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## HUMAN GENETICS

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The Length of Telomeres and the Baseline Level of Cytogenetic Damage in Leukocytes of Lung Cancer Patients

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**Abstract**---Despite the great efforts of basic and clinical research on lung cancer (LC), the prevention and treatment of this type of cancer show still far from satisfactory indicators. The complex mechanisms of the LC biology are already recognized, but there is a significant gap in the knowledge about the interaction of key biomarkers in the etiology and progression of this disease. The present work is aimed at studying the relationship between the telomere length (TL) and frequencies of chromosomal aberrations (CA) and micronuclei (MN) in leukocytes of primary lung cancer patients. In 67 men with lung cancer and 77 control donors (men), the baseline frequencies of CA and MN were determined in blood leukocytes, and TL was also studied. As a result, a significant increase in TL was found in lung cancer patients compared with control donors ( $25.86 \pm 2.13$  versus  $15.29 \pm 1.22$ , respectively,  $p = 0.00001$ ). In leukocytes of LC patients, a significant increase in the baseline level of structural aberrations of chromosomes, as well as micronuclei containing centromeres, was noted. Only in the sample of lung cancer patients were direct correlations of

TL with the frequency of chromosomal type aberrations and with the frequency of paired fragments revealed. In the same sample, significant inverse correlations of TL with the frequency of binucleated leukocytes containing nuclear protrusions and with the frequency of cells entering apoptosis were recorded. The study first showed that an increase in TL in lung cancer patients is associated with structural chromosomal damages, but not with the incidence of aneuploidies.

**Keywords:** lung cancer, leukocytes, genome instability, telomere length, chromosomal aberrations, micronuclei

Telomeres maintain the integrity of chromosomes by closing their ends and providing protection against degradation and fusion during the cell cycle. The structure of telomeres in humans consists of 1000–2000 repeats of TTAGGG sequences associated with complexes of six shelterins. With age, there is a gradual depletion of telomeres and a reduction in their length [1]. Shorter telomeres were shown to correlate with increased levels of inflammation and oxidative stress [2, 3].

Previously, correlations between the telomere length (TL) in leukocytes and various diseases such as atherosclerosis, heart failure, type 2 diabetes mellitus, chronic psychosocial stress, depression, dementia, Alzheimer's disease, and cancer were found [4–9]. There is an opinion that short telomeres in combination with other mechanisms of carcinogenesis can lead to genome instability, which is typical for many types of cancer [10]. Thus, TL in peripheral blood leukocytes appears to be a marker of the susceptibility to many types of cancer: breast, lung, bladder, head and neck, and pancreatic cancer [11–14]. However, recent epidemiological data showed that not only short but also long telomeres of lymphocytes may be associated with an increased risk of cancer [15, 16].

Correlations between TL and chromosomal aberrations (CA) were observed in blood leukocytes of healthy people [17], in cells of solid tumors [18], and in patients with different types of leukemia [19, 20]. Genome instability (point mutations, structural and numerical chromosome abnormalities) is one of the fundamental mechanisms of malignant transformation [21–23] and a significant biomarker of cancer risk [24, 25]. This is an early event in carcinogenesis, often found in cells of newly diagnosed (untreated) patients with various types of cancer [26–33]. It was shown that increased frequencies of CA and micronuclei (NM) are predictors of the risk of developing cancer in human populations [34–37]. Meta-analysis of MN frequencies in leukocytes of untreated patients with different types of cancer showed that 72% of the samples demonstrated an increase in the baseline MN level compared with control donors [38]. A similar increase in CA levels was recorded in cancer

patients before starting therapy [39]. However, the potential of both biomarkers for practical assessment of cancer risk and detection of genome instability during cancer progression has not yet been fully exploited.

Lung cancer (LC) is one of the most common malignant tumors and one of the leading causes of death from cancer worldwide. In particular, death from lung cancer in men accounts for about 1/3 of deaths from all malignant neoplasms [40]. The etiology of LC is characterized by aneuploidy and genome instability, which are observed in patients before the therapy, but also have a strong effect on the survival of already treated patients [41–44]. Thus, the study of genome instability in LC is of great importance for early diagnosis and recognition of high-risk patients, as well as for tracking the results of the therapy, especially depending on the type of LC, which was shown to have different biology [45]. In this study, we first compare the data on TL with the baseline frequencies of cytogenetic damage in blood leukocytes of untreated LC patients.

## MATERIALS AND METHODS

### *Characteristics of Studied Groups*

The length of telomeres and the baseline frequencies of CA and MN were studied in peripheral blood leukocytes of 60 patients with primary diagnosed lung cancer (only men, the average age of  $59.64 \pm 1.15$  years) admitted to the Kemerovo Regional Oncological Dispensary and in 77 healthy male donors, residents of Kemerovo (the average age of  $59.49 \pm 0.75$  years). There was no significant difference in age between LC patients and the control group ( $p = 0.09$ ). Among LC patients, 61.2% were active smokers, while in the control sample, approximately equal numbers of smokers and nonsmokers were registered (Table 1).

For this study, blood samples from LC patients were taken upon admission to the clinic prior to any diagnostic and therapeutic procedures. The sample included patients who met the following criteria: no infectious diseases at the time of sampling and no occupational exposure to ionizing radiation or known chemical mutagens/carcinogens. In total, 52.2% of patients were diagnosed with squamous cell lung cancer, 25.4% with adenocarcinoma, 10.5% with small cell lung cancer, and 11.9% with other types of lung cancer (non-small cell, large cell, neuroendocrine, mesenchymal). For each patient, the stage of the disease was determined on the basis of the TNM classification (tumor, node, metastases) in accordance with the Goldstraw recommendation [46]. On this basis, 41.9% of patients were in stages I–II, 58.1% were in stages III–IV, and 14.7% of patients had metastases to distant organs. The description of this group is presented in Table 1.

All participants were informed about the purpose, methodology, and potential risks of the study; an informed consent was signed by each donor. The study was carried out in accordance with the requirements of the Ethics Committee of Kemerovo State University.

#### *Telomere Length Measurement*

To study the relative telomere length, quantitative PCR (QPCR) was used according to the protocol [47]. In each sample, the ratio of the copy number of telomeric repeats to the copy number of a single gene (T/S) of the 36B4u ribosomal phosphoprotein, also known as *RPLPO*, was determined. This ratio is proportional to the average telomere length. The concentration of studied DNA was previously measured on a Quantus fluorometer (E6150, Promega) using the Quantiflor dsDNA system (E2670, Promega). The mix for each QPCR reaction contained 5  $\mu$ L of Syber Green Master Mix (iTag Universal SYBR green Supermix, Biorad), 0.9  $\mu$ L of nuclease-free H<sub>2</sub>O; 0.05  $\mu$ L of each primer, and 0.4 ng of DNA. To amplify telomeric sequences, primers telg 5'-CGG TTT GTT TGG GTT TGG GTT TGG GTT TGG GTT TGG GTT-3' and telc 5'-GGC TTG CCT TAC CCT TAC CCT TAC CCT TAC CCT TAC CCT-3' were used. Primers albc 5'-CAG CAA GTG GGA AGG TGT AAT CC-3' and albd 5'-CCC ATT CTA TCA TCA ACG GGT ACA A-3' were used to amplify the single copy 36B4u reference gene [47]. QPCR conditions were set at 95°C for 3 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min, followed by HRM from 65°C to 95°C in increments of 0.5°C. PCR was performed three times; average values were used for analysis. QPCR results were analyzed using Bio-Rad CFX384 Manager™ software. For each sample, a threshold amplification cycle, in which the fluorescence intensity exceeded the background level (Ct), was noted for both genes. Next, the  $\Delta$ Ct index as the difference in the threshold cycle for the telomeric sample and the reference sample of the same sample was calculated ( $\Delta$ Ct = Ct<sup>tel</sup> – Ct<sup>36B4</sup>). As a result, the relative number of telomeric repeats to the single copy 36B4u gene was determined by the formula  $T/S = 2^{(-\Delta Cq)}$ .

#### *Analysis of Chromosomal Aberrations*

Heparinized samples of peripheral whole blood were cultured in RPMI-1640 medium (PanEco) supplemented with 20% fetal calf serum (Gibco) and 2% phytohemagglutinin (PanEco) for 48 h at 37°C. Colchicine (0.05 mg/mL) (PanEco) was added to the cultures 2 h before fixation. After the end of incubation, the cell cultures were treated with a hypotonic solution of 0.55% KCl for 20 min at 37°C. The material was fixed in three changes of the cooled Carnoy's fixative (methanol and glacial acetic acid in the ratio of 3 : 1). The resulting

suspension was dropped onto clean glass slides, cooled and moistened with water. The preparations were encrypted and stained with a 2% Giemsa dye solution (Merck).

Consideration of metaphases included in the analysis and criteria for registration of cytogenetic damages were consistent with generally accepted recommendations [48]. The following indicators were considered: the proportion of aberrant metaphases, the frequency of chromatid-type aberrations (single fragments, chromatid exchanges), the frequency of chromosomal aberrations (paired fragments, dicentric and ring chromosomes, atypical monocentrics). Achromatic gaps were not included in the number of aberrations and were not considered.

#### *Analysis of Micronuclei in the Culture of Leukocytes with Cytokinesis Block*

Micronuclei and other cytogenetic disorders were determined according to the standard CBMN protocol [49] using modifications proposed by F.I. Ingel [50]. In a culture flask containing 3.8 mL of culture medium (RPMI-1640 medium + 20% bovine serum + 100 U/mL penicillin), 0.2 mL of whole blood was placed. Phytohemagglutinin (PanEco) was added to the flasks at the final concentration of 6 µg/mL and cultured for 44 h at 37°C. After 44 h from the start of incubation, 48 µL of cytochalasin B (Applichem GmbH) was added to each culture, and they were cultured for another 24 h at the same temperature. At the end of the cycle, the cells were fixed in accordance with the protocol, and final suspensions were dropped onto cooled glass slides. The preparations were encrypted and stained with a 2% Giemsa dye solution (Merck) for 15 min. The preparations were analyzed under a Nikon Eclipse 80i microscope at a magnification of ×1000.

The proliferative pool was analyzed in each preparation: 500 cells with different numbers of nuclei were counted, and cells at the stage of apoptosis and mitosis were also considered. Then, in other areas of the glass, another 1000 only binucleated lymphocytes were counted, in which various types of cytogenetic damage, such as frequencies of binucleated lymphocytes with MN, frequencies of cells with nucleoplasmic bridges, frequencies of cells with nuclear protrusions, were recorded. Additionally, the index of the number of mononuclear lymphocytes with MN was calculated.

#### *Analysis of Micronuclei in Leukocyte Culture Using Pancentromeric DNA Probes*

To identify centromere-positive (CEN+) or centromere-negative (CEN-) micronuclei in lymphocytes, the method of fluorescence in situ hybridization (FISH) with pancentromeric DNA probes was used. The final preparations were washed in 2x SSC three times for 5 min,

DNA denaturation was carried out for 5 min in a solution of 0.01% pepsin, and then they were fixed in 1% paraformaldehyde for 10 min and washed in 1x PBS for 5 min. The preparations were dehydrated by wiring in a battery of alcohols of ascending concentration (70, 90, 100%) for 2 min and dried at room temperature. Dry preparations were loaded with 10  $\mu$ L probes, covered with cover slips, and placed in a hybridizer (ThermoBrite), 5 min at 75°C, 20 h at 37°C. After hybridization, the cover slips were removed and washed in solutions WT1 (2 mL 20x SSC + 98 mL water + 300  $\mu$ L Nonidet) for 1 min and WT2 (10 mL 20x SSC + 90 mL water + 100  $\mu$ L Nonidet) for 2 min (cuvettes with solutions were placed in a water bath heated to 71–72°C). Then the slides were washed in water for 3 min and dried at room temperature in the dark. Dry slides were coated with 5  $\mu$ L of Prolong Gold antifade with DAPI and covered with cover slips. Contrast staining after washes was performed with DAPI mixed with Prolong Gold Antifade mounting medium (Thermo Fisher Scientific). The slides were stored in the dark at 4–10°C until microscopic analysis.

The analysis was performed using a fluorescence microscope (Axioskop-Zeiss) equipped with filters for DAPI (blue) and FITC (green) and connected to a computer equipped with ISIS software version 5.0 (MetaSystems) for collecting and analyzing objects with MN. A total of 1000 binucleated leukocytes were studied in 38 patients with lung cancer and 37 control donors.

### *Statistical Analysis*

Statistical processing of the results of the study was carried out using the StatSoft STATISTICA 7.0 program. The quantitative indicators were assessed by calculating the mean values ( $\mu$ ) and standard errors of the mean (SE). The frequency distribution of cytogenetic indicators was checked using the Kolmogorov–Smirnov test. Comparison of the groups was performed using the Mann–Whitney *U* test and Fisher’s exact test. The correlation between indicators for cases with abnormal data distribution was calculated with employment of nonparametric statistics using the Spearman’s correlation coefficient.

## RESULTS

### *Telomere Length in Leukocytes of Lung Cancer Patients and Control Donors*

Figure 1 shows the results of the TL measurement in leukocytes of lung cancer patients and control donors. Obviously, the mean T/S value is significantly higher in the patients compared with the control ( $25.86 \pm 2.13$  versus  $15.29 \pm 1.22$ ;  $p = 0.00001$ ). When comparing TL in subgroups of patients with different pathomorphological types of lung cancer, no

differences were found between them. In particular, the T/S values were  $24.63 \pm 3.05$  in patients with squamous cell LC,  $29.60 \pm 5.13$  in patients with adenocarcinoma, and  $27.52 \pm 5.72$  in patients with small cell LC. LC patients with stages I–II, according to the TNM classification, had an average value of the relative telomere length of  $26.6 \pm 3.39$ , and those with stages III–IV had  $25.81 \pm 2.74$  ( $p > 0.05$ ). There were also no differences in TL when comparing subgroups of patients with metastases to distant organs and without them ( $30.86 \pm 5.79$  versus  $25.54 \pm 2.35$ ;  $p > 0.05$ ). Finally, age and smoking status did not affect in any way the DL parameters either in the sample of patients or in the control.

*Baseline Frequencies of Chromosomal Aberrations in Leukocytes of Lung Cancer Patients and Control Donors*

LC patients had a significant increase in the proportion of aberrant metaphases compared with control donors ( $4.4 \pm 0.25\%$  versus  $2.11 \pm 0.14\%$ ;  $p < 0.0001$ ). They also showed a significant increase in the frequency of chromatid-type aberrations, such as single fragments ( $2.95 \pm 0.24\%$  versus  $1.46 \pm 0.11\%$ ;  $p < 0.0001$ ) and chromatid exchanges ( $0.1 \pm 0.03\%$  versus  $0.03 \pm 0.02\%$ ;  $p = 0.003$ ) compared with the control group. Finally, there is a significant increase in chromosomal aberrations, such as paired fragments ( $1.1 \pm 0.14\%$  versus  $0.46 \pm 0.06\%$ ;  $p < 0.0001$ ) and chromosomal exchanges ( $0.53 \pm 0.08\%$  versus  $0.26 \pm 0.05\%$ ;  $p = 0.002$ ) in LC patients compared with the control group (Table 2).

Correlation analysis showed that the Spearman's coefficient was not significant when comparing the frequency of CA with age ( $p = 0.27$  for patients and  $p = 0.73$  for the control). Also, no significant differences were found in the frequencies of CA between the subgroups of patients with different histological types of LC and stages of the tumor process. Finally, the proportion of aberrant metaphases was not significantly increased in leukocytes of smokers with LC compared with nonsmokers ( $4.63 \pm 0.32\%$  versus  $3.98 \pm 0.42\%$ ;  $p = 0.31$ ) and did not differ between smokers and nonsmokers in control donors ( $2.02 \pm 0.16\%$  versus  $2.2 \pm 0.23\%$ ;  $p = 0.67$ ).

*Baseline Frequencies of Micronuclei in Leukocytes of Lung Cancer Patients and Control Donors*

The results of micronucleus analysis of lymphocytes cultured under conditions of the cytokinetic block are summarized in Table 3. As in the case of the test on identification of CA, significant unidirectional differences in the level of cytogenetic markers were revealed between patients and controls. LC patients demonstrate an increased frequency of binucleated leukocytes with one MN ( $1.31 \pm 0.1\%$  versus  $0.78 \pm 0.05\%$ ;  $p < 0.0001$ ) and binucleated



leukocytes with two MN ( $0.14 \pm 0.02\%$  versus  $0.09 \pm 0.01\%$ ;  $p < 0.05$ ) and total frequency of binucleated cells with MN ( $1.49 \pm 0.11\%$  versus  $0.86 \pm 0.06\%$ ;  $p < 0.0001$ ) compared with the control.

The frequency of mononuclear leukocytes with MN was  $0.33 \pm 0.05\%$  in LC patients and  $0.23 \pm 0.03\%$  in the control group ( $p > 0.05$ ). There were also no differences in the frequency of cells with protrusions between the compared samples:  $1.77 \pm 0.14\%$  in patients and  $1.69 \pm 0.12\%$  in the control. The frequency of binucleated leukocytes with nucleoplasmic bridges was significantly increased in LC patients ( $0.2 \pm 0.02\%$ ) compared with the control ( $0.12 \pm 0.02\%$ ;  $p < 0.001$ ). In addition, in the total pool of analyzed cytological objects in patients, a significant decrease in the frequency of cells with signs of apoptosis was found in comparison with the control ( $1.32 \pm 0.89\%$  versus  $2.18 \pm 1.56$ ;  $p < 0.003$ ). The age of the subjects, as well as the smoking factor did not affect the results of MN analysis either in the sample of patients with LC or in the control group. Comparison of patient subgroups with three main pathomorphological forms of lung cancer (squamous cell, adenocarcinoma, small cell) did not reveal significant differences in the results of MN analysis.

In order to assess the differential contribution of CEN+ and CEN- micronuclei to the total MN index in leukocytes of 38 LC patients and 37 control donors, an additional cytogenetic analysis was performed using pancentromeric DNA probes, the results of which are presented in Table 4. The total frequency of MN was  $1.43 \pm 0.1\%$  in the group of LC patients and  $0.87 \pm 0.07\%$  in the control group ( $p = 0.00001$ ). At the same time, only CEN+ MN was significantly higher in leukocytes of patients compared with the control ( $0.83 \pm 0.08\%$  and  $0.32 \pm 0.03\%$ ;  $p = 0.00001$ ), while the frequencies of CEN- between the compared samples did not differ significantly.

#### *Relationship between the Telomere Length and Cytogenetic Damage in Leukocytes*

The relationship between TL and structural damage of chromosomes was assessed using the Spearman's correlation coefficient. There was no relationship between TL and the proportion of aberrant metaphases in both groups: in patients ( $p = 0.238$ ) and in the control ( $p = 0.662$ ). Also, there was no significant correlation between TL and the frequency of chromatid-type aberrations in LC patients ( $p = 0.903$ ) and in the control ( $p = 0.972$ ). However, Spearman's correlation coefficient for TL with chromosomal aberrations turned out to be significant in the sample of LC patients ( $R = 0.3$ ;  $p = 0.02$ ) and insignificant in the group of control donors (Fig. 2). Also the correlation of TL with paired fragments was significant in LC patients ( $R = 0.36$ ;  $p = 0.004$ ), while this was not observed in the control group (Fig. 3).

No relationship between frequencies of TL and MN in leukocytes of LC patients and in control donors was revealed ( $p > 0.05$ ). However, an inverse relationship was found between TL and the frequency of binucleated leukocytes with protrusions ( $R = -0.27$ ;  $p = 0.04$ ) in LC patients (Fig. 4). An inverse correlation was also found for TL and the frequency of cells with signs of apoptosis ( $R = -0.31$ ;  $p = 0.02$ ) in LC patients (Fig. 5). No significant correlations were found between these biomarkers for control donors.

## DISCUSSION

To date, the relationship between the telomere length and the risk of malignant tumors remains largely controversial and requires further study. There is an opinion that not only a decrease but also a lengthening of TL may be associated with the risk of developing cancer. In the present study, performed in accordance with the case--control design, LC patients had significantly longer telomeres in leukocytes compared with healthy donors. This fact is in line with previously published cohort studies of male smokers in Finland [51] and Asian nonsmoking women [52]. The association between longer telomeres in leukocytes with the risk of lymphoma and melanoma was also established [9, 51, 53–55].

There were no significant differences in TL between patients with different histological LC subtypes (squamous cell carcinoma, adenocarcinoma and small cell carcinoma), different cancer stages, or different smoking status. However, dividing the general sample into subgroups leads to a significant decrease in the number of compared cases, which may well affect the reliability of the results of comparison. It is obvious (Fig. 1) that dispersion of individual values of TL in LC patients is greater than in control donors, and this suggests that an increase in compared samples of patients with different histological types of LC at different stages of the disease in the future will reveal the relationship of TL to these factors.

The telomere length paradox has been widely discussed in recent years. It is known that older people, whose telomeres in somatic cells are shorter than in young people, have an increased susceptibility to major types of cancer [10]. However, constitutively long telomeres can provide additional rounds of cell division. This, in turn, increases the risk of accumulating somatic mutations that block the pathways to cell aging and apoptosis and promote tumorigenesis. Various mathematical hypotheses and models were proposed to resolve the telomere length paradox and its relationship with cancer [56–58]. For example, it was suggested that the role of TL varies depending on the stage of carcinogenesis [15]. According to this hypothesis, the first stage in a two-stage model of carcinogenesis (accumulation of mutations in multipotent dividing stem cells) occurs regardless of the length of individual telomeres. At the same time, the telomere length begins to play a key role in the progression

of cancer at its second stage, clonal expansion. Thus, this model emphasizes that constitutively long telomeres do not cause cancer, but may contribute to carcinogenesis.

Carcinogenesis is often considered as the result of a series of driver mutations, genomic instability, and selection of cells with a replicative advantage [59]. People with longer telomeres have an increased risk of clonal cell expansion with DNA damage.

Significantly increased baseline frequencies of MN and CA in leukocytes of LC patients shown in our study are consistent with the data of other authors on genome instability in this type of cancer [33, 42, 60–62]. At the same time, the use of FISH technology with pancentromeric DNA probes first showed that aneuploidies (CEN+ micronuclei) play a significant role in the total pool of cytogenetic disorders in lymphocytes of LC patients, which confirms the importance of genomic type mutations in the etiology and pathogenesis of this type of cancer [63].

The present study first showed the direct relationship between chromosomal aberrations, paired fragments, and TL, which was found only in patients with LC, but not in control donors. In addition, an interesting observation is the inverse correlation between TL and the frequency of leukocytes with nuclear protrusions, as well as the inverse correlation of TL with the frequency of cells entering apoptosis. It can be assumed that incomplete or insufficient repair of DNA double-strand breaks in LC patients is manifested by the accumulation of chromosomal aberrations and protrusions. As for the presence of a feedback between TL and apoptosis, it confirms the fact that constitutively long telomeres allow cells with accumulated damage to escape from the systems of programmed death.

The absence of a relationship between the frequency of MN and TL is probably associated with the fact that an increase in the frequency of MN in patients is mainly a consequence of aneuploidies arising from defects in the control point of the spindle assembly [64].

In the group of control donors, no associations were found between TL and any cytogenetic damage. Earlier studies of healthy men in Norway showed that an increased frequency of chromosomal aberrations in leukocytes is associated with a shorter telomere length [17]. It can be assumed that, in healthy control subjects, spontaneous chromosomal aberrations and TL are in equilibrium, which is disturbed when genome damage and other mechanisms of carcinogenesis trigger an irreversible process of neoplastic transformation.

Thus, our study shows that, in the complex LC biology, the genome damage of somatic cells is associated with longer telomeres. Further efforts in this direction should be focused on a comparative study of representative samples of patients with main histological forms of LC at different stages of the disease. It can be expected that the results of these studies will

provide a better understanding of the significance of TL as a biomarker of bronchogenic cancer progression.

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#### COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interest.* The authors declare that they have no conflict of interest.

*Statement of compliance with standards of research involving humans as subjects.* All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants involved in the study.

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## TABLES

**Table 1.** Characteristics of the studied groups

| Indicators                              | LC patients, $n = 67$    | Control, $n = 77$        |
|---|--------------------------|--------------------------|
| Age (years) ( $\mu \pm SE$ ) / min--max | 59.64 $\pm$ 9.38 / 22-73 | 59.49 $\pm$ 6.61 / 43-79 |
| Smoking status (%):                     |                          |                          |
| smokers                                 | 61.2                     | 52.0                     |
| nonsmokers                              | 38.8                     | 48.0                     |
| LC type* (%):                           |                          |                          |
| squamous cell cancer                    | 52.2                     |                          |
| adenocarcinoma                          | 25.4                     |                          |
| small cell cancer                       | 10.5                     |                          |
| other cancers                           | 11.9                     |                          |
| TNM** (%):                              |                          |                          |
| I, II                                   | 29.9                     |                          |
| III, IV                                 | 70.1                     |                          |
| Metastases – (%)                        | 86.6                     |                          |
| Metastases + (%)                        | 13.4                     |                          |

\*Pathomorphological diagnosis.

\*\*LC stages according to the tumor–node–metastasis classification.

**Table 2.** Frequencies of chromosomal aberrations in leukocytes of lung cancer patients and control donors

| Aberration type                   | Lung cancer ( <i>n</i> = 67) |         | Control ( <i>n</i> = 77) |         |
|-----------------------------------|------------------------------|---------|--------------------------|---------|
|                                   | $\mu \pm \text{SE}, \%$      | min–max | $\mu \pm \text{SE}, \%$  | min–max |
| Proportion of aberrant metaphases | $4,4 \pm 0,25^{**}$          | 0,5–9,5 | $2,11 \pm 0,14$          | 0–5     |
| Single fragments                  | $2,95 \pm 0,24^{**}$         | 0–8,5   | $1,46 \pm 0,11$          | 0–3,5   |
| Chromatid exchanges               | $0,1 \pm 0,03^*$             | 0–0,5   | $0,03 \pm 0,02$          | 0–1     |
| Paired fragments                  | $1,1 \pm 0,14^{**}$          | 0–5,5   | $0,46 \pm 0,06$          | 0–2,5   |
| Chromosomal exchanges             | $0,53 \pm 0,08^*$            | 0–4     | $0,26 \pm 0,05$          | 0–2     |

\* $p < 0.01$ , \*\*  $p < 0.0001$ , differ from the value for the control group.

**Table 3.** Frequencies of micronuclei and other cytogenetic damages ( $\mu \pm \text{SD}, \%$ ) in leukocytes of lung cancer patients and control donors

| Group       | Binucleated cells with MN |                   |                       | Binucleated cells with bridges | Binucleated cells with protrusions | Mononuclear cells with MN |
|-------------|---------------------------|-------------------|-----------------------|--------------------------------|------------------------------------|---------------------------|
|             | 1 MN                      | 2 MN              | total MN              |                                |                                    |                           |
| Lung cancer | $1,31 \pm 0,1^{***}$      | $0,14 \pm 0,02^*$ | $1,49 \pm 0,11^{***}$ | $0,2 \pm 0,02^{**}$            | $1,77 \pm 0,14$                    | $0,33 \pm 0,05$           |
| Control     | $0,78 \pm 0,05$           | $0,09 \pm 0,01$   | $0,86 \pm 0,06$       | $0,12 \pm 0,02$                | $1,69 \pm 0,12$                    | $0,23 \pm 0,03$           |

\* $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , differ from the value for the control group.

**Table 4.** Frequencies of centromere-positive (CEN+) and centromere-negative (CEN–) micronuclei ( $\mu \pm \text{SD}, \%$ ) in leukocytes of lung cancer patients and control donors

| Biomarker                 | Lung cancer ( <i>n</i> = 38) |                 |                  | Control ( <i>n</i> = 37) |                 |                 |
|---------------------------|------------------------------|-----------------|------------------|--------------------------|-----------------|-----------------|
|                           | MN +                         | MN –            | total MN         | MN +                     | MN –            | total MN        |
| Binucleated cells with MN | $0,83 \pm 0,08^*$            | $0,61 \pm 0,04$ | $1,43 \pm 0,1^*$ | $0,32 \pm 0,03$          | $0,58 \pm 0,05$ | $0,87 \pm 0,07$ |

\* $p = 0.00001$ , differs from the value for the control group.

**FIGURE CAPTIONS**

**Fig. 1.** The results of measuring telomere length.  $F = 20.24$ ;  $p = 0.00001$ .

Key:

Относительная длина теломер --> Relative telomere length;

Контроль --> Control;

Рак легких --> Lung cancer.

**Fig. 2.** The relationship of the telomere length to the frequency of chromosomal aberrations in leukocytes of lung cancer patients.

Key:

Относительная длина теломер --> Relative telomere length;

Аберрации хромосомного типа, % --> Chromosomal aberrations, %.

**Fig. 3.** The relationship of the telomere length to the frequency of paired fragments in leukocytes of lung cancer patients.

Key:

Относительная длина теломер --> Relative telomere length;

Парные фрагменты, % --> Paired fragments, %.

**Fig. 4.** The relationship of the telomere length to the frequency of nuclear protrusions in leukocytes of lung cancer patients.

Key:

Относительная длина теломер --> Relative telomere length;

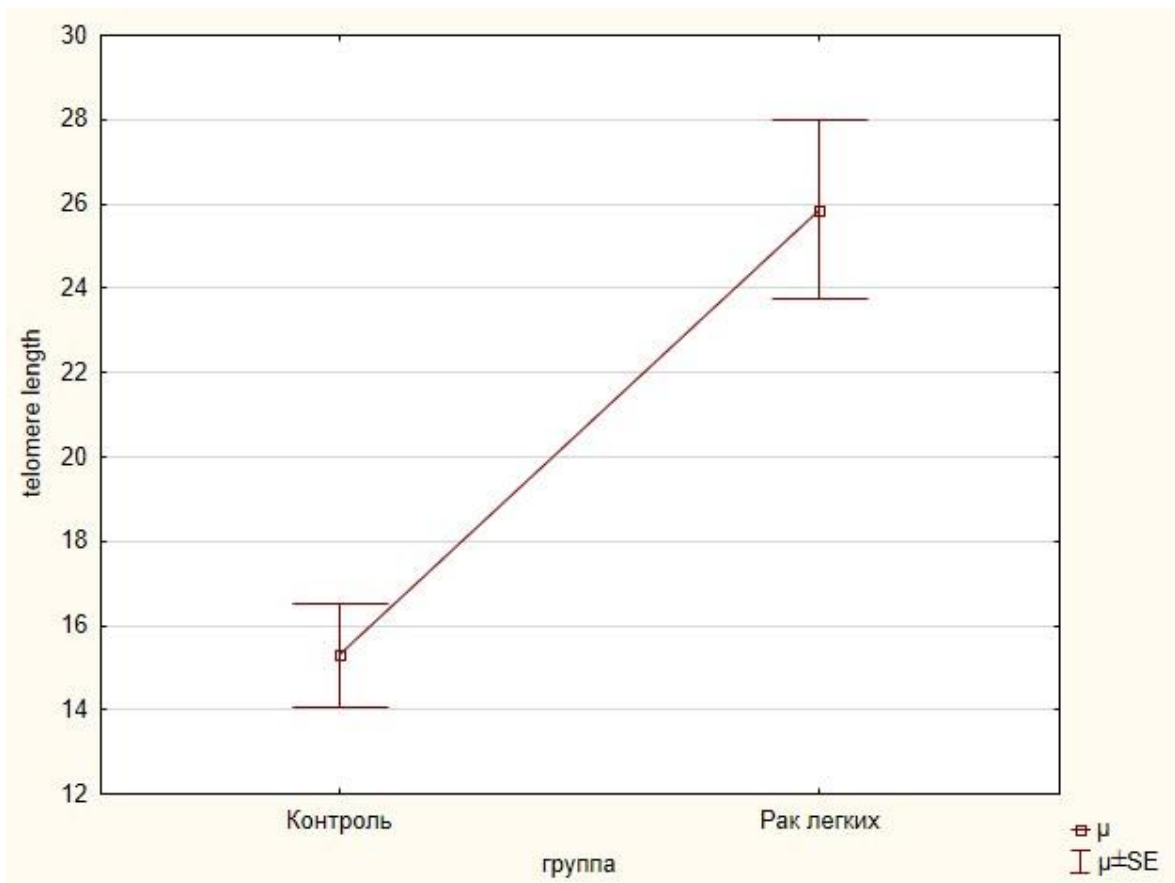
Двухъядерные лейкоциты с протрузиями, % --> Binucleated leukocytes with protrusions, %.

**Fig. 5.** The relationship of the telomere length to the frequency of apoptosis in leukocytes of lung cancer patients.

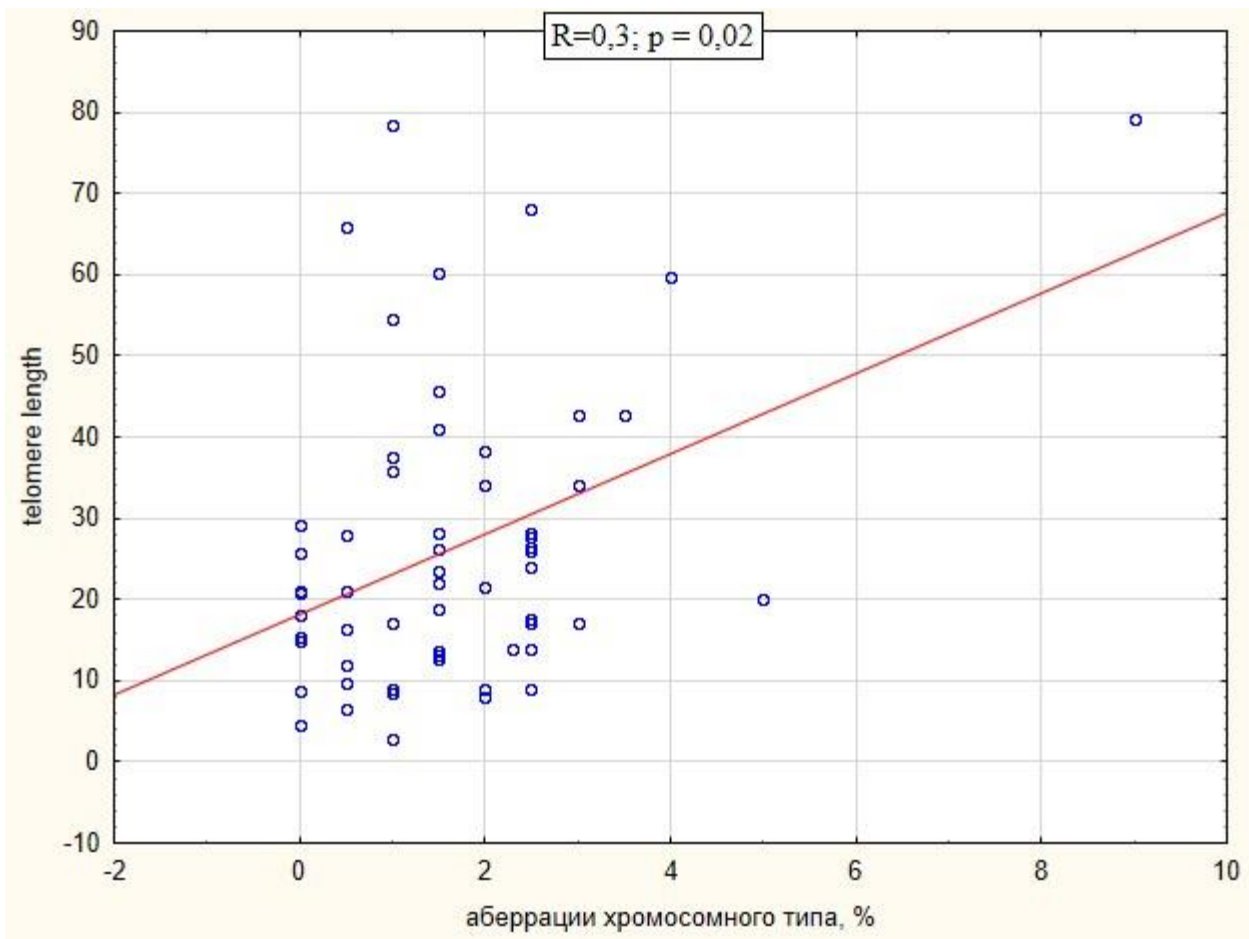
Key:

Относительная длина теломер --> Relative telomere length;

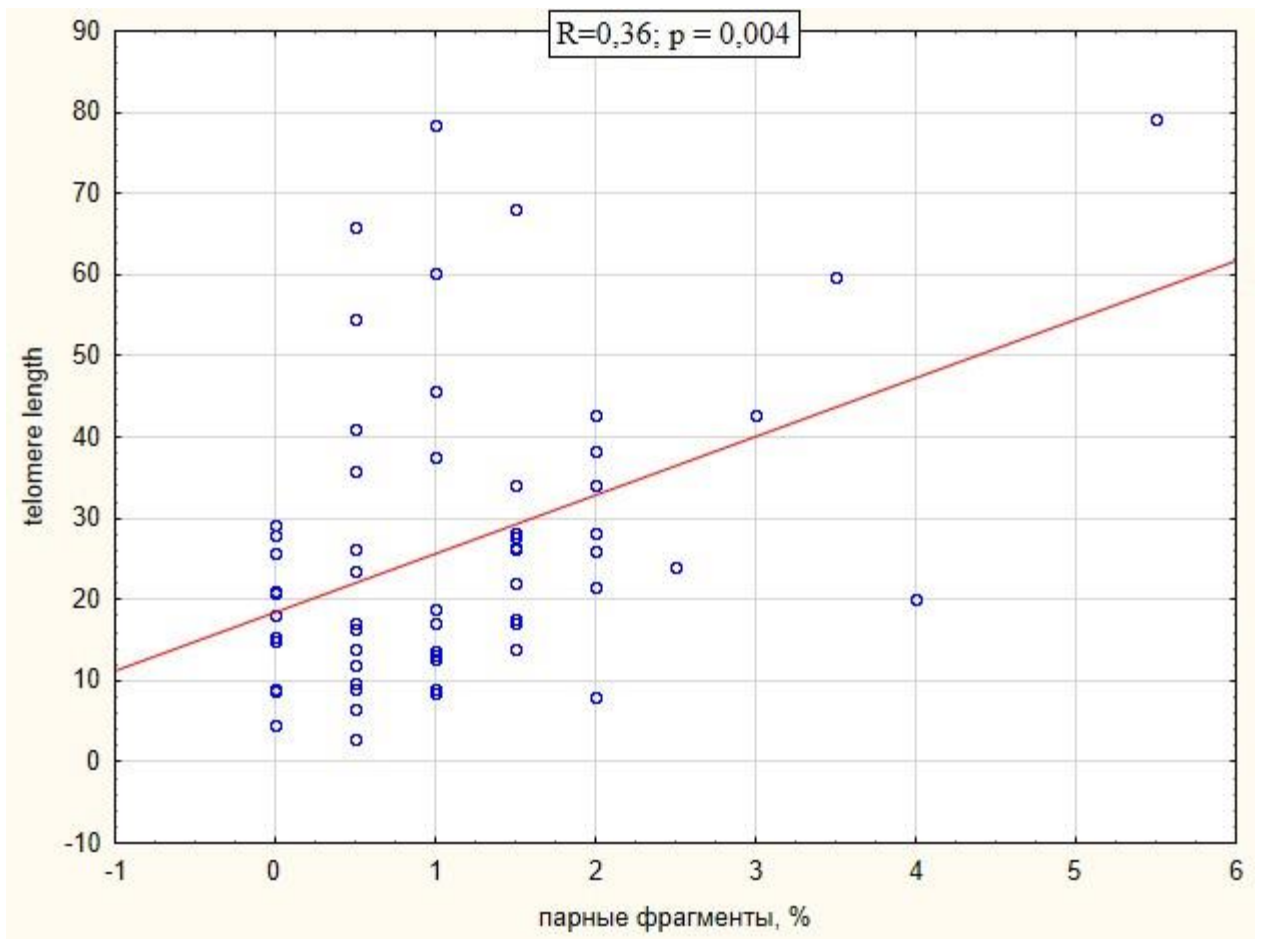
Апоптоз, % --> Apoptosis, %.



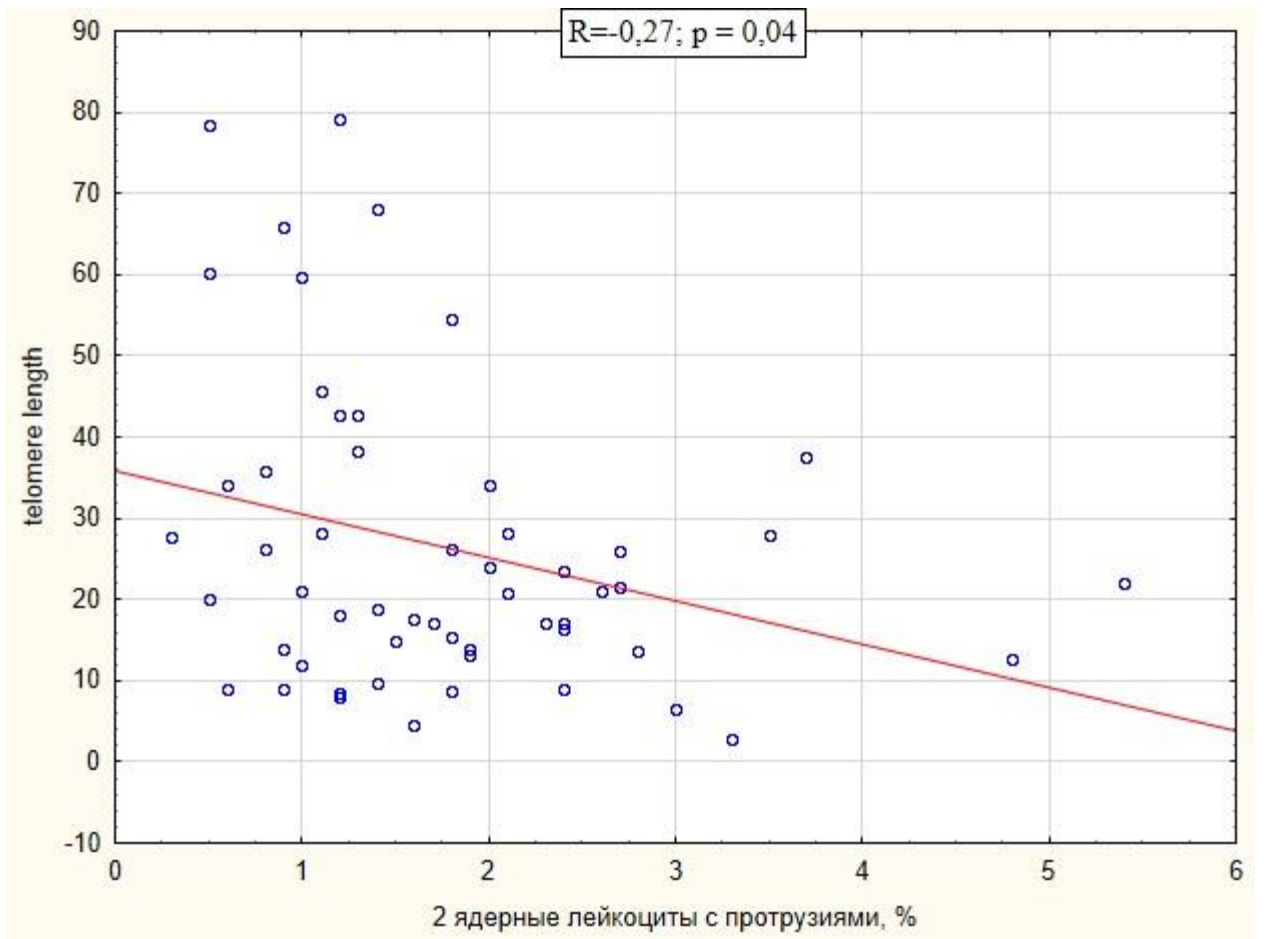
**Fig. 1.** The results of measuring telomere length.  $F = 20.24$ ;  $p = 0.00001$ .



**Fig. 2.** The relationship of the telomere length to the frequency of chromosomal aberrations in leukocytes of lung cancer patients.

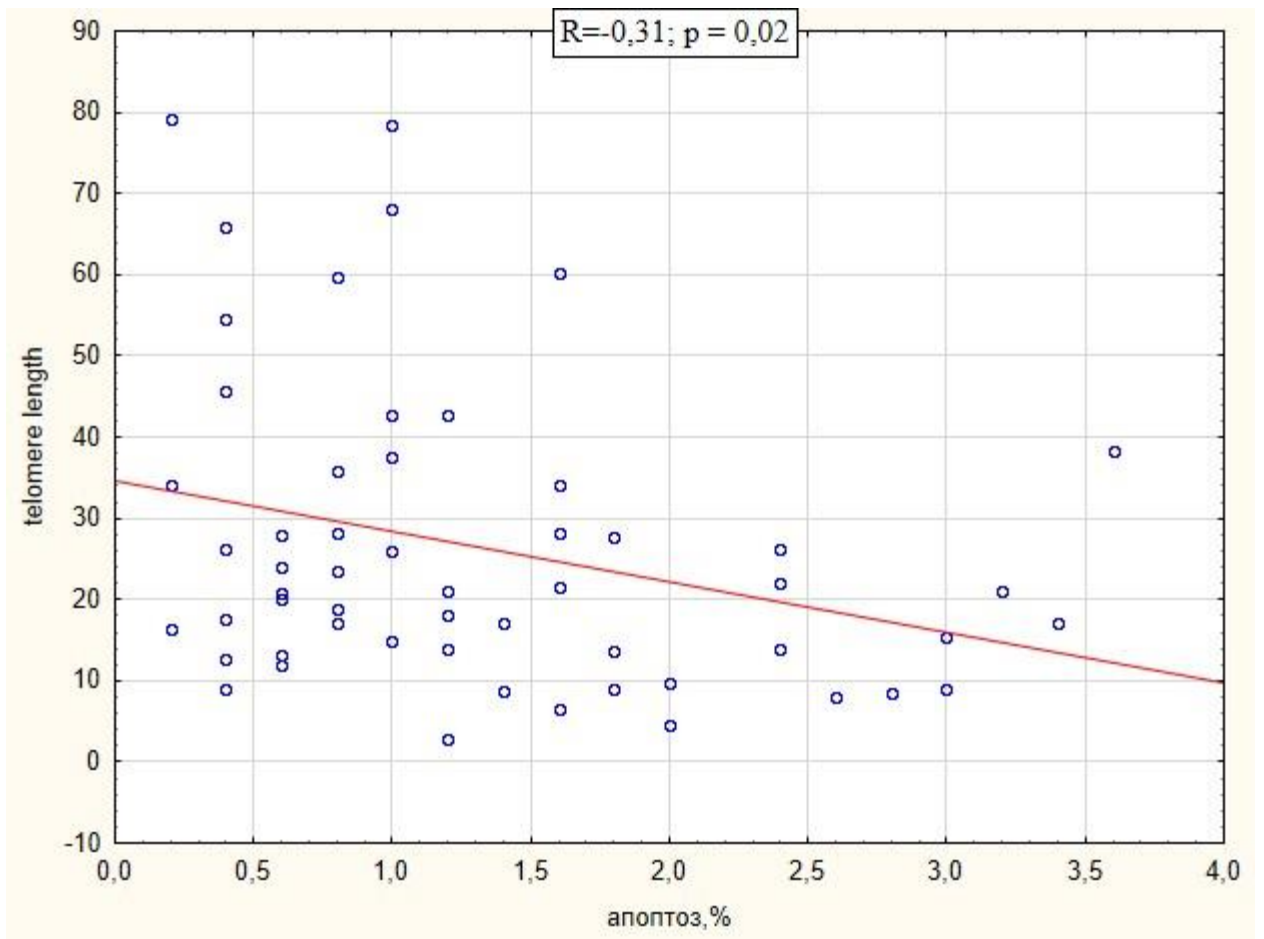


**Fig. 3.** The relationship of the telomere length to the frequency of paired fragments in leukocytes of lung cancer patients.



**Fig. 4.** The relationship of the telomere length to the frequency of nuclear protrusions in leukocytes of lung cancer patients.





**Fig. 5.** The relationship of the telomere length to the frequency of apoptosis in leukocytes of lung cancer patients.