Telomere length shortening associated with cataract in younger adults

Acortamiento de la longitud telomérica en adultos jóvenes con catarata

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Abstract

Purpose: A prospective observational study was performed that included 205 adults, 102 cases and 103 controls, classified into the age groups of ≤ 55 and ≥ 60 years old, to examine the prognostic value of telomere length in patients with or without cataract and to assess the effects of telomerase ribonucleic acid (RNA) gene variants (telomerase reverse transcriptase [TERC] and telomere RNA component [TERT]) on telomere length. **Methods:** Telomere length and telomerase polymorphisms were quantified in peripheral blood leukocytes by a quantitative polymerase chain reaction. TaqMan[®] probes were used for telomerase polymorphisms (TERT 1327C > T and TERC 63G > A) genotyping. **Results:** Regardless of age, patients with cataracts had shorter telomere lengths compared to healthy controls ($p < 0.01$). No association was detected between telomere shortening and TERC 63G > A in both cases and controls, and no correlation was observed between leukocyte telomere length and cardiovascular risk factors in patients with cataracts. **Conclusions:** Irrespective of age, telomeres in peripheral blood leukocytes of patients with cataracts were shorter compared to subjects without cataracts. Our findings suggest that the development of cataract is associated with peripheral blood cell aging.

Key words: Cataract. Diabetes. Hypertension. Telomere length. Polymorphism. Smoking.

Resumen

Objetivo: Se realizó un estudio prospectivo observacional con 205 adultos (102 casos y 103 controles) clasificados en grupos de ≤ 55 años y ≥ 60 años, con el fin de evaluar la longitud telomérica en pacientes con y sin catarata, y los efectos de los polimorfismos (TERC y TERT) de la telomerasa sobre dicha longitud. **Métodos:** La longitud telomérica y los polimorfismos de la telomerasa se cuantificaron en leucocitos de sangre periférica por reacción en cadena de la polimerasa. Se utilizaron sondas TaqMan[®] para genotipificar polimorfismos de telomerasa (TERT 1327C>T y TERC 63G>A). **Resultados:** Independientemente de la edad, los pacientes con catarata tenían longitudes teloméricas más cortas que los controles ($p < 0.01$). No se detectó asociación entre el acortamiento de los telómeros y TERC 63G>A en casos ni en controles, y no hubo correlación entre la longitud telomérica en los leucocitos y los factores de riesgo cardiovascular en los pacientes con catarata. **Conclusiones:** Sin que influyera la edad, los telómeros en leucocitos de sangre periférica de pa-

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cientes con catarata fueron más cortos que en aquellos sin catarata. Nuestros hallazgos sugieren que el desarrollo de cataratas está asociado con el envejecimiento de las células de la sangre periférica.

Palabras clave: Cataratas. Diabetes. Hipertensión. Longitud telomérica. Polimorfismo. Tabaquismo.

Introduction

Telomeres are nucleotide repeat sequences that cap the ends of chromosomes. With each cell division, telomeres shorten until they reach a critical length when the cell can no longer replicate, causing senescence or death. This mechanism leads to a decline in cell function. It has been suggested that short telomeres could be markers of low resistance to stress¹. Accordingly, telomere length has been proposed as an indicator of cell aging and longevity and has been associated with many diseases related to aging and lifespan^{2,3}.

To maintain telomere length, the enzyme telomerase is responsible for synthesizing GGTTAG repeats at the ends of chromosomes. Human telomerase mainly consists of telomerase reverse transcriptase (telomere ribonucleic acid component [TERT]), telomerase ribonucleic acid (RNA) (telomerase reverse transcriptase [TERC]), and dyskerin (DKC1). Common genetic variants, TERT 1327C > T rs27350940 and TERC 63G > A rs2293607, have been recently linked to reduced telomerase activity, causing premature telomere shortening^{4,5}. These genes are expressed in embryonic tissues or peripheral mononuclear cells but have not been identified in the human lens.

Further, telomere length has been associated with cardiovascular risk factors such as diabetes, high blood pressure, and smoking⁶. In fact, patients with diabetes have shorter telomeres than healthy individuals⁷, and individuals with short telomere lengths show more diabetic complications⁸. In addition, several studies have detected a faster telomere shortening in smokers, probably due to oxidative damage caused by tobacco⁹. In the Framingham Heart Study, hypertensive men were found to have significantly shorter age-adjusted leukocyte telomere lengths compared to their normotensive peers¹⁰. However, this association could not be confirmed in a mixed male and female cohort examined in the Cardiovascular Health Study¹¹. Thus, there could be a sex-related effect on leukocyte telomere length in humans, whereby telomere length is shorter in adult men compared to women¹².

The hypothesis that lens transparency could be related to leukocyte telomere length as a marker of aging is supported by observations in premature aging disorders such as Werner syndrome. In patients with this syndrome, features linked to normal aging, including cancer

and bilateral cataracts, appear early, and patients show accelerated telomere shortening¹³⁻¹⁵.

This study was designed to examine leukocyte telomere length in younger adults with cataract and assess the supposed association between TERT and TERC gene polymorphisms and premature cataracts. We also determined whether telomere length differed between subjects with and without cardiovascular risk factors to eliminate confounding factors (smoking, diabetes, and hypertension), possibly causing telomere shortening.

Patients and methods

Subjects

Blood samples were collected from 205 subjects after obtaining their informed consent. The study protocol received Institutional Review Board approval and adhered to the principles of the Declaration of Helsinki. Subjects were divided into four groups: 46 subjects with cataract aged ≤ 55 years matched with 47 subjects without cataracts of similar age, and 56 subjects with cataract aged ≥ 60 years matched with 56 subjects without cataract of similar age. Clinical and demographic characteristics are summarized in [table 1](#) (clinical and demographic characteristics of the subjects included in our study). Mean age of the subjects in the four groups was 48.5 ± 7.47 years (cataract ≤ 55), 43.8 ± 7.63 (cataract-free ≤ 55 years), 72.5 ± 7.6 years (cataract ≥ 60 years), and 66.5 ± 4.3 years (cataract-free ≥ 60 years).

All participants underwent a standard ophthalmologic examination, including best-corrected visual acuity (BCVA), refraction, intraocular pressure measurement, and ultrasound biometry. The examination included observation of the dilated anterior segment using a slit lamp to verify the presence or absence of cataract (nuclear, cortical, and posterior subcapsular) and ocular fundus evaluation. Lens transparency was graded according to the validated Lens Opacities Classification System (LOCS) III.

Inclusion criteria for patients were BCVA < 0.5 Snellen decimal after excluding any other ophthalmological pathology that could explain the loss of visual acuity and the presence of cataract confirmed by ocular examination. For controls, inclusion criteria were BCVA = 1 in the absence of cataracts according to the LOCS

Table 1. Clinical and demographic characteristics of the subjects included in our study

	Cases ≤ 55 years, n %	Controls ≤55 years, n %	Cases ≥ 60 years, n %	Controls ≥ 60 years, n %
Sample size	46	47	56	56
Gender				
Men	28 (60.9)	21 (44.7)	25 (44.6)	18 (32.1)
Women	18 (39.1)	26 (55.3)	31 (55.4)	38 (67.9)
Hypertension	9 (19.6)	4 (8.5)	32 (57.1)	30 (53.6)
Diabetes	10 (21.7)	7 (14.9)	10 (17.9)	13 (23.2)
Smoking	11 (23.9)	11 (23.4)	9 (16.1)	10 (17.9)

III. All subjects, cases and controls, met the same criteria in terms of place of residence or out/indoor job (to control environmental risks such as sun exposure).

Exclusion criteria were present or past treatment-related to the development of cataract (corticosteroids, vitreoretinal surgery, chemotherapy, and radiation), alcohol abuse, and any local (eye trauma, uveitis, and glaucoma) or systemic disease associated with cataract (Steinert disease, metabolic disorders, Marfan syndrome, and other skeletal muscle diseases).

DNA extraction

Genomic DNA was extracted from nucleated peripheral blood cells following the phenol-chloroform method, as previously reported¹⁶.

Telomere length analysis

Telomere length was measured by relative and comparative quantitative polymerase chain reaction (PCR). We compared mean telomere repeat sequence copy number with the reference single copy gene 36B4. For the each DNA sample, we performed six different experiments: 3 (for telomere repeat sequence) and 3 (for single-copy gene). The method was described by Gil and Coetzer in 2004¹⁷ using the same primers developed by Cawthon in 2002 for telomere length¹⁸ and the 36B4 gene as endogenous control¹⁹. The telomere-specific primers were forward, 5'-GGTTTTTGAGGGTGAG-GGTGAGGGTGA GGG TGAGGGT-3' and reverse, 5'-TCCCGACTATCCCTATCCCTATCCCTATCCC TATC-CCTA-3'. The 36b4-specific primers were forward, 5'-CAGCAAGTGGGAAGG TGTAATCC-3' and reverse, 5'-CCC ATTCTATCATCAACGGGTACAA-3'²⁰. The control gene 36B4 was amplified under the same PCR conditions as the telomere gene, thus reducing the risk of inter-plate variation.

The following was added to each well: 5 ng/μL of DNA, 5 mL of SYBR Green PCR Master Mix (Kapa Biosystems) and 4 mL of H₂O. We also added 1 μL of "forward" primer (5 μM) and 2.33 μL of "reverse" primer (5 μM) for the telomere amplification reaction, and 0.4 μL (5 μM) of each primer for the amplification of the 36B4 gene. The thermal cycling profile proceeded as follows: 10 min at 95°C followed by 35 cycles of 95°C for 15 s, 54°C for 2 min, and 72°C for 15 s²¹. qPCR amplification was performed using the Step-One Plus Real-time PCR system (Applied Biosystems). The RQ Manager Software version 1.2 was used to determine relative telomere length.

The Ct value, defined as the number of PCR cycles needed for the amplified DNA to cross a predefined threshold, was used to calculate the telomere repeat copy number to single-copy gene copy number ratio (T/S) using $T/S = 2^{2DCt}$. The relative telomere length was determined by normalizing the ratio (T/S) of each sample to the calibrator DNA to standardize sample values across all reaction plates. For ease of data manipulation, the natural log of this value was used²².

Telomerase polymorphism analysis

Telomerase gene DNA polymorphisms were measured using a real-time PCR allelic discrimination procedure conducted in a total volume of 12 μL per reaction. DNA (2.5 μL) was placed in a 9.5 μL reaction mixture containing 0.25 μL of oligonucleotide primers and TaqMan probes (VIC/FAM), 5 μL of TaqMan® Universal PCR Master Mix No AmpErase® UNG (Applied Biosystems), and made up with double-distilled H₂O to a final volume of 12 μL. Following the manufacturer's instructions for the Step-One Plus Real-time PCR system (Applied Biosystems), the cycling protocol consisted of an initial 10-min denaturation step at 95°C, followed by

Table 2. Sequences of VIC and FAM probes (5'-3') used for TERT-1327C > T and TERC-63G > A polymorphism detection by real-time PCR

Polymorphism	TaqMan Probe (Applied Biosystems)	Sequence 5'-3' (VIC/FAM)
TERT-1327C > T (rs2735340)	C_1839086_10	TCGCTCTGTAAATACTTAGGATTAC(A/G)GGTCGCTCTTCTAGAAATCCCTTA
TERC-63G > A (rs2293607)	C_16184740_10	AGGTTTGGGGGTTACAAAGCCCCA(C/T)TGCCGGCGAGGGGTGACGGATGCGC

40 cycles of denaturation at 95°C for 15 s, annealing at 65°C for 60 s, and an extension stage at 60°C for 30 s.

The sequences of VIC and FAM probes used for polymorphism detection are detailed in [table 2](#).

Statistical analysis

Statistical analysis was performed using SPSS Statistics for Windows software, version 2.0 and the GenEx 5.3.6 program developed to calculate telomere length. To confirm the GenEx software results, we used a Mann–Whitney U test.

Associations between leukocyte telomere length and TERT/TERC polymorphisms (single nucleotide polymorphisms [SNPs]) were modeled using a Chi-square test.

As some data followed a normal distribution and others (age) showed a non-parametric distribution, analysis of variance (ANOVA) or the Mann–Whitney U test was used to examine the influence of possible confounding variables (diabetes, smoking, hypertension, and age) on telomere length in patients with cataracts. We also performed a multivariate ANOVA (MANOVA) to compare these dependent variables together (diabetes, smoking, hypertension, and age) in the same groups.

Results

Significant differences were observed in relative telomere length between subjects with cataracts and those without cataract regardless of age ($p < 0.01$). As expected, according to the impact of age on telomere length, subjects without cataract aged 60 years or older had shorter telomeres than those 60 years or under ($p < 0.01$). However, we found significant differences ($p < 0.01$) in telomere length in subjects with cataract unrelated to age. Interestingly, the younger subjects with cataract had shorter telomeres than older individuals with cataract ([Table 3](#): leukocyte telomere lengths compared between study groups).

Table 3. Leukocyte telomere lengths compared between study groups

	Telomere length	p-value
Cases vs. Controls	2.88 ± 1.43 5.17 ± 1.58	P<0.01
Cases ≤ 55 years vs. Controls ≤ 55 years	2.36 ± 1.56 5.85 ± 1.58	P<0.01
Cases ≥ 60 years vs. Controls ≥ 60 years	3.30 ± 1.17 4.69 ± 1.30	P<0.01
Cases ≤ 55 years vs. Cases ≥ 60 years	2.36 ± 1.56 3.30 ± 1.17	P<0.01
Controls ≤ 55 years vs. Controls ≥ 60 years	5.85 ± 1.58 4.69 ± 1.30	P<0.01

The hypothesis that inter-individual variations in TERT and TERC telomerase genes could be associated with lesser telomerase activity and, therefore with a shorter leukocyte telomere length was not confirmed in any group of subjects included in our study ([Table 4](#): genotype frequencies of TERT and TERC SNPs in cases and controls).

Our analysis reveals that the relationship between telomere length and cataract was not affected by cardiovascular risk factors such as diabetes, smoking, and hypertension across all groups.

In patients with cataracts, MANOVA indicated no significant ($p > 0.05$) differences in leukocyte telomere length according to the factors hypertension, diabetes, smoking, and age. The p-value obtained in the ANOVA test for each of these cardiovascular risk factors was > 0.05 .

However, as age does not follow a parametric distribution, we used the Mann–Whitney U test and a significant effect of age on leukocyte telomere length was then observed ($p < 0.05$).

Discussion

Human cataractogenesis is characterized by the senescence of lens cells. This senescence is expedited by

Table 4. Genotype frequencies of TERT and TERC SNPs in cases and controls

SNP	Genotype	Cases n (%)	Controls n (%)	p-value
TERT rs2735940	TT	31 (3.4)	26 (25.2)	0.7
	TC	54 (52.9)	58 (56.3)	0.44
	CC	17 (16.7)	19 (18.4)	0.44
	TT	31 (3.4)	26 (25.2)	
	TC + CC	71 (69.9)	77 (74.8)	
	TT + TC	85 (83.3)	84 (81.55)	
	CC	17 (16.7)	19 (18.4)	
TERC rs2293607	AA	60 (58.8)	69 (67.7)	0.38
	AG	38 (37.3)	29 (28.2)	0.25
	GG	4 (3.9)	5 (4.9)	1
	AA	60 (58.8)	69 (67)	
	AG + GG	42 (41.2)	34 (33)	
	AG + AA	98 (96.1)	98 (95.1)	
	GG	4 (3.9)	5 (4.9)	

SNP: polymorphism; A: adenine; G: guanine; T: thymine; C: cytosine;
TERT: telomerase reverse transcriptase; TERC: telomerase RNA component.

oxidative stress and non-ionizing radiation in the form of ultraviolet light that induces DNA damage due to oxygen-derived free radicals. Due to the absence of telomerase activity in the lens, the oxidative attack by phospholipid hydroperoxides present in the aqueous humor and lens cell membrane damage likely produce telomere shortening^{1,6,23,24}. Therefore, increased oxidative stress and the lack of reductive detoxification of phospholipid hydroperoxides are the triggering mechanisms of telomere shortening and human cataractogenesis.

Here, we observed that greater lens transparency was related to a longer leukocyte telomere length, as has been reported by other groups^{6,13,23}.

To the best of our knowledge, this is the first study to examine leukocyte telomere length in young patients with cataract and to report relatively short lengths for the age range. We observed that younger persons with cataract had shorter telomeres than age-matched controls without cataract. Moreover, this group of patients also showed a shorter telomere length compared to older subjects with cataracts.

We speculate that this finding may reflect premature aging in this population. Accordingly, the onset of cataracts in young adults may serve as an *in vivo* marker of natural aging and could also warn of other conditions associated with telomere shortening and aging such as glaucoma, age-related macular degeneration²⁵, cardiovascular disease, or cancer. Telomeres could be therefore considered novel biomarkers of aging and also predictors of the risk of certain age-related degenerative diseases. Thus, telomere length could help monitor the efficiency of prevention or/and strategies aimed at modifying the aging process.

The lens has several real advantages as a marker of aging. For example, using noninvasive, nonhazardous, and quick imaging techniques, we can measure its transparency on a continuous scale. Moreover, the use of a grading system such as the LOCS III offers a reproducible and accurate way to determine lens transparency.

Our results also revealed that the TERT and TERC gene polymorphisms, rs2735940, and rs2293607 were not associated with telomere shortening in any group, including younger people with cataracts. This means that the telomere shortening observed in our study in young people with cataracts was not associated with these SNPs.

Several reports⁷⁻¹¹ inform a link between short telomere length and cardiovascular risk factors, including hypertension, diabetes, or smoking. We analyzed the relationship between potential confounders (diabetes, hypertension, and tobacco) and leukocyte telomere length. Our study participants were stratified by age, sex, and cardiovascular disease, as shown in Table 1. In patients with cataracts, no relationship emerged in our multivariate analysis between telomere length, age, and cardiovascular risk factors.

This indicates that the telomere shortening in young patients with cataracts observed in our study was not influenced by cardiovascular risk factors or telomerase polymorphisms, meaning that this shortening could be directly associated with cataracts. Future longitudinal studies are needed to confirm our hypothesis and strengthen the idea that early cataracts could indicate premature aging and the possible presence of age-related disease.

Several limitations should be considered to interpret our results. Telomere length in peripheral leukocytes may not reflect telomere length alterations in lens cells, although several studies have shown their good correlation^{12,13,24}. Furthermore, as we wanted to examine a cohort of subjects representative of the general population, women were not excluded from the study. However, it is well known that adult women have a longer leukocyte telomere length than men²⁶, although, at birth, telomere length is equivalent in boys and girls^{12,27}. Another limitation is that cigarette smoking accelerates telomere shortening and this has been linked to multiple negative health outcomes over a person's lifetime²⁸. Some studies have found a dose-response relationship between cumulative lifetime exposure to cigarette smoking and telomere length in adults²⁹. Nevertheless, to avoid these confounding factors, we balanced out smokers in all the groups included in our study.

Finally, other factors such as shelterin complex^{30,31} should be considered. Telomeres consist of tandem repeats of a DNA sequence connected to six-protein

components known as shelterins³⁰, being Telomere Repeat binding Factor 1 (TRF1) and Adrenocortical Dysplasia Protein Homolog (TPP1) the most relevant proteins in mammals. Shelterin proteins are specialized in protecting telomeres from DNA repair mechanisms and in regulating telomerase activity. Accordingly, the suppression of TRF1 and TPP1 results in the development of age-related and degenerative pathologies such as dyskeratosis congenita, aplastic anemia, and idiopathic pulmonary fibrosis in young mice. These diseases are associated with short/dysfunctional telomeres and a characteristic failure in the regenerative ability of tissues³¹. Consequently, there are other complexes such as shelterin, in addition to telomerase polymorphisms, that act on this enzyme conditioning telomere length, and therefore, they may participate in the appearance of age-related diseases such as cataracts.

While it is difficult to recruit young patients with cataracts, the link observed here between a short telomere length and premature cataract requires confirmation in larger patient series.

Conclusions

We report for the first time that younger people with cataract show a shorter leukocyte telomere length compared to subjects without cataract and that this relationship is not affected by cardiovascular risk factors or telomerase polymorphisms. If this link is confirmed in larger patient series, we hypothesize that the early development of cataracts and short telomere length could be a marker of premature aging.

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Conflicts of interest

The authors report no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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