levels were calculated using the Friedwald formula.

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. Multiple linear regression analysis was applied as described in the Results section. Results are expressed as mean \pm SD of the natural numbers. In all cases, significant results were declared to be those with a *P*-value of <0.05 . Analyses were performed using SPSS version 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Ox-LDL, TNF and IL-10 serum levels in HD patients and control group are shown in Table 1. LDL was not significantly different between HD patients and control group (102.8 ± 34.5 and 111.6 ± 20.1 , $P = 0.175$), but ox-LDL/LDL was significantly higher in HD patients than in the control group (0.9 ± 0.4 vs 0.6 ± 0.2 , $P < 0.001$). Ox-LDL was positively correlated to TNF, C ($r = 0.243$, $P = 0.029$).

Table 1 Ox-LDL, TNF and IL-10 serum levels in haemodialysis patients and in the control group

	Haemodialysis patients (n = 42)	Non-renal failure subjects (n = 39)	P
Ox-LDL (U/L)	91.2 ± 33.9	66.9 ± 26.4	0.001
TNF- α (pg/mL)	24.5 ± 6.1	17.4 ± 7.1	<0.001
IL-10 (pg/mL)	15.7 ± 35.4	10.7 ± 10.5	0.248

IL-10, interleukin-10; TNF- α , tumour necrosis factor- α ; ox-LDL, oxidized low-density lipoprotein.

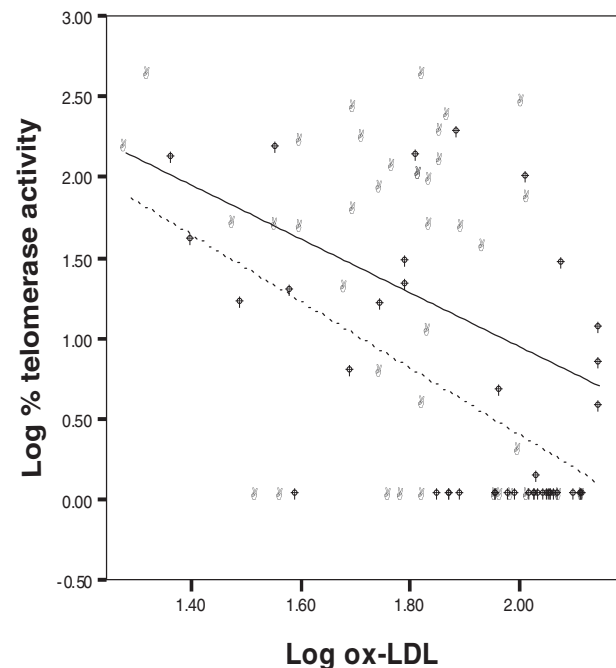


Fig. 1 Scatter plots of percentage telomerase activity in peripheral blood mononuclear cells by serum oxidized low-density lipoprotein (ox-LDL; both logarithmically transformed) in control group and haemodialysis patients. (#) control group ($R^2 = 0.11$); (*) renal failure patients ($R^2 = 0.31$).

Telomerase activity in PBMC was negatively correlated to ox-LDL in the whole group – HD patients and control group – included in the study ($r = -0.506$, $P = 0.000$). The same was valid when HD patients were considered separately ($r = -0.559$, $P = 0.000$) or when only the subjects of the control group were taken into consideration ($r = -0.335$, $P = 0.037$) (Fig. 1). TNF serum level was also negatively correlated to telomerase activity in PBMC when the 81 subjects, HD patients and control group were considered together ($r = -0.492$, $P = 0.000$) and when the control group was considered separately ($r = -0.439$, $P = 0.005$). The same correlation was also negative when HD patients were considered separately ($r = -0.281$, $P = 0.061$). (Fig. 2). Finally, IL-10 was positively but non-significantly correlated to telomerase activity in PBMC ($r = 0.107$, $P = 0.342$).

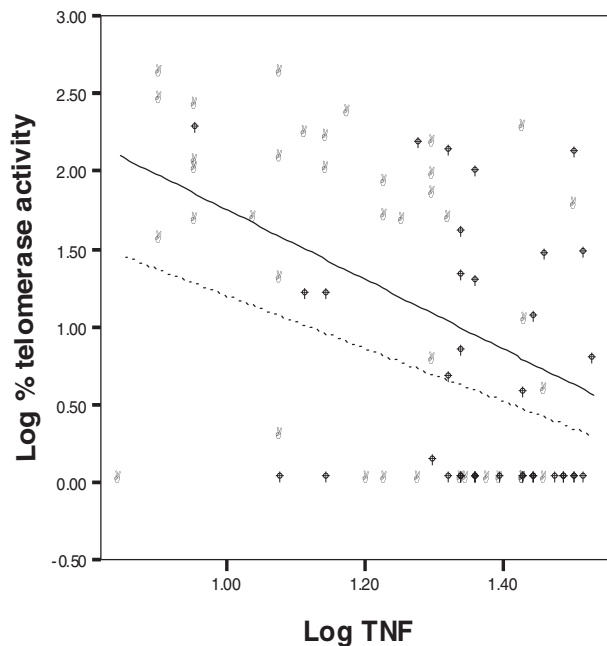


Fig. 2 Scatter plots of percentage telomerase activity in peripheral blood mononuclear cells by serum tumour necrosis factor (TNF; both logarithmically transformed) in control group and haemodialysis patients. (#) control group ($R^2 = 0.19$); (+) renal failure patients ($R^2 = 0.08$).

In stepwise multiple linear regression analysis, using percentage telomerase activity as the dependent variable and age, sex, body mass index, smoking, diabetes, hypertension or atherosclerosis presence, angiotensin converting enzyme inhibitors, angiotensin receptor antagonists, Ca channel blockers, aspirin use and treatment with sevelamer hydrochloride or not, vitamin D or not, erythropoietin or not, and i.v. iron or not as well as glomerular filtration rate ≥ 80 or < 80 mL/min, haemoglobin, white blood cells, percentage neutrophils, percentage lymphocytes, serum calcium, phosphorus, cholesterol, low-density lipoproteins, high-density lipoproteins, triglycerides, ferritin, serum, ox-LDL, TNF and IL-10 as potential predictors with entry factors at $P < 0.05$, and removing those factors no longer contributing at $P > 0.10$, only ox-LDL and TNF were significant predictors for the percentage telomerase activity in PBMC ($\beta = -0.421$, $t = -4.083$, $P = 0.000$ and $\beta = -0.381$, $t = -3.691$, $P = 0.000$, respectively).

Selecting only the control group and using the same parameters in stepwise multiple regression analysis, the only significant predictors for percentage telomerase activity in PBMC were TNF and ox-LDL ($\beta = -0.416$, $t = -2.942$, $P = 0.006$ and $\beta = -0.301$, $t = -2.140$, $P = 0.039$, respectively). Selecting only HD patients and using the same parameters plus HD duration in months as well as the type of HD membrane in stepwise multiple regression analysis, the only significant predictors for percentage telomerase activity in PBMC were ox-LDL and duration of HD in months ($\beta = -0.671$, $t = -4.709$, $P = 0.000$ and $\beta = -0.349$, $t = -2.447$, $P = 0.023$, respectively).

DISCUSSION

The main finding of this study is that serum ox-LDL is inversely correlated to telomerase activity in PBMC. This finding was valid for HD patients as well as for the control group included in the present study. The same reverse correlation, though not as strong as the former, seems to be valid for serum TNF level and telomerase activity in the same type of cell.

Oxidative stress is possibly implicated in premature cellular senescence. This phenomenon occurs in response to various physiological stresses independently of the number of cell divisions.¹ Telomerase activity in diverse types of cells is influenced by oxidative stress and seems to be related to 'stress-induced senescence' because of the crucial role this specialized enzyme has in maintaining telomeres function and structure.^{1,9}

Oxidative stress is increased in end-stage renal failure (ESRF) patients and, as we have previously described, telomerase activity in PBMC is decreased in this population.⁴ In the present study, a strong inverse correlation between these two parameters was established. Ox-LDL was measured as index of oxidative stress while at the same time as a well-known atherogenic factor. It was also shown recently that HD procedure promotes ox-LDL formation and subsequent endothelial cell damage in HD patients.¹⁰ Moreover, this oxidized molecule seems to be able to inactivate telomerase activity in endothelial cells and to induce senescence in endothelial progenitor cells.^{11,12} On the other hand, not only shorter telomere length but also low telomerase activity in leucocytes, reflecting the real biological age, have been proposed as a marker of increased – and premature – cardiovascular risk.^{13,14} Potentially cellular senescence – as detected in an early phase before telomere shortening by telomerase activity reduction – in some crucial cell types is a link between increased oxidative stress and accelerated – frequently premature – atherosclerosis observed in this population.

Low-grade inflammation is another characteristic of HD patients.¹⁵ In a previous study we did not find any correlation between C-reactive protein and IL-6 and telomerase activity in PBMC.⁴ In the present study, TNF- α was inversely associated with this enzyme activity. This divergence may be due to the different characteristics of the two pro-inflammatory cytokines (IL-6 and TNF) and may also parallel the more firm relationship between TNF and oxidative stress.¹⁶ In a cell type like PBMC, which is directly implicated in the inflammatory process, it is generally expected for a higher inflammatory activity to be associated with lower telomerase activity.¹⁷ It is worth noting that when only HD patients were included in statistical analysis, the only significant predictors for percentage telomerase activity in PBMC were ox-LDL and duration of HD and not TNF (see Results). This finding potentially means that duration of HD is more important than serum TNF level in prediction of telomerase activity in PBMC in this population. In addition, the importance of HD duration was already depicted in our previous study.⁴

Main limitations of the present study are the small number of subjects included as well as the single measurement of the oxidative stress and inflammatory indexes which may vary considerably in time.¹⁸

In conclusion, the inverse correlation of ox-LDL to telomerase activity in PBMC shows that increased oxidative stress in end-stage renal disease may be related to 'stress-induced cellular senescence' in circulating mononuclear cells of these patients. Future confirmation of our results in larger studies is needed in order to validate our assumption that premature cellular senescence which is potentially related to atherosclerosis is another consequence of oxidative stress in HD patients.

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