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Shorter leukocyte telomere length is associated with severity of COVID-19 infection.

Gabriel Arantes dos Santos [a,*], Ruan Pimenta [a], Nayara I. Viana [a,c], Vanessa R. Guimarães [a], Poliana Romão [a], Patrícia Candido [a], Juliana A. de Camargo [a], Diná M. Hatanaka [b], Paula GS. Queiroz [b], Alexandre Teruya [b], Katia R.M. Leite [a], Victor Srougi [a,b], Miguel Srougi [a], Sabrina T. Reis [a,b,c]

[a] Urology Department, Laboratory of Medical Investigation (LIM55), Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil
[b] Moriah Hospital, São Paulo, SP, Brazil
[c] Minas Gerais State University (UEMG), Passos, MG, Brazil

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ABSTRACT

The infection by COVID-19 is a serious global public health problem. An efficient way to improve this disease's clinical management would be to characterize patients at higher risk of progressing to critically severe infection using prognostic biomarkers. The telomere length could be used for this purpose. Telomeres are responsible for controlling the number of maximum cell divisions. The telomere length is a biomarker of aging and several diseases. We aimed to compare leukocyte telomere length (LTL) between patients without COVID-19 and patients with different clinical severity of the infection. Were included 53 patients who underwent SARS-CoV-2 PCR divided in four groups. The first group was composed by patients with a negative diagnosis for COVID-19 (n = 12). The other three groups consisted of patients with a confirmed diagnosis of COVID-19 divided according to the severity of the disease: mild (n = 15), moderate (n = 17) and severe (n = 9). The LTL was determined by Q-PCR. The severe group had the shortest LTL, followed by the moderate group. The negative and mild groups showed no differences. There is an increase of patients with hypertension (p = 0.0099) and diabetes (p = 0.0067) in moderate and severe groups. Severe group was composed by older patients in comparison with the other three groups (p = 0.0083). Regarding sex, there was no significant difference between groups (p = 0.6279). In an ordinal regression model, only LTL and diabetes were significantly associated with disease severity. Shorter telomere length was significantly associated with the severity of COVID-19 infection, which can be useful as a biomarker or to better understand the SARS-CoV-2 pathophysiology.

1. Introduction

The coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2 infection imposes a major threat to the world's healthcare systems and has caused thousands of deaths. Very little is known regarding molecular changes involved in the infection progression and severity. Leukocyte Telomere Length (LTL) is considered a risk factor to infections in general and could be associated with the clinical presentation of COVID-19 [1].

Telomeres are nucleoprotein structures formed by tandem repeats, at the ends of eukaryotic chromosomes, responsible for maintaining genomic stability and controlling the maximum number of cell divisions [2]. In each round of cell division, telomeres lose part of their genetic material. The telomerase is an enzyme responsible for preventing this process, but it is suppressed in the vast majority of somatic cells. Thus, telomeres shorten after every mitosis and when their size becomes critically short, the cell loses its viability [3]. That is why telomeres are considered molecular clocks and are used as biomarkers in various diseases.

In a recent study, Aviv et al. reported that shorter telomere length could predict a higher risk of dying from COVID-19 [4]. In the present study, we sought to test this hypothesis by comparing the LTL in patients with COVID-19, with different clinical severity.

* Corresponding author.
E-mail address: arantes_gabriel@hotmail.com (G.A. dos Santos).

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2. Methods

2.1. Patients

This study was approved by the IRB of the Medical School of the University of São Paulo under the number 4.113.149. All participants signed the informed consent form. All patients were selected from Moriah Hospital and HCFMUSP (Sao Paulo, Brazil) in a COVID-19 standard protocol.

The study comprised 53 patients divided into 4 groups according to their clinical presentation severity and pharyngeal swab RT-PCR test for SARS-CoV-2. Group I was formed by 12 symptomatic patients, without respiratory dysfunction, with a negative swab test. Group II was formed by 15 symptomatic patients, without respiratory dysfunction, with a positive swab test (no hospitalization required). Group III consisted of 17 patients with respiratory dysfunction, who needed hospitalization, with a positive swab test. Group IV was formed by 9 patients (with a positive swab test) hospitalized in an intensive care unit (ICU), who present severe respiratory dysfunction and needed mechanical ventilation. They agreed to participate in the study through their representatives. Respiratory dysfunction was defined as any need for oxygen support. Blood samples were collected from patients with active infection for LTL analysis.

2.2. DNA extraction

Genomic DNA was extracted from blood using PureLink Genomic DNA Mini Kit (Applied Biosystems, CA, USA) according to the manufacturer's recommendations. The DNA concentration and quality was measured in a spectrophotometer NanoDrop ND-1000 (Thermo-Scientific, MA,USA).

2.3. Leukocyte telomere length

LTL was determined by the Q-PCR technique as described by Cawthon [5]. Briefly, this method compares the telomere repeat sequence copy number (T) to a reference single-copy gene copy number (S) in each sample. This methodology uses the (T/S) ratio to compare the relative telomere length (by ΔΔCt method) between the samples.

The primers set were from ScienCell's Relative Human Telomere Length Quantification qPCR Assay Kit (CA, USA). The telomere length was determined in a 10 μL reaction containing 5 μl 2× qPCR Sygreen Mix (PCR Biosystem PA, USA) 0.5 μl primer and 3.5 μl H_2O. Each reaction contained 5ng of DNA. All reactions were performed in duplicates, with controls included in the assay.

The Q-PCR was performed in an ABI 7500 Fast Real-Time PCR System (Applied Biosystems, CA, USA). The PCR cycling conditions were: 2 minutes at 50 °C, 10 minutes at 95 °C, and 35 cycles of 20 seconds at 95 °C, 20 seconds at 52 °C and 45 seconds at 72 °C.

2.4. Analysis of results

The graphs and the statistical analysis were performed using GraphPad Prism 8 software. The D'Agostino & Pearson test was applied to test whether the data were parametric or non-parametric. Age was parametric, therefore, assessed using the ordinary one-way ANOVA test. To compare the LTL (non-parametric) between groups, we utilized the Kruskal-Wallis test. Differences in sex, hypertension and diabetes between groups were assessed using the chi-square contingency analysis.

Ordinal regression was performed on SPSS software (23.0). In this analysis, we used the three positive groups for COVID-19. We ordered the groups according to their severity (i.e. Group II = 1, Group III = 2 and Group IV = 3 in the regression). For diabetes and hypertension, we classify according to absence = 0 or presence = 1. For sex we consider male = 1 and female = 0. For telomere length and age, we segregate samples using median as a cutoff. Younger/longer LTL patients were classified as "0" and older/shorter LTL patients were classified as "1". The result was considered significant when $p \leq 0.05$.

3. Results

Fig. 1 illustrates the LTL differences between groups. Group IV presented a shorter LTL than group I ($p < 0.0001$), group II ($p < 0.0001$) and group III ($p = 0.04$). Group III presented significantly shorter telomeres than group I ($p = 0.01$). Group III also presented shorter LTL than group II, but with a marginal p-value ($p = 0.09$). There was no difference between groups I and II ($p > 0.99$).

Table 1 represents the groups' composition regarding age, sex, hypertension and diabetes. There is a relative increase of patients with hypertension ($p = 0.0099$) and diabetes ($p = 0.0067$) in group III and IV. Group IV was composed by older patients in comparison with the other three groups ($p = 0.0083$). Regarding sex, there was no significant difference between groups ($p = 0.6279$), but groups III and IV showed a greater proportion of males.

Next, we performed an ordinal regression considering COVID-19 severity, i.e., only including group II, III and IV (Table 2). Both diabetes and LTL fit into our model and were associated with COVID-19 severity ($p < 0.05$). Sex, age and hypertension ($p > 0.05$) do not show significant association with disease severity in our regression model.

4. Discussion

Telomere length is an important biomarker for aging and several diseases [6]. The elderly present shorter telomeres when compared to youths [7]. Shorter telomeres were reported to be associated with a higher risk for cardiovascular diseases and diabetes [8–10]. Studies also suggest that male had shorter telomere than females [11]. Of note, all these conditions are concerning risk factors for clinical severity and

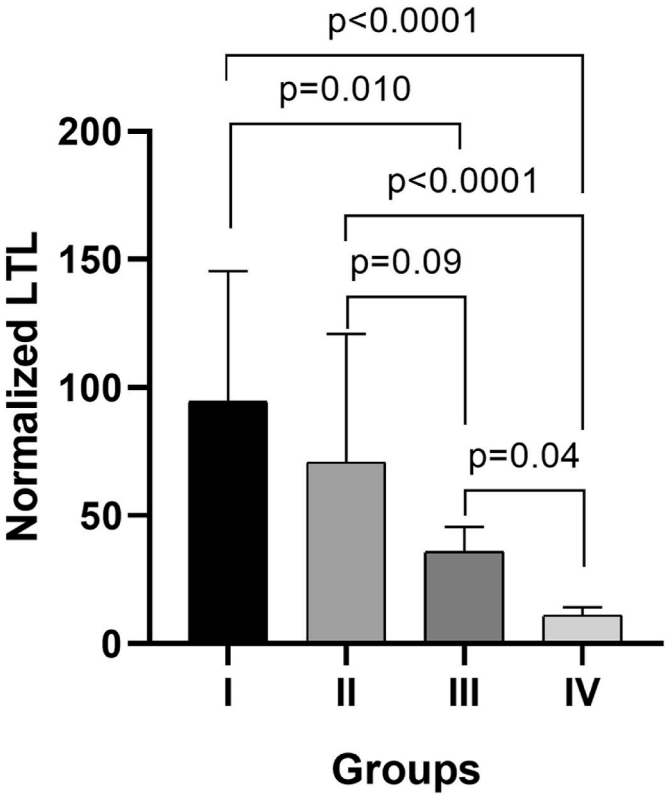

Fig. 1. Comparison between LTL of groups I, II, III and IV. The telomere length was normalized by a single copy gene. The y axis is in arbitrary unit. The p value is determined by Kruskal-Wallis test.

Table 1
Patients characteristics.

Group	I	II	III	IV	p-value
			Age (mean)[a]		
	39.8 (±11.37)	47.47 (±11.62)	40.47 (±11.34)	56.11 (±11.72)	**0.0083**
			Sex (n)[b]		
Female	6	7	5	3	0.6279
Male	6	8	12	6	
			Hypertension (n) [b]		
No	11	13	13	3	**0.0099**
Yes	1	2	4	6	
			Diabetes (n)[b]		
No	12	15	12	5	**0.0067**
Yes	0	0	5	4	

[a] Ordinary one-way ANOVA.

[b] Chi-square.

Table 2
Ordinal regression model between risk factors and COVID-19 severity.

Parameter Estimates			
Group*	Parameter	Odds Ratio	p-value
III	Sex	0.114	0.82
	Age	12.87	0.54
	LTL	0.043	**0.033**
	Diabetes	1.88×10^{-10}	**<0.0001**
	Hypertension	2.118	0.691
IV	Sex	0.105	0.116
	Age	3.327	0.458
	LTL	0.002	**0.003**
	Diabetes	1.25×10^{-10}	**<0.0001**
	Hypertension	0.225	0.36

This reduced model is equivalent to the final model because omitting the effect does not increase the degrees of freedom. * The reference category is group II.

death of SARS-COV-2 [12].

In the present study, we showed a progressive decrease in telomere length according to the severity of the disease, as suggested by Aviv [4]. The author hypothesizes that people with shorter telomeres deplete their T lymphocytes quicker, hampering the immune response to COVID-19 infection. Interestingly, T lymphocytes have low telomerase activity and shorter telomeres than other leukocytes [13,14].

Inflammation could also influence telomere stability by generating reactive oxygen species (ROS), which are particularly harmful to telomeres DNA [15]. Therefore, inflammation could accelerate telomere shortening. Additionally, critically short telomeres induce cell senescence that perpetuates inflammation and generates a positive feedback continuous cycle [16].

It is known that patients with critically severe presentations of COVID 19 have intense systemic inflammatory responses [17]. This could partially explain the differences between the groups I and III/IV: people with naturally shorten telomeres have complications in the course of COVID 19 infection, which promotes a great inflammatory process which shorten telomeres even more.

Of note, telomere length is influenced by genetic and environmental factors [18,19]. For example, smokers have shorter telomeres than nonsmokers and, as demonstrated in a recent study, there are an association between COVID-19, smoking and telomere maintenance [20,21].

The great limitation of this study is that shorter LTL could not be directed associated with the severity of the disease, but explained by a third factor. Age is one of the main determinants of telomere length, and the group IV was composed by older patients compared to the other three groups. As our cohort was small, age adjustments were not viable. In any case, when applying an ordinal regression model, both LTL and diabetes are associated with the severity of the disease, which suggests that LTL may be useful in predicting the vulnerability of a severe course of COVID-19 infection. In addition, as previously discussed, LTL is known to be a risk factor for diabetes (and other comorbidities related to SARs-CoV-2). However, it is important to emphasize that although age, sex and hypertension are not statically associated with the severity of the disease, we have a higher concentration of older, male and hypertensive patients in the most severe groups (III and IV) which probably influenced the infection severity.

Here we need to consider that, with the exception of LTL, all other parameters studied are known to be risk factors for the severity of COVID-19 [22]. This raises the question whether the telomere length merely reflects age and age-related diseases or can independently predict COVID-19 clinical course. Our regression model indicates that LTL can be an independent factor for the severity of the disease, conclusion reinforced by recent studies [23–25].

The use of LTL in clinical practice is still limited and controversial [26]. For example, the Q-PCR technique used in this work had the advantage of being easy to perform; requiring a small amount of initial sample, had many population-based studies for comparisons and presents commercial kits. But, variations between the different laboratories and the fact that it provides only LTL as a relative ratio (and not the absolute telomere length) still limit the application of this method [27]. Either way, although LTL measurement is not yet clinically applicable, it could provide useful insights in diseases progression and a recent study confirms that leukocyte telomeres are representative of the length of the body's telomeres [6,28,29].

It is also important to highlight that, to be considered a potentially applicable biomarker, the variable needs to be technically accessible and easy to interpret, which can advocate against the use of Q-PCR for measuring telomere length, since it has relatively low accuracy and does not yet have a clinically useful cut-off point [30]. Despite this, the other methodologies that measure telomere length (usually based on fluoroscopy, labeled probes or cytometry) also have their limitations, especially on the high cost/time per assay [27]. In addition, studies reinforce a great correlation between the telomere length measured by different techniques, which reinforces that Q-PCR may be the technique of choice for telomere length in clinical use [24]. For example, in a scenario where other clinical parameters and comorbidities may leave doubts about whether the patient should be observed or recover at home, collecting a blood sample and performing a simple PCR assay may be useful. Either way, if the telomere length can be used as a biomarker (standalone or in conjunction with other parameters) or just provides insights into the pathophysiology of COVID-19 needs to be further investigated.

Despite the low number of patients, we aimed to provide a short communication regarding the role of the telomere length in the severity of COVID-19 infection. We present a novel finding that could be used to stratify patients with a greater risk of severe illness. The present study is part of a prospective larger trial with the purpose of providing a definitive answer to this question.

In summary, we present an experimental report that correlated shorter telomeres with the severity of COVID-19 infection. Telomere length could be explored as a prognostic factor in such disease. Future studies on this topic should include more patients and analyze telomere maintenance pathways, which may influence LTL.

Declaration of competing interest

The authors declare that they have no conflict of interest.

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Data Sharing

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate: This study was submitted and approved by the Research Ethics Committee of the University of Sao Paulo Medical School under the number 4.113.149. All participants signed the informed consent form. Availability of data and materials: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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Author statement

Ethics approval and consent to participate: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

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