Analysis of Relative Average Length of Telomeres in Leukocytes of Women with COVID-19

D. S. Krasnienkov[a], O. V. Gorodna[b], T. M. Kaminska[c], V. V. Podolskiy[d], Vl. V. Podolskiy[d], M. V. Nechyporenko[b], Yu. G. Antypkin[d], and L. A. Livshits[b, *

[a] Chebotarev Institute of Gerontology, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
[b] Institute of Molecular Biology and Genetics, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[c] Kyiv City Children's Clinical Hospital of Infectious Diseases, Kyiv, Ukraine
[d] Lukyanova Institute of Pediatrics, Obstetrics and Gynecology, National Academy of Medical Sciences of Ukraine, Kyiv, Ukraine
*e-mail: livshits@edu.imbg.org.ua
Received July 5, 2022; revised July 13, 2022; accepted November 8, 2022

Abstract—Coronavirus disease (COVID-19) is an acute infectious disease of the respiratory tract caused by a new SARS-CoV-2 coronavirus. A global vaccination program against SARS-CoV-2 continues, and the incidence of COVID-19 worldwide is significantly decreasing. However, among millions of those who survived COVID-19, numerous groups will need assistance due to increased clinical consequences after COVID-19. Currently, there is a need to search for molecular biomarkers for monitoring the onset and progression of post-COVID syndrome. For this purpose, the relative average length of chromosome regions was studied in the groups of women of reproductive age: in the group of patients ($n = 64$) recovered from COVID-19 and in the control group ($n = 42$) of women of the same age. The analysis was carried out using a method of multiplex monochrome quantitative real-time PCR on DNA samples isolated from the peripheral blood leukocytes. According to the results of the study, it was established that the relative average length of chromosomes in the peripheral blood leukocytes was statistically significantly lower in the group of patients with COVID-19 than in the control group ($p < 0.05$). The results obtained allow one to state that the observed shortening of the relative average length of telomeres in the group of patients that recovered from COVID-19 can indicate that SARS-CoV-2 infection can directly cause the erosion of telomeres in the blood cells, particularly, in leukocytes. Thus, the determination of the relative average length of telomeres can be an informative prognostic marker for estimating the risk of the severity of COVID-19 disease and the development of post-COVID syndrome.

INTRODUCTION

Coronavirus disease (COVID-19) is an acute infectious disease of the respiratory tract caused by a new coronavirus (SARS-CoV-2) and now, according to the order of the International Committee on Taxonomy of Viruses, is called severe acute respiratory syndrome coronavirus 2 (Velavan et al., 2020). This disease became known first on December 31, 2019, when WHO was informed about the cases of pneumonia of undetermined microbial etiology in Wuhan, Hubei Province, China. After the first reported case of the disease in China, the virus spread rapidly to other countries in Asia, Central and Northern Europe, and America. In a matter of weeks, a local outbreak became threatening and turned into a global problem. On January 30, 2020, WHO declared COVID-19 a major emergency in the field of public health (Ren et al., 2020). As of May 1, 2021, the number of SARS-CoV-2 infected worldwide reached almost 170 million and 3.5 million lethal outcomes.

A global vaccination program against SARS-CoV-2 continues, and the incidence of COVID-19 worldwide is significantly decreasing. However, among millions of those who survived COVID-19, numerous groups will need assistance due to increased clinical consequences after COVID-19 determined as a post-COVID syndrome (Oronsky, 2021; Walitt, 2021). Despite a number of manifestations associated with post-COVID syndrome, there is a lack of potentially significant molecular biomarkers to monitor the onset and progression of post-COVID syndrome.

In the previous studies, data on the effect of viral and bacterial infections on the estimation of biological age determined as DNAmAge (performed using the analysis of the length of telomeric regions of chromosomes) were obtained (Horvath and Levine, 2015; Kananen et al., 2015; Sabry et al., 2016). It was estab-

lished that shorter telomeres are associated with the risk of the development of more severe symptoms of COVID-19 (Froidure et al., 2020). It is important to note that there was a significant shortening of telomeric regions of chromosomes in the group of patients with post-COVID syndrome as compared with such index in the control group. It should be noted that the differences were most significant among the population under 60 years old (Mongelli et al., 2021).

The possibility that changes in telomere length characterizing a high risk of the development of severe course of COVID-19 disease can overlap with those associated with aging is discussed (Mongelli et al., 2021). Therefore, the aim of this study was to analyze the association between the length of telomeric regions of the chromosomes of peripheral blood leukocytes and past SARS-CoV-2 infection in women of reproductive age from Ukraine.

MATERIALS AND METHODS

Analysis of Clinical Data and Sample Formation. As a part of this work, biological samples of the peripheral blood were collected. Study group one (64 patients) consisted of women of reproductive age who were treated in the infectious disease department of the hospital (Kyiv, Ukraine). The diagnosis of COVID-19 was confirmed using PCR test. The control group consisted of healthy women (42 individuals) with a negative result of PCR test for COVID-19. All individuals of this group are residents of Kyiv (Ukraine).

According to the basic rules of bioethics, when using a human as an object of study, we obtained informed consent to conduct this study from all test subjects, and the nomenclature of peripheral blood samples, which included a numerical code, was introduced.

DNA Samples. Genomic DNA samples were isolated from the venous blood with a reagent kit for the isolation of nucleic acids DNA-TECHNOLOGY "PROBA-NK-PLYUS" according to the manufacturer's protocol.

The quality of DNA preparations was determined by spectral characteristics, and an ND-1000 Spectrophotometer instrument (NanoDrop, United States) in the range $\lambda 220 - \lambda 300$ was used.

Determination of the Length of Leukocyte Telomeres using a Method of Multiplex Monochrome Quantitative Polymerase Chain Reaction. To estimate the relative average length of telomeres, a quantitative monochrome multiplex real-time polymerase chain reaction was performed using a method proposed by R. Cawthon (2009).

To carry out this reaction, a "real-time PCR kit" in the presence of EvaGreen intercalating dye (Solis BioDyne production) was used in accordance with the manufacturer's recommendations on a Bio-Rad Chromo4 amplifier. Per a single sample, 10 ng DNA, as well as a pair of primers telg and telc (final concen-

tration, 450 nmol each), as well as a pair of albumin primers albu and albd (final concentration of each, 250 nmol) were added to 18 µL of the reaction mixture to amplify in vitro the albumin reference gene. The composition of the reaction mixture and temperature and time regimes of multiplex monochrome quantitative PCR for estimating the relative average length of telomeric regions of the chromosomes were described in detail in our previous studies (Khalangot et al., 2020).

After the completion of thermal cycling, fluorescence data on each PCR cycle were obtained using an Opticon Monitor 3 program. For each well/eppendorf, the Richardson function was adjusted with subsequent calculation of Cy0 (Guescini et al., 2008). This is a non-threshold method, which allows us to increase significantly the accuracy of quantitative PCR by taking into account the reaction kinetics in each test tube. Subsequently, the standard curves were constructed: one curve for a telomeric signal (T) and another for a signal from a single-copy gene (S). By Cy0, the amount of telomeric DNA relative to reference DNA was determined using a calibration line; similarly, data for the amount of albumin DNA were obtained. The values of relative average length of telomeres were obtained by T/S ratio.

Statistical Analysis. Shapiro−Wilk test was used to verify the normality of the distribution of the obtained indices. Nonparametric Mann−Whitney test (U test) was used to estimate the difference in the distribution of indices. Statistical processing was carried out in a Statistica 10 software (StatSoft).

RESULTS AND DISCUSSION

According to the results of molecular genetic analysis, characteristics of the relative average length of chromosomes (value of T/S ratio) were obtained from study groups one and two for each individual. It should be noted that these indices in the group of women that recovered from COVID-19 (study group one) varied within $0.01-8.2$ (T/S), while the value $0.14-4.65$ (T/S), respectively, was observed in the control group. We estimated the distribution of the relative average length of telomeres in both studied groups. According to the results of testing the normality of distribution of variables using the Shapiro−Wilk test, evidence was obtained that the distribution of telomere length in the studied groups (Table 1) does not correspond to normal ($p < 0.000001$).

A comparative analysis of the relative average length of telomeres in the group of women that recovered from COVID-19 and female individuals from the control group was carried out using the Mann−Whitney U test. It was established that women that recovered from COVID-19 had statistically significantly shorter telomeres as compared from individuals of the control group ($p < 0.01$). The characteristics of T/S distribution (relative average length of leukocyte telo-

Table 1. Parameters of regularities of the distribution of T/S indices (relative average length of leukocyte telomeres) among women that recovered from the COVID-19 disease and healthy female individuals from the control group

Study group	N	T/S ratio (relative average length of telomeres)				
		minimum	Q1 (quartile)	median	Q3 (quartile)	maximum
1	64	0.01	0.19	0.46	1.03	4.65
2 (control)	42	0.47	0.8	1.49	2.55	8.2

meres) in women that recovered from COVID-19 and the control group are given in Fig. 1.

Our observations are consistent with preliminary data indicating an increase in the risk of complications with COVID-19 in patients with short telomeres, which can be caused by reduced regenerative potential (Sanchez-Vazquez et al., 2021). It is important to note that shorter telomeres were also observed among patients with a respiratory distress syndrome. It was demonstrated that shorter telomeres in the peripheral blood leukocytes were observed in patients with higher mortality (Albrecht et al., 2014; Dos Santos et al., 2021).

In the studies carried out by a group of authors, it was hypothesized that not a genetic component but exposome is a decisive factor in the association of shorter telomeres with the regenerative potential of an individual (Jiang et al., 2022). At the same time, a number of researchers noted that the length of telomeres by its nature should be a more powerful informative marker of complications with COVID-19 than the gender or the presence of hypertension in a patient with COVID-19. It is also important to note the interesting results obtained regarding telomere-dependent lymphopenia in elderly patients, which increases the probability of complications in case of infection with SARS-CoV-2 (Benetos et al., 2021). A rapid decrease in the ability to clonal expansion of leukocytes in

patients over 60 years was caused by a short telomere length, which determined a decrease in the efficiency of clonal expansion of T lymphocytes by ten times in the age range of 60−70 years old. Clonal expansion is critical for the efficient control of infectious diseases; accordingly, the significance of estimation of telomere length for the stratification of patients relative to this index with COVID-19 and other viral diseases is obvious (Anderson et al., 2022). A decrease in the relative average length of telomeres that we noted in female patients that recovered from COVID-19 is consistent with the results on an increase in biological age in patients that recovered from COVID-19 based on data on methylation of the promoter regions of a number of genes (Mongelli et al., 2021).

The presence of shorter in length telomeres in individuals can also be a molecular biomarker of the formation of SARS-CoV-2 antigen-specific/effector memory T cells removing viral particles from the organism (Aviv, 2021). Thus, T cells with shorter telomeres can cause a limitation of the adaptive immunity against SARS-CoV-2.

The results obtained in our study allow us to state that the observed shortening in the relative average length of telomeres in the group of patients that recovered from COVID-19 can indicate that SARS-CoV-2 infection can directly cause the erosion (a change in the spatial structure of telomeres) in the blood cells, particularly, in leukocytes, and, consequently, affect the epigenetic regulation of the expression of a number of genes due to a change in the activity of chromatin remodulation proteins that are localized in telomeric regions of the chromosomes (Tyagi et al., 2016; Magas-Acosta and Valadez-Graham, 2020). Thus, according to the results of the conducted studies, it can be concluded that the relative average length of telomeres can be an informative prognostic marker for estimating the risk of the severity of COVID-19 disease and the development of post-COVID syndrome and possible cause of premature aging in women of reproductive age.
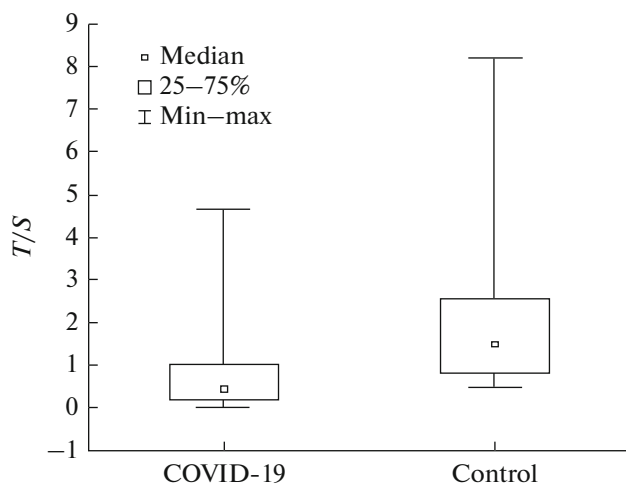

Fig. 1. Characteristics of T/S distribution (relative average length of leukocyte telomeres) in women that recovered from COVID-19 and the control group.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest. The authors declare that they have no conflicts of interest.

Statement of compliance with standards of research involving humans as subjects. The ethical approval of the study was obtained from the Bioethics Committee of the Lukyanova Institute of Pediatrics, Obstetrics and Gynecology, National Academy of Medical Sciences of Ukraine, protocol no. 28 of October 15, 2020. Informed consent was obtained from the subjects.

REFERENCES

Albrecht, E., Sillanpää, E., Karrasch, S., Alves, A.C., Codd, V., Hovatta, I., Buxton, J.L., Nelson, C.P., Broer, L., Hägg, S., Mangino, M., Willemsen, G., Surakka, I., Ferreira, M.A., Amin, N., Oostra, B.A., Bäckmand, H.M., Peltonen, M., Sarna, S., Rantanen, T., Sipilä, S., Korhonen, T., Madden, P.A., Gieger, C., Jörres, R.A., Heinrich, J., Behr, J., Huber, R.M., Peters, A., Strauch, K., Wichmann, H.E., Waldenberger, M., Blakemore, A.I., de Geus, E.J., Nyholt, D.R., Henders, A.K., Piirilä, P.L., Rissanen, A., Magnusson, P.K., Viñuela, A., Pietiläinen, K.H., Martin, N.G., Pedersen, N.L., Boomsma, D.I., Spector, T.D., van Duijn, C.M., Kaprio, J., Samani, N.J., Jarvelin, M.R., Schulz, H., Telomere length in circulating leukocytes is associated with lung function and disease, *Eur. Respir. J.*, 2014, vol. 43, no. 4, pp. 983−992.
https://doi.org/10.1183/09031936.00046213

Anderson, J.J., Susser, E., Arbeev, K.G., Yashin, A.I., Levy, D., Verhulst, S., and Aviv, A., Telomere-length dependent T-cell clonal expansion: A model linking ageing to COVID-19 T-cell lymphopenia and mortality, *EbioMedicine*, 2022, vol. 78, p. 103978.
https://doi.org/10.1016/j.ebiom.2022.103978

Aviv, A., Short telomeres and severe COVID-19: The connection conundrum, *EbioMedicine*, 2021, vol. 70, p. 103513.

Benetos, À., Lai, T.-P., Toupance, S., Labat, C., Verhulst, S., Gautier, S., Ungeheuer, M.-N., Perret-Guillaume, Ch., Levy, D., Susser, E., and Aviv, A., The nexus between telomere length and lymphocyte count in seniors hospitalized with COVID-19, *J. Gerontol., Ser. A*, 2021, vol. 76, no. 8, pp. e97−e101.
https://doi.org/10.1093/erona/glab026

Cawthon, R.M., Telomere length measurement by a novel monochrome multiplex quantitative PCR method, *Nucleic Acids Res.*, 2009, vol. 37, no. 3, p. e21.

Dos Santos, G.A., Pimenta, R., Viana, N.I., Guimarães, V.R., Romão, P., Candido, P., de Camargo, J.A., Hatanaka, D.M., Queiroz, P.G., Teruya, A., Leite, K.R.M., Srougi, V., Srougi, M., and Reis, S.T., Shorter leukocyte telomere length is associated with severity of COVID-19 infection, *Biochem. Biophys. Rep.*, 2021, vol. 27, p. 101056.
https://doi.org/10.1016/j.bbrep.2021.101056

Froidure, A., Mahieu, M., Hoton, D., Laterre, P.-F., Yombi, J.C., Koenig, S., Ghaye, B., Defour, J.-P., and Decottignies, A., Short telomeres increase the risk of severe COVID-19, *Aging*, 2020, vol. 12, no. 20, pp. 19911−19922.

Guescini, M., Sisti, D., Rocchi, M.B., Stocchi, L., and Stocchi, V., A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition, *BMC Bioinformatics*, 2008, vol. 30, no. 9, p. 326.
https://doi.org/10.1186/1471-2105-9-326

Horvath, S. and Levine, A.J., HIV-1 Infection accelerates age according to the epigenetic clock, *J. Infect. Dis.*, 2015, vol. 212, pp.1563−1573.

Jiang, L., Tang, B.S., Guo, J.F., and Li, J.C., Telomere length and COVID-19 outcomes: A two-sample bidirectional mendelian randomization study, *Front. Genet.*, 2022, vol. 23, no. 13, p. 805903.
https://doi.org/10.3389/fgene.2022.805903

Kananen, L., Nevalainen, T., Jylhävä, J., Marttila, S., Hervonen, A., Jylhä, M., and Hurme, M., Cytomegalovirus infection accelerates epigenetic aging, *Exp. Gerontol.*, 2015, vol. 72, pp. 227−229.

Khalangot, M.D., Krasnienkov, D.S., and Vaiserman, A.M., Telomere length in different metabolic categories: Clinical associations and modification potential, *Exp. Biol. Med.*, 2020, vol. 245, no. 13, pp. 1115−1121.

Magaña-Acosta, M. and Valadez-Graham, V., Chromatin remodelers in the 3D nuclear compartment, *Front. Genet.*, 2020, vol. 11, p. 600615.
https://doi.org/10.3389/fgene.2020.600615

Mongelli, A., Barbi, V., Zamperla, M.G., et al., Evidence for Biological Age Acceleration and Telomere Shortening in COVID-19 Survivors, *Int. J. Mol. Sci.*, 2021, vol. 22, no. 11, p. 6151.
https://doi.org/10.3390/ijms22116151

Oronsky, B., Larson, C., Hammond, T.C., Oronsky, A., Kesari, S., Lybeck, M., and Reid T.R., A Review of Persistent Post-COVID Syndrome (PPCS), *Clin. Rev. Allergy Immunol.*, 2021, vol. 20, pp. 1−9.
https://doi.org/10.1007/s12016-021-08848-3

Ren, L.L., Wang, Y.M., Wu, Z.Q., et al., Identification of novel coronavirus causing severe pneumonia in human: a descriptive study, *Chin. Med. J.*, 2020, vol. 133, no. 9, pp. 1015−1024.
https://doi.org/10.1097/CM9.0000000000000722

Sabry, D., Ahmed, R., Abdalla, S., Fathy, W., Eldemery, A., Elamir, A., *Braf, Kras* and *Helicobacter pylori* epigenetic changes-associated chronic gastritis in Egyptian patients with and without gastric cancer, *World J. Microbiol. Biotechnol.*, 2016, vol. 32, p. 92.

Sanchez-Vazquez, R., Guío-Carrión, A., Zapatero-Gaviria, A., Martínez, P., and Blasco, M.A., Shorter telomere lengths in patients with severe COVID-19 disease, *Aging* (Albany), 2021, vol. 13, no. 1, pp. 1−15.
https://doi.org/10.18632/aging.202463

Tyagi, M., Imam, N., Verma, K., Patel, A.K., Chromatin remodelers: We are the drivers!!, *Nucleus*, 2016, vol. 7, no. 4, pp. 388−404.
https://doi.org/10.1080/19491034.2016.1211217

Velavan, T.P. and Meyer, C.G., The COVID-19 epidemic, *Trop. Med. Int. Health*, 2020, vol. 25, no. 3, pp. 278−280.
https://doi.org/10.1111/tmi.13383

Walitt, B. and Bartrum, E., A clinical primer for the expected and potential post-COVID-19 syndromes, *Pain Rep.*, 2021, vol. 6, p. 887.

Translated by A. Barkhash