https://doi.org/10.47183/mes.2025-384

EVALUATION OF THE FREQUENCY OF ULTRA-SHORT AND ULTRA-LONG TELOMERES IN CHRONICALLY EXPOSED WOMEN

Yana V. Krivoshchapova✉, Yulia R. Akhmadullina

Southern Urals Federal Research and Clinical Center for Medical Biophysics, Ozersk, Russia

Introduction. Telomere length is considered a potential biomarker for individual human radiosensitivity and radioresistance. Radiation exposure can both increase and decrease the average telomere length in cells, while the lengths of individual telomeres vary widely. The assessment of ultra-short and ultra-long telomere frequency may indicate alterations in the replicative potential of cells and radiation-induced genomic instability.
Objective. To investigate the relative telomere length using the Q-FISH method in exposed individuals and to identify the proportion of ultra-short and ultra-long chromosomal telomeric regions in this cohort.
Materials and methods. The study involved 43 volunteer donors (women) from different age groups (21–28; 60–67; and 71–83 years). At stage I, an investigation of the dose-dependence of relative telomere length was performed in the control group. The donors were divided into groups: younger (n = 4) — non-exposed women aged 21–28 years; middle-aged (n = 12) — women aged 60–67 years; older (n = 5) — women aged 71–83 years. At stage II, the reference for average telomere length was established using donors (n = 5) from the older age group. At stage III, considering the established reference values, telomere length was studied in exposed individuals (n = 22), including analysis based on age and bone marrow dose. Cytogenetic preparations were obtained according to a protocol that includes cell culturing to the metaphase stage, hypotonic treatment, fixation of metaphase plates, and chromosome slide preparation. Telomeres were fluorescently stained using probes (DAKO, Denmark) in accordance with the manufacturer's protocol. Standard methods of descriptive and comparative statistics were used.
Results. In exposed individuals, the median telomere length was statistically significantly higher than that in the comparison group (10.3% vs. 5.8%, p = 0.0001). Concurrently, this group exhibited a reduced frequency of ultra-short telomeres (1.6% vs. 5%) and an increased frequency of ultra-long telomeres (19.5% vs. 5%, $p < 0.0001$). A case-control study confirmed this pattern for the individuals with medium and high bone marrow doses. A statistically significant decrease in median telomere length was observed in donors with a high bone marrow dose compared to those with medium doses (11.9% vs. 10.6%, p = 0.0001). An increase in the bone marrow dose led to an exponential decrease in the frequency of ultra-short telomeres (R^2 = 0.23, p = 0.0036).
Conclusions. A decrease in relative telomere length was observed in non-exposed individuals with an increase in age. In the group of young donors aged 20–28 years, the median telomere length was 31.0%, comprising 13.0% and 5.8% (p = 0.0001) in the 60–67 and 71–83 age groups, respectively. The reference range for ultra-short telomeres in the 71–83 year group was 0–0.7%, being 25.6% and above for ultra-long telomeres. In exposed individuals, the median telomere length was statistically significantly higher than in the comparison group (p = 0.0001). Exposed individuals exhibited a reduced frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres with respect to the comparison group (p = 0.004). A non-linear regression dependence of the frequency of ultra-short telomeres on bone marrow dose manifested in an exponential decrease in frequency with an increase in dose was noted.

Keywords: telomeres; telomere length; short telomeres; long telomeres; women; chronic radiation exposure; the Techa River

For citation: Krivoshchapova Ya.V., Akhmadullina Yu.R. Evaluation of the frequency of ultra-short and ultra-long telomeres in chronically exposed women. *Extreme Medicine.* 2026;28(1):69–78. https://doi.org/10.47183/mes.2025-384

Funding: the study was conducted within the framework of the state assignment of the Federal Medical and Biological Agency "Long-term cytogenetic effects of chronic radiation exposure in residents of the Southern Urals" (No. 388-03-2025-085, code: Cytogenetic Effects).

Compliance with ethical principles: the study was approved by the Ethics Committee of the Southern Urals Federal Research and Clinical Center for Medical Biophysics (Minutes No. 6, of 19.12.2022). Written informed consent was obtained from all participants prior to their inclusion in the study.

Potential conflict of interest: the authors declare no conflict of interest.

✉ Yana V. Krivoshchapova Yana_ho@mail.ru

Received: 28 Aug. 2025 **Revised:** 1 Nov. 2025 **Accepted:** 12 Nov. 2025 **Online first:** 27 Dec. 2025

УДК 614.876:539.1.04:575.224.232

ОЦЕНКА ЧАСТОТЫ СВЕРХКОРОТКИХ И СВЕРХДЛИННЫХ ТЕЛОМЕР У ХРОНИЧЕСКИ ОБЛУЧЕННЫХ ЖЕНЩИН

Я.В. Кривощапова✉, Ю.Р. Ахмадуллина

Южно-Уральский федеральный научно-клинический центр медицинской биофизики, Озерск, Россия

Введение. Длина теломер рассматривается как потенциальный биомаркер индивидуальной радиочувствительности и радиорезистентности человека. Радиационное облучение может как увеличивать, так и уменьшать среднюю длину теломер в клетках, при этом показатели длин отдельных теломер варьируют в широком диапазоне. Оценка частоты сверхкоротких и сверхдлинных теломер может указывать на изменения репликативного потенциала клеток и на радиационно-индуцированные нарушения генома.
Цель. Изучить относительную длину теломер методом Q-FISH у облученных лиц, а также выявить у них долю сверхкоротких и сверхдлинных теломерных участков хромосом.

Материалы и методы. Исследование проведено с участием 43 доноров-добровольцев (женщин) различных возрастных групп (21–28, 60–67, 71–83 года). На первом этапе в контрольной группе проведено исследование зависимости относительной длины теломер от возраста. Доноры были разделены на группы: младшая (n = 4) — не подвергавшиеся облучению женщины в возрасте 21–28 лет; средняя (n = 12) — женщины в возрасте 60–67 лет; старшая (n = 5) — женщины в возрасте 71–83 года. На II этапе у доноров (n = 5) старшей возрастной группы был определен референс средней длины теломер. На III этапе, учитывая установленные референсные значения, изучали длину теломер у облученных лиц (n = 22), в том числе в зависимости от возраста и дозы облучения красного костного мозга (ККМ). Цитогенетические препараты получали согласно протоколу, который включает культивирование клеток до стадии метафазы, гипотоническую обработку, фиксацию метафазных пластинок и получение препаратов хромосом. Флуоресцентная окраска теломер проводилась зондами (DAKO, Дания) в соответствии с протоколом производителя. В работе использовали стандартные методы описательной и сравнительной статистики.

Результаты. У облученных лиц медианная длина теломер статистически значимо выше, чем в группе сравнения (10,3% против 5,8%, p = 0,0001), при этом у них снижена частота сверхкоротких теломер (1,6% против 5%) и повышена частота сверхдлинных (19,5% против 5%, p < 0,0001). Исследование методом «случай — контроль» подтвердило данную закономерность для лиц со средней и высокой дозой облучения ККМ. Наблюдалось статистически значимое снижение медианной длины теломер в группе доноров с высокой дозой облучения ККМ относительно лиц со средними дозами (11,9% против 10,6%, p = 0,0001). С увеличением дозы облучения ККМ частота сверхкоротких теломер экспоненциально уменьшалась (R^2 = 0,23, p = 0,0036).

Выводы. Отмечено снижение относительной длины теломер у необлученных лиц с увеличением возраста, в группе молодых доноров 20–28 лет медианное значение длины теломер составило 31,0%, в группе 60–67 лет — 13,0%, в группе 71–83 года — 5,8% (p = 0,0001). Референсное значение сверхкоротких теломер для возрастной группы 71–83 года составило 0–0,7%, а сверхдлинных — от 25,6% и выше. У облученных лиц медианная длина теломер статистически значимо выше, чем в группе сравнения (p = 0,0001). У облученных лиц снижена частота сверхкоротких теломер и выше частота сверхдлинных теломер относительно группы сравнения (p = 0,004). Отмечена нелинейная регрессионная зависимость частоты сверхкоротких теломер от дозы облучения ККМ: с увеличением дозы облучения частота экспоненциально уменьшается.

Ключевые слова: теломеры; длина теломер; короткие теломеры; длинные теломеры; женщины; хроническое радиационное воздействие; река Теча

Для цитирования: Кривощапова Я.В., Ахмадуллина Ю.Р. Оценка частоты сверхкоротких и сверхдлинных теломер у хронически облученных женщин. *Медицина экстремальных ситуаций.* 2026;28(1):69–78. https://doi.org/10.47183/mes.2025-384

Финансирование: работа выполнена в рамках государственного задания Федерального медико-биологического агентства «Отдаленные цитогенетические эффекты хронического облучения у жителей Южного Урала» (№ 388-03-2025-085, шифр: «Цитогенетические эффекты»).

Соответствие принципам этики: исследование одобрено комитетом по этике ФГБУН ЮУрФНКЦ МБ ФМБА России (протокол № 6 от 19.12.2022). Всеми участниками подписано добровольное информированное согласие на участие в исследовании.

Потенциальный конфликт интересов: авторы заявляют об отсутствии конфликта интересов.

✉ Кривощапова Яна Владимировна Yana_ho@mail.ru

Статья поступила: 28.08.2025 **После доработки:** 01.11.2025 **Принята к публикации:** 12.11.2025 **Online first:** 27.12.2025

INTRODUCTION

According to current understanding, telomeres are essential terminal regions of chromosomes that perform a multitude of functions, with the primary one being the maintenance of genomic integrity. It has been noted that telomere length varies widely not only among individuals but even within a single cell and across chromosomal pairs, which can be explained by uneven telomere shortening [1, 2]. This phenomenon of telomere length distribution along the arms of individual chromosomes is referred to as the “chromosomal telomere profile” [3]. The telomere profile can serve as a potential biomarker for predicting the risk of certain diseases and for assessing the impact of adverse external factors, including ionizing radiation [4]. Consequently, research aimed at studying the influence of radiation exposure on telomere length is highly relevant. Exposure of individuals due to atomic bombings in Hiroshima and Nagasaki to doses conventionally categorized as low (5–700 mSv) and high (over 700 mSv) led to telomere shortening; these alterations persisted even in the long term (50–68 years post-exposure) in the high-dose group [5]. Conversely, some studies have reported an increase in telomere length in exposed individuals, which has been associated with elevated telomerase expression [6].

An assessment of relative telomere length in nuclear industry workers exposed to internal alpha (0.05 Gy; 0.05–0.1 Gy; > 0.1 Gy) and external gamma (1 Gy; 0.1–1.5 Gy; > 1.5 Gy) radiation found this parameter to decrease only after low-dose exposure, for both external gamma radiation (at doses < 1 Gy) and internal alpha radiation (at doses < 0.05–0.1 Gy). At higher doses, this parameter did not differ from the values in the non-exposed control group [7]. A study involving individuals chronically exposed at a wide dose range (0–4.7 Gy) revealed a decrease in relative telomere length under chronic exposure (0.6–4.7 Gy) compared to the control group for specific chromosomal arms of meta- and acrocentric chromosomes. No monotonic decrease in telomere length was observed for chromosomes within a single cell or across different arms of the same chromosome [1]. This indicates that telomere length is a dynamic structure determined not merely by chronological age but rather by the combined influence of numerous endogenous and exogenous factors.

At present, researchers are increasingly addressing the relationship between telomere length and cell and

body radiosensitivity [8]. For instance, certain diseases characterized by clinical hyper-radiosensitivity (ataxia-telangiectasia, Fragile X syndrome, Fanconi anemia) were shown to be associated with impairments in telomere maintenance [9]. Investigations into the radiosensitivity of human fibroblasts exposed to X-ray radiation in vitro demonstrated that cell clones that retained viability after a 4 Gy irradiation dose exhibited increased telomere length compared to non-irradiated clones. The authors advanced a hypothesis that cells with longer telomeres are more radioresistant, and that radiosensitivity correlates with telomere loss rather than with their average length [4].

A number of studies have shown that the quantity of ultra-short telomeres, rather than their average length, serves as the most informative predictive marker for the development of certain diseases [10]. For instance, an association between the frequency of ultra-short telomeres and various human pathologies, such as chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis, has been established [11].

All existing methods for studying chromosome telomeres can be broadly distinguished into two groups: molecular and molecular-cytogenetic. The molecular-cytogenetic method of quantitative fluorescence in-situ hybridization (Q-FISH) on metaphase chromosomes is the most suitable approach for the individual assessment of telomeric regions. This method employs fluorescently labeled peptide nucleic acids (PNA), which specifically hybridize with denatured telomeric DNA. Using the Q-FISH method, telomeric regions can be examined in each chromosome in every cell, since chromosome karyotyping is performed during the analysis [1].

In this study, we focuse on telomere length in individuals chronically exposed to a wide dose range due to residence in radionuclide-contaminated areas, during long-term follow-up (60–65 years post-exposure). The radiation exposure was combined: internal, i.e., due to the intake and accumulation of ^{89,90}Sr radionuclides in the body, and external — from γ-radiation. The critical organ for radiation exposure was the red bone marrow (RBM). Doses to the RBM were predominantly accumulated before 1960 [12].

Our aim was to investigate telomere length using the Q-FISH method in exposed individuals and to identify the proportion of ultra-short and ultra-long chromosomal telomeric regions in this cohort.

MATERIALS AND METHODS

Study design

The study involved 43 female donors from different age groups (21–28; 60–67; 71–83 years). The inclusion criteria were the absence of a history of autoimmune or oncological diseases, and chronic inflammatory diseases in the acute phase. Individuals taking cytostatics or antibiotics were excluded. Health status information for the exposed individuals was obtained from the Database "Chelovek" (Man); individual doses were calculated using TRDS-2016 in the Biophysics Laboratory. Information on cancer history in the examined individuals was provided by an Epidemiology Laboratory [13].

At the first stage, an investigation of the age-dependence of relative telomere length was conducted in a control group (donor characteristics are presented in Table 1). Three age groups of donors were formed:

- younger age group — 4 unexposed women aged 21–28 years (mean age 24 ± 2.9 years);
- middle-aged group — 12 donors aged 60–67 years (mean age 63.8 ± 1.8 years);
- older age group — 5 donors aged 71–83 years (mean age 76.4 ± 5 years).

Although donors from the older age group resided in radionuclide-contaminated areas, their accumulated bone marrow dose did not exceed 0.05 Gy over their lifetime. Consequently, hereafter (in stages II and III of the study), these donors will be referred to as non-exposed individuals or the comparison group.

At stage II, the reference for average telomere length was established in the comparison group. At stage III, taking into account the established reference values, telomere length was studied in exposed individuals, including analysis based on age and bone marrow dose.

With the purpose of assessing the influence of ionizing radiation on telomere length, two groups of exposed

Table 1. Characteristics of donors at the first study stage

Parameter	Age group of donors		
	younger age	middle-aged group	older age
Number of donors, N	4	12	5
Age, years, M (min–max)	24 (21–28)	63.8 (60–67)	76.4 (71–83)
Bone marrow dose, Gy	0	0–0.05	0–0.05
Number of telomere region measurements, abs.	4,331	22,311	9,732

Table compiled by the authors based on their original data

Note: age is presented as the mean value along with the maximum and minimum values.

donors were formed: those exposed to doses ranging 0.14–0.86 Gy (10 individuals aged 69–80 years) constituted the medium-dose exposure group; the high-dose exposure group comprised 12 individuals aged 71–84 years with bone marrow doses of 1.04–3.1 Gy (Table 2).

Furthermore, a case-control analysis was conducted to eliminate the influence of the age factor. To that end, potential controls for each donor ("case") from the medium-dose exposure group were selected based on the criterion of matching the age at the time of examination. The bone marrow dose was considered the influencing factor. Four pairs of donors of similar age were formed. In each pair, one donor had an established accumulated bone marrow dose ranging 0.61–0.86 Gy, while the other donor in the pair either had not been accidentally exposed or had a dose of ≤ 0.05 Gy (Table 3).

For the case-control study within the high bone marrow dose range, five pairs of donors of similar age were selected. One donor in each pair had a dose exceeding 1 Gy, and the other donor either had not been accidentally exposed or had a dose of ≤ 0.05 Gy (Table 3).

Methodology for metaphase chromosome preparation

The subject of the study was the nuclear chromatin of T-lymphocytes from peripheral blood stimulated with phytohaemagglutinin (PHA). T-lymphocytes were chosen due to the ease of obtaining the source material and their sufficiently high concentration. T-lymphocytes do not divide in peripheral blood and reside in the G0 phase of the cell cycle, representing a naturally synchronized cell population in the body. Lymphocyte precursor cells are irradiated directly in the RBM.

Blood was drawn into vacuum plastic blood collection tubes containing lithium heparin.

The culture mixture was prepared from the following components: 2 mL of whole heparinized blood, 5 mL of RPMI-1640 medium (PanEco, Russia), 0.5 mL of

Table 2. Characteristics of exposed donors

Parameter	All exposed individuals	Medium doses 0.14–0.86 Gy	High doses 1.04–3.1 Gy
Age, years, M (min–max)	74.6 (69–84)	73.4 (69–80)	75.7 (71–84)
Number of donors, N	22	10	12
Bone marrow dose, Gy	0.14–3.1	0.14–0.86	1.04–3.1
Number of telomere region measurements, abs.	38,416	18,852	19,564

Table compiled by the authors based on their original data

Note: age is presented as the mean value along with the maximum and minimum values.

Table 3. Characteristics of donors in the case-control study

Pair No. in the case-control study	Donor age, years		Bone marrow dose, Gy		Number of measurements, abs.	
	case	control	case	control	case	control
Exposed to medium doses (0.14–0.86 Gy)						
1	80	80	0.64	0.003	1,748	1,456
2	74	75	0.8	0.05	3,810	1,458
3	72	73	0.86	0.008	3,208	2,630
4	71	71	0.61	0	1,918	2,744
Exposed to high doses (1.04–3.1 Gy)						
1	84	83	1.04	0.03	1,610	1,444
2	81	80	1.25	0.003	1,850	1,456
3	71	71	1.57	0	1,836	2,744
4	73	73	1.93	0.008	1,922	2,630
5	74	75	2.73	0.05	1,840	1,458

Table compiled by the authors based on their original data

selected fetal bovine serum (PAA Laboratories, Austria). PHA was added to a final concentration of 20 μg/mL (PanEco, Russia). Cells were cultured in a CO_2 incubator (SANYO MCO-18AIC, Japan) at 37.5 °C for 54 h. Three hours prior to the end of cultivation, colchicine was added to achieve the final concentration of 0.1 μg/mL (PanEco, Russia).

Subsequently, metaphase cells underwent hypotonic treatment, fixation of metaphase plates (using a 3:1 mixture of 95% medical-grade ethanol and glacial acetic acid), and chromosome slide preparation. For fluorescent staining using the Q-FISH method, slides were treated with RNAse (100 ng/mL) and pepsin solutions, followed by denaturation of the probe and specimen DNA. Hybridization was performed in accordance with the manufacturer's protocol, which was supplied with the original reagent solutions.

To assess the length of chromosomal telomeric regions, DAKO probes (Denmark) were used. Centromeric probes (Metasystems; Germany) were applied to stain the centromeric region of chromosome 2, which served as a reference signal. Analysis of fluorescently stained specimens was performed using an AxioImager Z2 fluorescence microscope (Zeiss; Germany) equipped with DAPI and SpO (spectrum orange) filters.

The intensity of the recorded fluorescent signal was measured using the Isis specialized image analysis software. For each subject, 20–30 cells were analyzed. Results were obtained separately for the short (p-) and long (q-) arms of each of the 46 chromosomes. Telomere length was relative, being expressed as a percentage of the telomeric (T) signal length relative to the centromeric (C) signal length — (T/C%). The method for assessing telomeric region length is described in detail in [14].

Standard methods of descriptive statistics were used to process the data obtained, including calculation of the median, its 95% confidence interval (95% CI), and the 5th and 95th percentiles (%). Confidence intervals were calculated using a bootstrap procedure with n = 1000 resamples. Intergroup comparisons were performed using the non-parametric Mann–Whitney U test.

The threshold for ultra-short telomeres was defined as the value below which the lowest 5% of all telomere length measurements in the non-exposed group fall. Similarly, the reference value for ultra-long telomeres was calculated as all values exceeding the 95th percentile of the measurement distribution. The obtained reference values were used for comparative analysis with data on the relative telomere length in individuals exposed at various dose ranges.

When comparing the frequency of ultra-short and ultra-long telomeres in the case-control study and against the reference value, the χ^2 test was used. Multivariate analysis was performed to determine the association of telomere length with age and dose. Spearman's correlation coefficient and regression analysis were employed to assess the relationship between the dose and the number of ultra-short and ultra-long telomeres per individual. Statistical analysis was conducted using the following software packages: Past 4.01 (Oyvind Hammer; UK), Statistica 10 (StatSoft, USA), and SigmaPlot (SYSTAT Software, USA).

RESULTS

During the study, data on telomere length measurements were obtained for three age groups of non-exposed donors (Table 4).

In the donor group aged 20–28 years, 4331 measurements of telomeric regions were performed. The minimum recorded telomere length was 0%, the maximum was 900.0%, and the median telomere length was 31.0%.

Simultaneously, in the middle-aged donor group (60–67 years), 22,311 measurements of telomeric regions were performed. The telomere length range (min–max) was 0–967.9%, and the median value was 13.0%. In the donor group aged 71–83 years, 9732 measurements were obtained. The minimum telomere length was 0%, the maximum was 141.2%, and the median was 5.8%.

The values for ultra-short telomeres in the younger donor group ranged 0–6.3%; for donors aged 60–67 and 71–83 years the values were 0–1.8 and 0–0.7%, respectively. Meanwhile, the ranges for ultra-long telomeres were 221.2–900.0% for donors aged 20–28 years, 105.9–967.9% for donors aged 60–67 years, and 25.6–141.2% for donors aged 71–83 years.

Table 4. Length of chromosomal telomeric regions in non-exposed donors by age (T/C %)

Relative length of chromosomal telomeric regions, T/C %	Age group of donors		
	younger age (21–28 y.o.)	middle-aged (60–67 y.o.)	older age (71–83 y.o.)
min–max values	0–900.0	0–967.9	0–141.2
Median [95% CI] 5–95% percentiles	31.0* [28.5–32.6] 6.3–221.2	13.0* [12.8–13.3] 1.8–105.9	5.8* [5.6–5.9] 0.7–25.6

Table compiled by the authors based on their original data

Note: * statistically significant differences between groups (Mann–Whitney U test), p = 0,0001; y.o. — years old

It was established that median telomere length decreased with an increase in donor age. Pairwise comparison of values in the studied control groups revealed statistically significant differences (p = 0.0001). Regression analysis demonstrated a statistically significant ($p < 0.001$; $R^2 = 0.07$), albeit extremely weak, linear dependence between relative telomere length and donor age:

$$y = 86.1 - 26.7\,x, \quad (1)$$

y — relative telomere length, x — age of the examined individuals.

For the subsequent analysis of telomere length in exposed individuals, a reference value of 0.7% was adopted for ultra-short telomeres, corresponding to the lower bound of the 90% telomere length range in non-exposed donors aged 71–83 years. Ultra-long telomeres were defined using the upper bound of the 90% telomere length range — 25.6%.

Table 5 presents the telomere length measurement data for exposed donors. The comparison group consists of non-exposed donors aged 71–83 years.

It can be seen that the median telomere length for all exposed individuals was 10.3%, which is statistically significantly higher than that in the comparison group (5.8%; p = 0.0001). At the same time, the 90% interval of telomere length ranged 1.4–56.5%.

Considering the reference value for ultra-short telomeres, within the group of exposed donors (0.14–3.1 Gy), 616 telomere measurements were classified as ultra-short, constituting 1.6% of all measurements. Within the reference range for ultra-long telomeres, exposed donors had 7491 measurements, accounting for 19.5%. Thus, exposed individuals exhibited a lower number of ultra-short telomeres ($\chi^2 = 397.03$; $p < 0.0001$) and a higher number of ultra-long telomeres ($\chi^2 = 1184$; $p < 0.0001$) relative to the comparison group.

In the medium-dose exposure group (0.14–0.86 Gy), the median telomere length was 11.9% (min–max: 0–643.4%). For donors exposed to high doses, the median was 10.6% (min–max: 0–332.1%), which is statistically significantly lower than in the medium-dose group (p = 0.0001).

Relative to the reference values, the frequency of ultra-short telomeres in donors exposed to medium doses was 1.3%, and 1.4% in those exposed to high doses The frequency of ultra-long telomeres was 24% in the medium-dose group and 19.5% in the high-dose group, which was statistically significantly different from the values in the comparison group (p = 0.0001). Thus, in exposed individuals with a high accumulated bone marrow dose, the frequency of ultra-short telomeres is similar to that in the medium-dose group. At the same time, in the former group, the number of ultra-long telomeres is higher.

The relationship between telomere length, age, and bone marrow dose was established by a two-factor analysis (general linear model). A statistically non-significant model where telomere length in the entire sample of exposed individuals did not depend on age or bone marrow dose was obtained.

Table 6 presents data on the pairwise comparison for the case-control analysis, where the “cases” are individuals with medium (0.14–0.86 Gy) and high (1.04–3.1 Gy) accumulated bone marrow doses.

In all examined pairs from the medium dose group, a statistically significant increase in telomere length was detected in exposed donors compared to their controls.

In the group of individuals exposed to high doses, a statistically significant increase in telomere length in exposed donors compared to their controls in four out of five pairs was found. In Pair No. 1, a statistically significant decrease in telomere length was observed in the exposed donor relative to their control. However, in this specific case-control pair, the frequency of ultra-short telomeres did not differ from the control values (0.8% vs. 0.7%; $\chi^2 = 0.03$; p = 0.86), while the frequency of ultra-long telomeres was significantly lower (1.4% vs. 2.9%; $\chi^2 = 8$; p = 0.005) (Table 7).

Table 5. Length of chromosomal telomeric regions in exposed donors (T/C %)

Relative length of chromosomal telomeric regions, T/C %	Groups of exposed donors		
	All exposed individuals	Medium doses (0.14–0.86 Gy)	High doses (1.04–3.1 Gy)
Min–max values	0–643.4	0–643.4	0–332.1
Median [95% CI]	10.3 [10.2–10.4]	11.9 [11.7–12.1]*	10.6 [10.4–10.8]
5–95% percentiles	1.4–56.5	1.6–61.7	1.4–57.7
Share of ultra-short telomeres, %	1.6	1.3	1.4
Share of ultra-long telomeres, %	19.5	24*	19.5

Table compiled by the authors based on their original data

Note: * — statistically significant difference from the high-dose exposure group, $p \le 0.0001$.

Table 6. Pairwise comparison of telomeric region length in the case-control study for medium doses (0.14–0.86 Gy) and high doses (1.04–3.1 Gy)

Pair No. in the case-control study	Relative length of chromosomal telomeric regions, T/C %								Mann–Whitney *U* test
	Min–max		Median		5%		95%		
	case	control	case	control	case	control	case	control	
Medium doses (0.14–0.86 Gy)									
1	0–143.3	0–66.2	17.0	3.5	3.4	0.1	54.5	17.9	$p = 0.0001$
2	0–267.1	0–119.1	16.9	6.6	3.7	0.06	68.6	32.6	$p = 0.0001$
3	0.13–71.7	0–65.8	3.9	3.6	0.8	0.8	16.8	10.7	$p = 0.0001$
4	0–643.4	0–141.2	20.1	9.9	4.6	1.5	110.5	32.4	$p = 0.0001$
High doses (1.04–3.1 Gy)									
1	0.3–50.5	0–61.2	4.1	8.1	1.3	1.9	15.1	23.5	$p = 0.0001$
2	0–121.6	0–66.2	12.4	3.5	3.3	0.1	36.8	17.9	$p = 0.0001$
3	0–248	0–141.2	27.1	9.9	5.9	1.5	98.8	32.4	$p = 0.0001$
4	0–227.7	0–65.8	8.7	3.6	2.4	0.8	35.1	10.7	$p = 0.0001$
5	0–174.3	0–119.1	11.3	6.6	2.3	0.06	41.7	32.6	$p = 0.0001$

Table compiled by the authors based on their original data

Table 7. Frequency of ultra-short and ultra-long telomeres in the case-control study

Pair No.	Ultra-short telomeres, %		χ^2	*p*-value	Ultra-long telomeres, %		χ^2	*p*-value
	case	control			case	control		
Medium doses								
1	0.8	1.1	147.0	<<< 0.0001	31	2.0	460	<<< 0.0001
2	0.5	11	351	<<< 0.0001	33	9.7	294	<<< 0.0001
3	3.3	4	1.7	0.19	1.8	0.2	32.3	<<< 0.0001
4	0.47	1.9	17.8	<<< 0.0001	39	9.7	566.2	<<< 0.0001
High doses								
1	0.8	0.7	0.03	0.86	1.4	2.9	8.0	0.005
2	1	11	150	<<< 0.0001	3.2	2	135	<<< 0.0001
3	0.38	1.9	20	<<< 0.0001	14	9.7	20.7	<<< 0.0001
4	0.4	4	57.2	<<< 0.0001	9	0.2	227.7	<<< 0.0001
5	0.5	11	179.6	<<< 0.0001	13.5	9.7	11.2	0.0008

Table compiled by the authors based on their original data

Note: *p*-value — level of statistical significance of the differences; χ^2 — Pearson's chi-squared test.

Table 7 presents the frequencies of ultra-short and ultra-long telomeres for each case-control pair (based on reference values) among individuals with medium and high bone marrow doses.

The study yielded statistically significant data on changes in the frequency of ultra-short telomeres in three out of four pairs ($p < 0.0001$), with the exception of Pair No. 3 ($p = 0.19$). At the same time, in all examined pairs,

the frequency of occurrence of ultra-short telomeres in exposed donors was statistically significantly lower than that in the controls. Furthermore, the frequency of ultra-long telomeres in all pairs of donors exposed to doses of 0.14–0.86 Gy was statistically significantly higher compared to the control group ($p < 0.0001$).

It can be seen that for individuals exposed in the dose range of 1.04–3.1 Gy, the frequency of ultra-short telomeres is statistically significantly lower relative to the control in four out of five pairs. No statistically significant differences in the frequency of ultra-short telomeres were observed in Pair No. 1. Also, in this same pair, the exposed donor had a lower frequency of ultra-long telomeres compared to the control. In the remaining four examined pairs, an increase in the frequency of ultra-long telomeres was observed in exposed individuals relative to the controls.

Subsequently, for the donors selected into the case-control study, Spearman's rank correlation coefficient was used to assess the relationship between the proportion of ultra-short telomeres and the dose. The results found no correlation between ultra-long telomeres and bone marrow dose; however, a trend towards a decrease in the frequency of ultra-short telomeres with an increase in bone marrow dose was observed. A statistically significant inverse correlation of moderate strength was established between the frequency of ultra-short and ultra-long telomeres in exposed donors ($r_s = -0.654$, $p = 0.004$).

Subsequently, the most suitable regression model describing the dependence of ultra-short telomere frequency on bone marrow dose was fitted (Figure).

The Figure shows that an increase in dose is associated with an exponential decrease in the number of ultra-short telomeres.

$$y = 4.3\ e^{-1.5D}\ [R^2 = 0.23,\ p = 0.0036], \qquad (2)$$

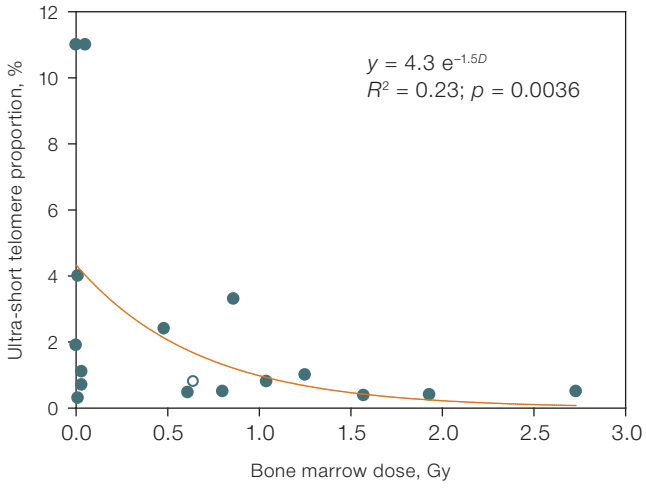

Figure prepared by the authors

Fig. Regression dependence of the percentage of ultra-short telomeres on bone marrow dose

y — frequency of ultra-short telomeres, D — bone marrow dose, Gy.

Thus, it was demonstrated that exposed individuals exhibit a reduced frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres relative to the comparison group. An exponential decrease in the frequency of ultra-short telomeres with an increase in bone marrow dose was observed.

DISCUSSION

The present study continues a project investigating the effects of chronic radiation exposure on chromosomal telomeric regions. As part of this long-term project, the length of telomeric regions in individual chromosome pairs in chronically exposed individuals was examined, and the frequency of inversions involving telomeric regions and the frequency of telomeric region loss in metaphase chromosomes from cultured peripheral blood T cells of exposed residents in the Southern Urals were assessed [14]. To that end, a method of fluorescent staining of chromosomal telomeric regions was proposed and validated. The use of the Q-FISH method is justified by its capacity to investigate telomeric regions in each chromosome within a donor's cells. This enables the characterization of the telomere profile.

The present study is the first of its kind to conduct an analysis of the influence of both radiation and non-radiation factors (age) on the frequency of ultra-short and ultra-long telomeres. The frequency of ultra-short and ultra-long telomeres in exposed individuals was assessed based on reference values for telomeric region length, which were calculated for a comparison group of a similar age range. The reference range for telomere length in the comparison group (age 71–83 years) was 0.7–25.6%. Telomeres with a length < 0.7% were classified as ultra-short, while those above 25.6% were classified as ultra-long.

A statistically significant increase in median telomere length was demonstrated in the group of exposed individuals compared to the comparison group. At the same time, wide variability in telomere length was observed in both exposed individuals and the comparison group, which is consistent with previously obtained data [1, 15]. This variability may indicate the circulation of T cells with different replicative ages in the peripheral blood.

According to the results obtained, in the overall sample of exposed individuals, the frequency of ultra-short telomeres was lower, and that of ultra-long telomeres was higher relative to the comparison group. These findings were further supported by an assessment using the case-control method with age-matched donors. The presence of longer telomeres in exposed individuals may indicate molecular-genetic features associated with either increased telomerase activity

[16] or epigenetic changes in other enzymes regulating telomere replication and repair [17, 18], as well as the selection of more radioresistant cells and the death of radiosensitive ones [4]. The donor selection criteria for the cytogenetic study may have also played a role, since the sample inherently comprised the healthiest individuals relative to the general population. This is a necessary selection criterion, since excluded medical conditions, therapeutic procedures, and medications can distort cytogenetic data [1].

Furthermore, a statistically significant decrease in median telomere length was observed in the donor group with a high bone marrow dose compared to the group exposed to medium doses. This difference was primarily associated with a reduced frequency of ultra-long telomeres in the high-dose group, while the frequency of ultra-short telomeres remained at the same level as in individuals with medium doses. At the same time, the frequency of ultra-long telomeres did not depend on the bone marrow dose, whereas the frequency of ultra-short telomeres decreased exponentially with an increase in dose. The presented patterns require further in-depth investigation. Provided that subsequent studies confirm the relationship between the frequency of ultra-short telomeres and radiation dose, this parameter could be used as an indicator of radiation exposure or a biomarker of cell radiosensitivity [19, 20].

CONCLUSION

The present study provides data on the influence of chronic radiation exposure on telomere length in women, obtained using the Q-FISH method. This method enables the determination of relative telomeric region length by calculating the ratio of the telomeric signal fluorescence intensity to the centromeric signal fluorescence intensity of chromosome 2, expressed as a percentage (T/C, %). Ultra-short and ultra-long telomeres in exposed individuals were identified relative to the reference telomere length value calculated in the comparison group.

Our results revealed a statistically significant increase in median telomere length in exposed individuals relative to the comparison group. The increase in telomere length is associated with a decreased frequency of ultra-short telomeres and an increased frequency of ultra-long telomeres in exposed individuals. The study conducted using a case-control design with age-matched donors confirmed that this pattern persists independently of age, underscoring the significance of the radiation factor. The dependence of ultra-short telomere frequency on bone marrow dose is described by a non-linear regression manifested in an exponential decrease in the frequency of ultra-short telomeres with an increase in dose. This phenomenon highlights the importance of further investigation into the role of ultra-short telomeres as a potential marker of radiation exposure.

References

1. Akhmadullina YuR, Vozilova AV, Krivoshchapova YV. The effect of chronic exposure on the parameters of cytogenetic markers of senescence in the residents of the Techa riverside settlements. *Extreme Medicine.* 2024;26(2):56–66 (In Russ.). https://doi.org/10.47183/mes.2024.018
2. Baird DM, Rowson J, Wynford-Thomas D, Kipling D. Extensive allelic variation and ultrashort telomeres in senescent human cells. *Nature Genetics.* 2003;33(2):203–7. https://doi.org/10.1038/ng1084
3. Graakjaer J, Londono-Vallejo JA, Christensen K, Kølvraa S. The pattern of chromosome-specific variations in telomere length in humans shows signs of heritability and is maintained through life. *Annals of the New York Academy of Sciences*. 2006;1067:311–6. https://doi.org/10.1196/annals.1354.042
4. Berardinelli F, Nieri D, Sgura A, Tanzarella C, Antoccia A. Telomere loss, not average telomere length, confers radiosensitivity to TK6-irradiated cells. *Mutation Research.* 2012;740(1–2):13–20. https://doi.org/10.1016/j.mrfmmm.2012.11.004
5. Lustig A, Shterev I, Geyer S, Shi A, Hu Y, Morishita Y, et al. Long term effects of radiation exposure on telomere lengths of leukocytes and its associated biomarkers among atomic-bomb survivors. *Oncotarget*. 2016;7(26):38988. https://doi.org/10.18632/oncotarget.8801
6. Reste J, Zvigule G, Zvagule T, Kurjane N, Eglite M, Gabruseva N, et al. Telomere length in Chernobyl accident recovery workers in the late period after the disaster. *Journal of Radiation Research.* 2014;55(6):1–12. https://doi.org/10.1093/jrr/rru060
7. Scherthan H, Sotnik N, Peper M, Schrock G, Azizova T, Abend M. Telomere Length in Aged Mayak PA Nuclear Workers Chronically Exposed to Internal Alpha and External Gamma Radiation. *Radiation Research.* 2016;185(6):658–67. https://doi.org/10.1667/RR14271.1
8. Slijepcevic P. Is there a link between telomere maintenance and radiosensitivity? *Radiation Research.* 2004;161(1):82–6. https://doi.org/10.1667/rr3093
9. Fiesco-Roa MÓ, García B, Leal-Anaya P, van 't Hek R, Wegman-Ostrosky T, Frías S, et al. Fanconi anemia and dyskeratosis congenita/telomere biology disorders: Two inherited bone marrow failure syndromes with genomic instability. *Frontiers in Oncology.* 2022;12:949435. https://doi.org/10.3389/fonc.2022.949435
10. Hemann MT, Strong MA, Hao LY, Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell.* 2001;107(1):67–77. https://doi.org/10.1016/s0092-8674(01)00504-9
11. Cagsin H, Uzan A, Tosun O, Rasmussen F, Serakinci N. Tissue-Specific Ultra-Short Telomeres in Chronic Obstructive Pulmonary Disease. *International Journal of Chronic Obstructive Pulmonary Disease.* 2020;15:2751–7. https://doi.org/10.2147/COPD.S267799
12. Akleev AV, ed. *The consequences of radioactive contamination of the Techa River.* Chelyabinsk: Kniga; 2016 (In Russ.).
13. Shishkina EA, Napier BA, Preston DL, Degteva MO. Dose estimates and their uncertainties for use in epidemiological studies of radiation-exposed populations in the Russian Southern Urals. *PLoS One.* 2023;18(8):e0288479. https://doi.org/10.1371/journal.pone.0288479

14. Krivoshchapova YV. Estimation of the impact of chronic radiation exposure on telomere loss in women's T lymphocytes. *Bulletin of RSMU*. 2024;6:172–8 (In Russ.). https://doi.org/10.24075/brsmu.2024.055
15. Krivoshchapova YaV, Vozilova AV. The study of the telomere length of the chromosomes in T-lymphocytes of the exposed individuals. *Radiation Safety Problems*. 2022;3(107):71–96 (In Russ.). EDN: FPBYYE
16. Yasumoto S, Kunimura C, Kikuchi K, Tahara H, Ohji H, Yamamoto H, et al. Telomerase activity in normal human epithelial cells. Oncogene. 1996;13(2):433–9.
17. Drosopoulos WC, Deng Z, Twayana S, Kosiyatrakul ST, Vladimirova O, Lieberman PM, et al. TRF2 Mediates Replication Initiation within Human Telomeres to Prevent Telomere Dysfunction. *Cell Reports*. 2020;33(6):108379. https://doi.org/10.1016/j.celrep.2020.108379
18. Wang C, Zhao L, Lu S. Role of TERRA in the regulation of telomere length. *International Journal of Biological Sciences*. 2015;11(3):316–23. https://doi.org/10.7150/ijbs.10528
19. Mirjolet C, Boidot R, Saliques S, Ghiringhelli F, Maingon P, Créhange G. The role of telomeres in predicting individual radiosensitivity of patients with cancer in the era of personalized radiotherapy. *Cancer Treatment Reviews*. 2015;41(4):354–60. https://doi.org/10.1016/j.ctrv.2015.02.005
20. Stanley SE, Rao AD, Gable DL, McGrath-Morrow S, Armanios M. Radiation Sensitivity and Radiation Necrosis in the Short Telomere Syndromes. *International Journal of Radiation Oncology, Biology, Physics*. 2015;93(5):1115–7. https://doi.org/10.1016/j.ijrobp.2015.08.048

Authors' contributions. All authors confirm that their contributions meet the ICMJE criteria for authorship. The primary contributions are distributed as follows: Yana V. Krivoshchapova — research concept and design, conducting laboratory investigations, acquisition of primary data, statistical analysis of results, drafting the article; Yulia R. Akhmadullina — statistical analysis and interpretation of data, writing, editing, and final preparation of the manuscript.

AUTHORS

Yana V. Krivoshchapova
https://orcid.org/0000-0002-2555-2616
yana_ho@mail.ru

Yulia R. Akhmadullina, Cand. Sci. (Biol.)
https://orcid.org/0000-0003-4394-2228
akhmadullina.yul@yandex.ru