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**THE AGING ASPECTS
OF HUMANS PROTRACTEDLY
EXPOSED TO IONIZING
RADIATION**

Summary of the Doctoral Thesis
Speciality – Occupational and
Environmental Medicine

Riga, 2013

RĪGAS STRADIŅA
UNIVERSITĀTE

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Doctoral thesis was elaborated in the Centre of Occupational and Radiological Medicine of Pauls Stradins Clinical University Hospital (CUH) and Institute of Occupational Safety and Environmental Health of Rīga Stradiņš University (RSU) in collaboration with RSU Institute of Oncology, the Department of Molecular Biology and Genetics of Pauls Stradins CUH Joint Laboratory, RSU Interdepartmental Laboratory of Immune Genetics and Clinical Immunology, and Latvian Institute of Organic Synthesis.

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ieguldījums tavā nākotnē!

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1. TOPICALITY OF THE PROBLEM

Ionizing radiation health effects have received much attention in recent decades due to growing usage of nuclear power as energy source and due to several heavy accidents with release of radioactive elements into environment that have recently happened. The use of ionizing radiation is progressively growing in various industries (medicine, security, science, *etc.*) and concurrently the number of exposed humans is growing as well. Nuclear power plant damage may create radioactive pollution of wide area and expose a huge amount of people to ionizing radiation as it was seen in Chernobyl and Fukushima accidents [Akiba, 2012; Anzai *et al.*, 2011; Ohnishi, 2012; Yablokov *et al.*, 2009]. The irradiation doses received by victims of such accidents in most cases do not exceed 1 Gy as it was observed in Chernobyl disaster [UNSCEAR, 2011; WHO, 2006] inversely to those in A-bomb explosions when high doses are achieved [Shigematsu *et al.*, 1995]. The most of the radionuclides released in nuclear power plant accidents have long period of half-decay, which increases the risk of protracted irradiation for inhabitants. Thereby due to exposure of large number of people to low doses of ionizing radiation for a long time (for some of them during the whole their life), effective health care programmes need to be developed for combating the consequences. It is important to realize the late after-effects of ionizing radiation exposure and understand in details the mechanisms of them for timely resolving and prevention of radiation induced health problems.

The effect of high doses is clearly seen immediately or some time after exposure as an acute radiation sickness, which is highly dangerous with high mortality of irradiated people, while low-dose radiation exposure is not so evident and initially does not show any visible clinical symptoms and does not decrease longevity obviously [Mettler and Upton, 2008]. In low-dose ionizing radiation effects on human health still remain uncertain. Nearly the only clearly

proved effect of long-term low-dose radiation exposure is cancer development [National Research Council, 2006]. A number of studies conducted to reveal health effects of exposure to ionizing radiation show probable irradiation-dependent development of such non-cancer diseases as coronary heart disease, myocardial infarction, stroke, arterial hypertension, mental disorders, cataract, *etc.*, but evidence of that is still limited [UNSCEAR, 2008; National Research Council, 2006]. Many of these diseases are aging-associated. Some signs of premature aging were shown in studies of persons exposed to radiation [Yablokov *et al.*, 2009], but the evidence of ionizing radiation effects on human aging is still insufficient. The exact mechanisms of probable radiation induced premature senescence remain unclear. It is generally accepted that certain amount of active free radicals is produced in the organism under the ionizing radiation exposure. Ionizing radiation may damage important biological structures (*e.g.*, DNA, cell membranes and proteins) directly or through the action of free radicals. Irradiation induced defects usually are repaired by healing mechanisms of the body (*e.g.*, DNA repair machinery) shortly after the exposure, but some injuries may stay unrepaired and cumulate. In case of frequent repeated exposure to ionizing radiation the repairing systems are protractedly activated and overloaded, which may cause higher probability of unrepaired damages and accumulation of defects, which may result in growing mistakes in functioning and impairment of the functions of the organism as a whole. All this may lead to faster aging of the chronically irradiated organism. There are a lot of similar features in biological effects of ionizing radiation and normal aging process. In the development of normal aging process free radicals and accumulation of mistakes take an important place, too. On the other hand, aging is a very complicated and multifactor process involving all organs and systems of the organism. There are a lot of different theories of aging which try to explain its mechanisms, but until nowadays there is no single universal theory of aging, which could elucidate all processes occurring in senescence. At

the same time there is no any simple universal measurement of aging, which could clearly indicate the extent of biological age of the person. Taking into account the complicated nature of aging, which cannot be characterized by one certain parameter, several aspects of irradiated humans' senescence have been described in the doctoral thesis.

Objective of the Thesis

The objective is to establish, if persons protractedly exposed to small doses of ionizing radiation age faster and in a different way than chronically non-exposed humans.

Tasks of the Thesis

1. To characterize the varied signs of aging in humans protractedly exposed to small doses of ionizing radiation, including the analysis of epidemiological parameters and biological substrate measurements that describe aging intensity.
2. To compare aging signs of chronically exposed humans with aging features of persons, who did not receive excessive ionizing radiation exposure earlier in their life, with a purpose of clarifying differences in the process of aging.
3. On the basis of study findings to develop practical recommendations for health care improvement for persons protractedly exposed to ionizing radiation.

Hypothesis of the Thesis

1. The humans, who underwent protracted ionizing radiation exposure, are likely to age faster than non-exposed persons that may manifest with higher morbidity with age-dependent diseases and death at younger age.

2. Humans under various kinds of long-term irradiation might age with different intensity: Chernobyl nuclear power plant (CNPP) accident clean-up workers, due to continuous internal irradiation from incorporated long-living radionuclides are likely to age faster than people, who receive external intermittent exposure to ionizing radiation (radiologists, assistants of radiologists, *etc.*).

Scientific Novelty of the Thesis

1. The aging processes of humans protractedly exposed to ionizing radiation were analysed, simultaneously including relative telomere length measurements, assessment of factors participating in telomere length regulation (transforming growth factor β), and binding the results with precise data about health condition of the irradiated persons, that has not been done previously.
2. During current research paradoxically longer telomeres were found among persons, who have received heavier long-term irradiation, unlike telomere shortening, expected due to ionizing radiation exposure. Telomere lengthening might be significant carcinogenesis factor in persons exposed to radiation. In doctoral thesis, feasible explanation was proposed – telomere lengthening appears as a result of telomerase expression activation due to permanent DNA damage under long-term ionizing radiation exposure, and this requires further profound investigation in the future.
3. Ionizing radiation effects on human aging for the first time were compared simultaneously between two groups of differently exposed persons: humans exposed to continuous internal irradiation from incorporated radionuclides (Chernobyl nuclear power plant accident clean-up workers having radionuclides with long period of half-decay accumulated in their body) and persons chronically receiving small doses of radiation at work (external

intermittent irradiation with exposure discontinuation due to pace of work duties, rest time, *etc.*, in radiologists, assistants of radiologists and other occupations).

Practical Significance of the Thesis

1. Main features of aging in protractedly irradiated humans have been identified in the doctoral thesis; these findings help to clarify ionizing radiation effects and give opportunity for elaboration of effective preventive measures against unfavourable radiation impact on human health.
2. Taking into account progressively growing number of persons protractedly exposed to small doses of ionizing radiation and necessity of their long-term qualitative health care, research findings of specific features in irradiated persons' health problems enable adequate and timely planning of measures for treatment and prevention of diseases.
3. The thesis provides elaborated complex of practical recommendations for improvement of health care for protractedly irradiated persons.

Volume and Structure of the Thesis

The doctoral thesis "The Aging Aspects of Humans Protractedly Exposed to Ionizing Radiation" consists of eight parts: Introduction, Literature Review, Materials and Methods, Results, Discussion, Conclusions, Practical Recommendations, and Bibliography. The thesis is comprised by 153 pages with 40 figures, 39 tables, 317 references and 11 annexes. The thesis is written in the Latvian language. There are 16 publications in connection with the topic of doctoral thesis.

2. MATERIALS AND METHODS

The doctoral thesis summarizes the results of the study, which was conducted during the time period from 2008 till 2013. The study was designed to evaluate the effect of previous long-term ionizing radiation exposure on human aging processes. It was retrospective cohort study with elements of cross-sectional study. Several **study populations** were chosen for research:

1) Chernobyl nuclear power plant (CNPP) accident clean-up workers from Latvia;

2) aging employees older than 40 years, which were exposed to ionizing radiation at work for at least 5 years (radiologists, assistants of radiologists, *etc.*);

3) control group – for previous two groups age and gender matched Latvian inhabitants, who were not exposed to excessive ionizing radiation except natural background and rare small medical X-ray examinations.

Study participants were randomly selected from the persons examined in the Centre of Occupational and Radiological Medicine inpatient and outpatient departments of Pauls Stradins Clinical University Hospital (the Centre) in Riga. The study was approved by the Regional Committee for Medical Research Ethics. From every study participant obligatory informed written consent was received.

Characterization of study population “CNPP clean-up workers from Latvia” – all study participants were males. These men participated in CNPP clean-up works in 1986–1991 for one to three months long period. They were among those 6000 Latvian inhabitants sent by the Soviet Union to Chernobyl for combating consequences of the disaster. The main tasks during work in Chernobyl were: deactivation of contaminated radioactive objects; soil decontamination by digging and mechanical removal; transportation of people and contaminated materials; construction; encampment supporting activities

(food supply, *etc.*). None of the study participants has developed acute radiation sickness symptoms either in Chernobyl or after returning to Latvia. CNPP clean-up workers have regularly undergone clinical examination in the Centre since 1994. The frequency of examinations has been at least once in two or three years, thus these persons have been very well examined, receiving information about their health status for more than 15-year period. Additional information on health status, participation time in clean-up works, job tasks during stay in Chernobyl and documented irradiation dose was obtained from the Latvian State Register for Persons with Occupational Diseases and Persons Exposed to Ionizing Radiation in Chernobyl (the Register). All information on diseases revealed by regular examination was stored in the Register using the International Classification of Diseases, 10th Revision (ICD-10). Unfortunately only 58% of CNPP clean-up workers from Latvia had information about the dose of radiation exposure. The documented dose range was 0.1–500.0 mSv, median dose was 113 mSv and interquartile range – 80–190 mSv. Taking into account inaccurate radiation dose assessment in Chernobyl (only external irradiation was measured for calculations of documented doses; internal irradiation was not evaluated), documented doses were considered unreliable. Therefore participation time and performed tasks during stay in Chernobyl were employed for evaluation of radiation exposure in CNPP clean-up workers.

All evaluations during the study were made in age context of the explored persons, comparing data of exposed humans with non-exposed ones. Real value of control group usage for evaluation of analysed effects of radiation was disputable due to limited information about health status in controls. For achieving more precise assessment of radiation impact on health it was decided to separate CNPP clean-up workers with relatively high exposure from less exposed accident liquidators and compare aging signs in these subgroups. For comparative analysis **CNPP clean-up workers** were divided into several **subgroups** according to:

1) the year of participation time in clean-up works in Chernobyl: during 1986; from 1987 till 1991;

2) job tasks during clean-up works: persons, who performed deactivation of radioactive objects and digging with high risk to be contaminated with radioactive materials and potentially higher dose of irradiation; persons, who performed another job tasks (drivers, builders, cooks, *etc.*);

3) combining all above-mentioned factors among CNPP recovery workers, persons, who participated in 1986, performed deactivation and digging tasks and were exposed to higher radiation doses, were marked out as a high risk group; all other persons were graded as a low risk group.

Considering multiform signs of aging and complicated structure of its mechanisms, which make impossible the evaluation of aging by one certain parameter, several **methods** were used for achieving the aim of the thesis:

1) **epidemiological data analysis:**

- mortality rate of CNPP clean-up workers from Latvia with calculation of years of potential life lost (YPLL);
- the morbidity structure of CNPP clean-up workers, focusing on age-dependent diseases, including non-oncologic diseases and malignancies;

2) **measurements** and analysis **of biological samples** from the study participants:

- relative telomere length (RTL) measurement in peripheral blood leukocytes (PBL) by real time quantitative polymerase chain reaction (q-PCR);
- detection of transforming growth factor β (TGF β) level in blood serum by immune fermentative ELISA method;

- detection of nitrogen monoxide (NO) and iron levels in hair by electron paramagnetic resonance (EPR).

Data of several Latvian registers were utilized for calculations:

- Latvian State Register for Persons with Occupational Diseases and Persons Exposed to Ionizing Radiation in Chernobyl;
- The Central Statistical Bureau;
- Latvian Population Register;
- The Register for Patients with Certain Diseases of the Centre for Disease Prevention and Control.

Appropriate statistical methods were applied according to the shape of data distribution. The models were adjusted for age. Significance level was set at 0.05. All calculations were completed by software Microsoft Excel and IBM SPSS Statistics Version 20.

The structure of the study is summarized in Table 1.

3. MAIN RESULTS AND DISCUSSION

3.1. Mortality of CNPP Clean-Up Workers

In total 1018 out of 6004 CNPP clean-up workers died from April 26th 1986 till January 1st 2010, *i.e.* 17% in less than 24 years. The mortality of CNPP clean-up workers gradually increased from 0.2 per 1000 people in 1987 to 18.6 cases per 1000 in 2009, *i.e.* it increased 93 times. Mean age of CNPP accident recovery workers at participation time in clean-up works was 32.09 ± 7.36 years, but in 2009 their mean age was already 53.93 ± 7.07 years. The age of CNPP clean-up workers at the moment of death in 1986–2010 was between 24 and 89 years (mean age 51.03 ± 9.11 years; Figure 1), while in 1987–1999 the mean age was 40–45 years. Of CNPP clean-up workers 84.5% died younger than 59.

Table 1

Summary of the research structure

Analysed parameters	Method	Study populations	Size of group	Methods of statistical analysis
<i>Epidemiological data analysis</i>				
Mortality: standardized mortality rate (SMR), mortality rate per 1000 people, odds ratio (OR), 95% confidence interval (95% CI), years of potential life lost (YPLL)	Retrospective cohort study (1986-2009), descriptive analysis (1986-1998), comparative analysis (1999-2009)	1) CNPP clean-up workers from Latvia; 2) Age matched general Latvian male population	6004	Indirect standardization, independent samples <i>t</i> -test, χ^2 test, linear regression
Non-oncologic morbidity: prevalence per 1000 persons, odds ratio (OR), 95% confidence interval (95% CI)	Cross-sectional study (01.01.2011.), descriptive and comparative analysis between subgroups (1986-2010)	CNPP clean-up workers from Latvia divided in subgroups	3993	Estimation of the odds for disease development
Oncologic morbidity: standardized incidence rate (SIR), odds ratio (OR), 95% confidence interval (95% CI), prevalence per 1000 persons, incidence per 1000 persons	Retrospective cohort study (1986-2010), descriptive analysis (1986-1997), comparative analysis (1998-2010)	1) CNPP clean-up workers from Latvia; 2) Age matched general Latvian male population	5950	Indirect standardization, independent samples <i>t</i> -test, χ^2 test

Continuation of Table 1

Analysed parameters	Method	Study populations	Size of group	Methods of statistical analysis
<i>Measurements of biological samples</i>				
Relative telomere length (RTL) in peripheral blood leukocyte fraction cells and mononuclear cells	Real time quantitative polymerase chain reaction (q-PCR) [Cawthon, 2002]; cross-sectional study	1) CNPP clean-up workers from Latvia divided into subgroups;	584	Independent samples <i>t</i> -test, χ^2 test, Mann-Whitney test, linear regression, correlation analysis
		2) Employees exposed to ionizing radiation at work;	14	
		3) Control group	236	
Transforming growth factor β (TGFβ) level in blood serum	Immune fermentative ELISA method; cross-sectional study	1) CNPP clean-up workers from Latvia;	108	Independent samples <i>t</i> -test, χ^2 test, Mann-Whitney test, linear regression, correlation analysis
		2) Employees exposed to ionizing radiation at work;	36	
		3) Control group	103	
Nitrogen oxide (NO) and iron level in hair	Electron paramagnetic resonance (EPR); cross-sectional study	1) CNPP clean-up workers from Latvia;	58	Independent samples <i>t</i> -test, χ^2 test, linear regression
		2) Employees exposed to ionizing radiation at work;	29	
		3) Control group	87	

Comparing 1986-year CNPP clean-up workers with those from 1987–1991, similar odds for dying were found (OR 0.93 and 95% CI (0.80; 1.08)), moreover, significant differences in mean age of died persons were not revealed. The analysis of mortality by age groups disclosed noticeable differences: the highest odds for dying were in 1986-year young recovery workers, who participated in clean-up works at age under 29 years (OR 1.14 and 95% CI (0.81; 1.61)), between 30 and 39 years (OR 1.16 and 95% CI (0.94; 1.43)), and 40–49 years (OR 1.17 and 95% CI (0.83; 1.65)). By contrast, for older recovery workers, who were above 50 years at the moment of

participation in clean-up works, the odds to die were just opposite – 2.8 times higher for 1987-1991 year participants (OR 0.36 and 95% CI (0.14; 0.87), comparing 1986 with 1987-1991 year participants).

Figure 1. Distribution of CNPP clean-up workers died in 1987–2009 by age groups (percentage of total number died)

The time period from 1999 till 2009 was analysed in details. The median age of death of CNPP clean-up workers progressively increased by years and statistically significantly correlated with time (regression equation was $y = 0.907x - 1764.177$, Pearson's coefficient $r = 0.345$, $p < 0.001$). Comparing total mortality of CNPP clean-up workers with general Latvian male population by method of indirect standardization, it was found that the mortality rate in both groups during this period is very similar (SMR 1.00 and 95% CI (0.99; 1.18)), but main differences were between various age groups of CNPP recovery workers. The highest mortality was among 45–59 years old CNPP clean-up workers, that exceeded age and gender matched general population parameters (SMR 1.10, 95% CI (0.99; 1.18)). In addition, SMR changed by years in different age groups (Table 2).

Table 2

**Standardized mortality rates (SMR) of CNPP clean-up workers
comparing with age and gender matched general Latvian population in
1999–2009 by age groups**

Age group (years)	Years											At total in period
	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	
30-34	-	1.5	-	-	-	-	-	-	-	-	-	0.4
35-39	1.1	0.5	1.1	1.2	1.2	-	1.0	2.0	-	-	-	0.9
40-44	0.6	0.7	0.9	0.8	1.2	1.5	0.8	0.7	1.1	1.3	0.6	0.9
45-49	0.7	1.3	1.2	0.6	1.6*	1.5	0.8	1.1	1.2	1.6*	0.2*	1.1
50-54	1.4	0.7	1.3	1.2	1.3	1.1	0.8	1.0	1.0	1.1	1.5*	1.1
55-59	0.8	1.6	0.7	1.1	1.1	1.2	1.2	1.0	1.2	0.9	1.0	1.1
60-64	0.3	-	0.9	0.9	0.7	0.8	1.1	1.1	0.7	0.6	1.0	0.8*
65-69	0.5	0.5	0.9	-	1.2	0.5	1.1	0.7	1.0	0.4	1.2	0.8
70-74	-	-	-	-	0.5	1.7	0.8	1.0	0.6	0.3	1.0	0.6
75-79	-	-	-	-	-	-	1.8	-	0.5	1.6	-	0.6
80+	-	-	-	-	3.2	-	-	-	-	0.6	0.5	0.4
At total for all age groups	0.8	0.9	1.0	0.9	1.2*	1.2	1.0	1.0	1.0	0.9	1.0	1.0

* statistically significant differences

In total the mortality rate of CNPP recovery workers exceeded the parameters of general population mainly in young age groups, but in older groups SMR was significantly below Latvian population parameters. It is worth to say that mortality of CNPP clean-up workers in recent years, exceeded parameters of general population mostly in older age groups, comparing with previous years, and reflected mean age of the whole group of CNPP clean-up workers. The differences by years might be explained with aging of CNPP clean-up workers, because they are closed group, which gradually ages and dies-out. At the same time the mean age of CNPP clean-up workers is still

under 60 and this fact might explain the low mortality in age groups above 70 years comparing with general male population.

Simultaneously, years of potential life lost (YPLL) were analysed, *i.e.*, number of years, which a person could live till age of 65 years, if he wouldn't die due to any reason or disease. It was found that during 1999–2009 CNPP clean-up workers lost 9702 years comparing with 9530 years expected (calculated for age and gender matched general Latvian population; SIR 1.0 and 95% CI (1.00; 1.04)). The analysis of YPLL by years and age groups revealed the same trends as in SMR analysis, but YPLL gave possibility to find statistically significant differences between groups.

Figure 2. Mortality rate of CNPP clean-up workers by main causes of death in 1999–2009 (number of cases per 1000 persons)

The structure of mortality by causes of death has changed significantly during time (Figure 2). Death due to exogenous reasons took first place among CNPP clean-up workers early after Chernobyl accident; however in 2009 cardiovascular diseases already occupied the first place, oncologic diseases – the second, but exogenous reasons – the third. In other words, in late period

after the disaster age-related cases of death came to the fore. Comparing the mortality of CNPP clean-up workers by causes of death and age groups with general population, it was found that total mortality due to cardiovascular disorders and oncologic diseases was very similar to that in general population (SMR 0.9, 95% CI (0.81; 1.02) and SMR 0.9, 95% CI (0.77; 1.80) accordingly). The same was observed for deaths due to exogenous reasons (SMR 1.0 and 95% CI (0.86; 1.15)). Mortality due to oncologic diseases was lower than in general population in all age groups that may reflect efficient health care of this group. It is important to note that mortality due to cardiovascular diseases exceeded parameters of general population in age group of 35-44 years (SIR for YPLL was 1.3 and 95% CI (1.19; 1.37)), while at age above 55 years it was significantly lower than in general population. This fact shows tendency of CNPP clean-up workers to die at young age due to cardiovascular disorders like myocardial infarction and acute coronary syndrome, which are generally accepted as age-dependent causes of death.

Summarizing the analysis of mortality in CNPP clean-up workers, the conclusion can be drawn that this group of people dies at younger age (especially due to cardiovascular disorders) than general Latvian population, the reason for which might be premature aging because of complex of factors affected them in Chernobyl, *e.g.* previous exposure to ionizing radiation.

3.2. Oncologic Morbidity of CNPP Clean-Up Workers

In total data of 5950 CNPP clean-up workers from Latvia were analysed. Their mean age at participation time in clean-up works was 32.09 ± 7.36 years. Of them 55% participated in 1986, but the rest 45% participated in 1987–1991 years (mainly in 1987–1988). Between 5950 CNPP clean-up workers from Latvia totally 347 cases of oncologic diseases have been registered from 1986 till 2010. In other words, malignancy was found in every

17th person. At least 40% of all these workers have already died. Eight persons developed two histologically independent malignancies within time interval of several years. Mean age of affected persons at the moment of diagnosis was 52.5 ± 8.1 years. Their mean age at the moment of participation in CNPP disaster works was 36.8 ± 7.3 years. The dominant age of primary revealed oncologic patients gradually increased with time and it correlated with latent period since exposure to ionizing radiation (Pearson's coefficient $r = 0.502$, $p < 0.001$). Slight morbidity increase started four years after exposure, but significant oncologic morbidity rise was observed after 16 years latent period.

Incidence of malignant tumours increased gradually from 0.84 per 1000 in 1990 to 6.68 per 1000 in 2009 with significant augmentation since 2003. Morbidity achieved maximum in 2005, when it was 8.37 per 1000. Prevalence of malignancies among CNPP clean-up workers, evaluated on 01.01.2011., was 49 cases per 1000 people. Moreover the prevalence among 1986-year participants was higher (53 cases per 1000) comparing with 1987–1991 year participants (45 cases per 1000) – OR 1.17 (95% CI (0.88; 1.57)). Malignancies of urogenital system (prostate – 78 cases, kidney – 22, urinary bladder – 13, testis – 5 cases) took first place in morbidity structure (34.1% of all neoplasms). Noticeable increase in incidence of urogenital malignancies was observed since 2000.

Documented radiation dose received during stay in Chernobyl was available only for 51.5% of workers with neoplasms. Dose median was 110.0 mSv (IQR: 72.2, 180.0). 53.4% of workers with malignancies participated in CNPP clean-up works in 1986. 40.9% of 1986-year participants took part immediately after disaster during so-called iodine period. Radiation dose median for persons with neoplasms, who participated in 1986, was 155.0 mSv (IQR: 107.5, 200.0) and it was significantly higher ($p < 0.001$) than in 1987–1991 (median 84.35 mSv; IQR: 46.4, 100.0).

Odds ratio for malignancy development in 1986-year participants comparing with 1987-1991 was 1.05 and 95% CI (0.84; 1.31). Odds analysis of different localization tumour occurrence in CNPP clean-up workers by participation time in most cases showed higher risk for persons participated in 1986 than in 1987–1991 (Table 3), but differences were not statistically significant. Interestingly that odds for colon and rectum malignant tumour development was just opposite and it was significantly lower in 1986 participants (OR 0.39, 95% CI 0.16; 0.95).

Table 3

Odds ratios (OR) and 95% confidence intervals (95% CI) of occurrence of malignant neoplasm of different localization in 1986-year CNPP clean-up workers comparing with 1987–1991 year participants

Localization of malignant neoplasm	Absolute number		OR	95% CI
	1986	1987–1991		
Brain	8	5	1.33	0.44-4.08
Gastrointestinal tract:	46	42	0.91	0.60-1.39
mouth, tongue, lips, pharynx	15	11	1.14	0.52-2.48
stomach	17	14	1.01	0.50-2.05
liver	4	1	3.33	0.37-29.84
pancreas	3	1	2.50	0.26-24.04
colon, rectum	7	15	0.39	0.16-0.95
Respiratory system:	35	28	1.04	0.63-1.72
lungs	26	21	1.03	0.58-1.84
larynx	9	7	1.07	0.40-2.88
Urogenital system:	66	51	1.08	0.75-1.56
kidneys	13	8	1.35	0.56-3.27
urinary bladder	8	5	1.33	0.44-4.08
prostate	41	35	0.98	0.62-1.54
testes	2	2	0.83	0.12-5.91
penis	2	1	1.67	0.15-18.38
Thyroid gland	6	2	2.50	0.50-12.40
Haematological malignancies	11	4	2.29	0.73-7.21
Metastases without known origin	6	2	2.50	0.50-12.40
Total	187	149	1.05	0.84-1.31

During the time period between 1998 and 2010 total oncologic morbidity of CNPP clean-up workers was very similar to that of general

Latvian male population (SIR 0.91 and 95% CI (0.81; 1.03), but it is important to notice that 78% of all malignancies in accident recovery workers were diagnosed at age under 60 years and only 22% at age above 60 years, while in general Latvian male population incidence of cancers starts to grow significantly after the age of 55 years with maximum at 65–74 years. The highest oncologic morbidity of CNPP clean-up workers was found in age group of 40–54 years, when it significantly exceeded parameters of age and gender matched general population (SIR 1.22 and 95% CI (1.03; 1.44)). On the other hand oncologic morbidity of accident recovery workers at age above 60 years was significantly lower than in general population (in age group 60–64 – SIR 0.64 and 95% CI (0.45; 0.90), in age group above 70 years SIR was even lower – 0.34 and 95% CI (0.14; 0.71)). This fact may be explained with relatively young mean age of CNPP clean-up workers and small number of old recovery workers due to their high mortality at young age.

Detailed analysis of oncologic morbidity by localization sites of tumours in CNPP clean-up workers comparing with general Latvian male population, using indirect standardization, is shown in Table 4. The most frequently diagnosed malignancy among accident recovery workers was prostate cancer, which significantly exceeded morbidity in age matched Latvian male population (SIR 2.44 and 95% CI (1.93; 3.06)). Moreover in CNPP clean-up workers of age 45–49 years the prostate cancer morbidity exceeded the parameters of general male population more than 6 times (SIR 6.25 and 95% CI (2.01; 14.59)). On the other hand, in age groups over 60 years the morbidity with prostate cancer in CNPP clean-up workers was lower than in general population. It is important to highlight that prostate cancer is an age-dependent disease, whose incidence increases progressively with age (in general Latvian male population significant morbidity growth is observed starting with 60 years with maximum at 65–74 years). Our finding clearly indicates that prostate

cancer in CNPP clean-up workers appears much earlier than in general population. Good health care in this group of persons may also may have some effect, which allows doctors to diagnose cancers at earlier stage. High incidence of genitourinary neoplasms was prominent among CNPP clean-up workers of age 45–54 years since 2000, which may be interpreted as excretion result of incorporated long-living radionuclides (such as strontium, caesium, and plutonium) via kidneys.

Table 4

Comparison of oncologic morbidity between CNPP clean-up workers and general Latvian male population by tumour localization site

Localization of the tumour	ICD-10 code	Number of observed cases in CNPP clean-up workers	Number of expected cases in CNPP clean-up workers	SIR	95% CI
Prostate	C61	77	31.5	2.44*	1.93-3.06
Urine bladder	C66, C67	11	14.5	0.76	0.38-1.36
Kidneys	C64, C65	19	17.8	1.07	0.64-1.67
Thyroid gland	C73	4	1.9	2.10	0.56-5.33
Oral cavity	C00-C06	12	7.0	1.70	0.89-3.01
Large intestine	C18-C21	17	24.2	0.70	0.41-1.12
Stomach	C16	25	24.1	1.04	0.67-1.53
Lungs	C33, C34	35	62.6	0.56*	0.39-0.78
Larynx	C32	8	11.5	0.70	0.30-1.37
Central nervous system	C70-C72	5	7.3	0.70	0.22-1.60
All localizations		267	292.8	0.90	0.81-1.03

* statistically significant difference

The next most frequently diagnosed neoplasms are pulmonary malignant tumours, but morbidity with them was significantly lower than in general population in all age groups (SIR 0.56 and 95% CI (0.39; 0.78)). One of the reasons for this fact might be timely health care of CNPP clean-up workers, while the most cases of pulmonary cancers in general male population are

detected at late stages. Similar situation was observed with stomach cancers in CNPP clean-up workers (SIR 1.04 and 95% CI (0.67; 1.53)), but in age group of 40–49 years maximum of morbidity was observed (SIR 2.39 and 95% CI (1.19; 4.28)). Probable explanation may be the effect of radionuclides ingested with water, food, dust and secrets from upper parts of gastrointestinal and respiratory tract, which irradiated stomach.

Quite high morbidity with malignant neoplasms of oral cavity was observed among CNPP clean-up workers during the analysed period of time between 1998 and 2010 (SIR 1.72 and 95% CI (0.89; 30.1)). Incidence of the oral cavity cancer has started to grow significantly since 2003 (in 2003 SIR was 5.88 and 95% CI (1.18; 17.19)). Totally during the time period from 2003 till 2010 morbidity with malignant tumours of the oral cavity was more than two times higher than in age and gender matched general Latvian male population (SIR 2.47 and 95% CI (1.28; 4.32)). One of the contributing factors for cancer development in the oral cavity is smoking, but there is no information whether CNPP clean-up workers are heavier smokers than other males in Latvia. Our probable explanation of high incidence of the oral cavity cancers in CNPP clean-up workers is that it may be due to anatomical features of the oral cavity, surrounded by bones (similarly as pelvis in case of prostate cancer); bones are those structures which accumulate long-living radionuclides and might irradiate actively dividing tissues of the oral cavity for a long time. On the other hand, the brain is also surrounded by bones, but morbidity with brain malignancies was lower than in general population (SIR 0.69 and 95% CI (0.22; 1.60)). This might be explained with low division rate of cells in nervous tissues and quite low sensitivity of the brain to direct ionizing radiation damage.

The total morbidity with thyroid cancer during the time period from 1998 till 2010 was also higher than in general population (SIR 2.08 and 95% CI (0.56; 5.33)), but in age group 40–44 years it was even higher (SIR 14.29 and

95% CI (2.87; 41.74)). In general Latvian male population thyroid cancer is quite rare pathology and high incidence of it in CNPP clean-up workers may be the result of contact with radioactive substances in Chernobyl (radioactive iodine, which is absorbed by thyroid gland).

3.3. Non-oncologic Morbidity of CNPP Clean-Up Workers

The analysis included CNPP clean-up workers, alive on January 1st, 2011, who in previous years underwent medical examination in the Centre of Occupational and Radiological Medicine. The median age of 3993 evaluated persons was 54 years (IQR: 48, 59). 2171 of them participated in Chernobyl accident recovery works in 1986 year. The median age of this group was 53 years (IQR: 48, 59). Other 1822 participated in 1987–1991 and their median age was 54 years (IQR: 49, 59). Of all analysed CNPP clean-up workers 1694 performed digging and deactivation tasks, but 2299 performed from radiological contamination point of view other “cleaner” tasks. The age structure of these two groups was similar. The prevalence of the most frequent non-oncologic pathologies among CNPP clean-up workers is shown in Table 5.

As can be seen in the table, disorders of the nervous system took the first place in the non-oncologic morbidity structure of CNPP clean-up workers (941 cases per 1000 persons), the second – musculoskeletal disorders (933 per 1000), but the third – mental disorders (922 per 1000). Almost all CNPP clean-up workers had several disorders simultaneously. The biggest differences between subgroups of CNPP clean-up workers were observed comparing subgroups by performed tasks. It is important to notice that CNPP clean-up workers, who participated in 1986 and performed more “dirty” tasks, got ill with age-dependent disorders (*e.g.*, cardiac ischemia, musculoskeletal disorders and others) more frequently and at younger age than other CNPP clean-up workers ($p < 0.05$). This fact may be explained with heavier exposure to

ionizing radiation during works in 1986 and larger amount of long-living radionuclides incorporated due to digging and deactivation tasks. Such long-term irradiation might cause more severe damage of cells and tissues and disturb normal aging processes resulting in accelerated aging.

Table 5

Prevalence of the most frequent non-oncologic diseases in CNPP clean-up workers (evaluating on 01.01.2011.) comparing by performed tasks (prevalence per 1000 persons, OR and 95% CI)

Disease	ICD-10 code	Prevalence per 1000 persons			OR [†]	95% CI
		total	digging / deactivation tasks	other tasks		
Endocrine disorders:	E01-E90	629	606	645	0.84*	0.74-0.96
thyroid gland benign disorders	E01-E07	411	402	417	0.94	0.83-1.07
diabetes mellitus	E10-E16	71	74	68	1.10	0.87-1.41
insulin-independent diabetes	E11	61	61	61	1.00	0.77-1.30
Mental disorders:	F00-F99	922	929	916	1.18	0.94-1.50
cognitive impairment	F06.7	454	465	445	1.08	0.95-1.23
depression	F32-F33	35	41	31	1.35	0.97-1.89
Disorders of nervous system:	G00-G99	941	956	930	1.63*	1.23-2.15
non-inflammatory polyneuropathy	G62	595	645	557	1.45*	1.27-1.64
encephalopathy	G93.4	880	891	871	1.21	0.99-1.47
hand mononeuropathy	G56	306	322	294	1.14*	1.0-1.31
Disorders of organs of sense:	H00-H95	853	858	849	1.07	0.90-1.28
senile cataract	H25	268	250	282	0.85*	0.74-0.98

Continuation of Table 5

Disease	ICD-10 code	Prevalence per 1000 persons			OR [†]	95% CI
		total	digging / deactivation tasks	other tasks		
retinopathy, retinal angiopathy	H35	814	821	808	1.09	0.93-1.28
hearing impairment	H90-H91	46	45	47	0.95	0.71-1.29
Cardiovascular disorders:	I00-I99	720	717	723	0.97	0.84-1.11
arterial hypertension	I10-I15	562	550	572	0.92	0.81-1.04
cardiac ischemia	I20-I25	193	210	180	1.22*	1.04-1.42
angina pectoris	I20	145	155	137	1.14	0.96-1.37
myocardial infarction	I21	15	17	13	1.27	0.77-2.12
chronic coronary heart disease	I25	164	174	156	1.14	0.97-1.35
chronic coronary heart disease and angina pectoris	I20+I25	186	200	175	1.18*	1.01-1.39
heart failure	I50	125	128	124	1.04	0.86-1.25
cerebral infarction	I63	4	4	5	0.74	0.27-2.00
other cerebro-vascular disorders	I67	186	186	185	1.00	0.85-1.18
atherosclerosis	I70	116	104	124	0.82	0.67-1.00
Disorders of respiratory organs	J00-J99	651	685	626	1.30*	1.14-1.49
Disorders of digestive organs	K00-K93	852	875	835	1.38*	1.15-1.66
Skin disorders	L00-L99	134	152	121	1.31*	1.09-1.57
Musculoskeletal disorders:	M00-M99	933	953	919	1.79*	1.36-2.34
osteoporosis	M80-M81	78	85	72	1.19	0.95-1.51
Disorders of urogenital organs:	N00-N99	381	414	356	1.28*	1.13-1.46
benign prostate hyperplasia	N40	122	132	114	1.17	0.97-1.42

[†] comparing digging/deactivation task performers with other task performers

* statistically significant difference

3.4. The Results of Telomere Length Measurements

Totally, telomere length of 834 persons was evaluated. High inter-individual variability even in persons of one age was observed in all groups. A surprising finding was that slightly higher relative telomere length was observed in CNPP clean-up workers than in control group, and the difference appeared to be statistically significant ($p = 0.001$). Median RTL in Chernobyl accident workers was 1.23 U (IQR: 1.19, 1.27 U) vs. 1.21 U (IQR: 1.16, 1.26) found in controls. Despite relatively bigger number of CNPP clean-up workers ($n = 584$) comparing with controls ($n = 236$), the age structure of the investigated in the present study CNPP clean-up workers did not differ significantly from the age distribution of the control group (independent samples t -test didn't reveal significant differences, $t = -1.084$, $p = 0.279$). Mean age of CNPP clean-up workers was 54.95 ± 6.78 (age range: 42–81 years), but in controls – 55.55 ± 8.07 years (age range: 44–78 years).

The results of RTL measurements in various CNPP clean-up workers' subgroups comparing to each other and to controls are summarised in Table 6. Taking into consideration participation time in Chernobyl clean-up works there were no significant differences in RTL between 1986 and 1987–1991 year participants ($p > 0.05$), but median RTL of both subgroups was still longer than in controls ($p < 0.01$). In addition, longest telomeres were found in 1986-year participants. So in 1986-year liquidators median RTL was found 1.24 U (IQR: 1.18, 1.27), in 1987–1991 year workers – 1.23 U (IQR: 1.19, 1.26), while in controls – only 1.21 U (IQR: 1.16, 1.26).

Evaluating RTL difference between various job task performers, in deactivation and digging workers minimally longer telomeres were found comparing with other job workers ($p = 0.156$), at the same time the age structure of both subgroups was very similar and did not differ markedly ($p = 0.777$). Comparing these subgroups with controls, RTL in both task groups was

significantly longer than in controls ($p < 0.05$), moreover “dirty” task performers had noticeably longer telomeres, than controls did ($p = 0.001$).

Table 6

Comparison of relative telomere length (median RTL (IQR) and mean ranks of RTL) between CNPP accident clean-up workers’ subgroups and control group

Comparable groups	Number of participants in corresponding groups	Median RTL (IQR) in corresponding groups, U	Mean ranks ^a of RTL in corresponding groups	p -value ^b
1986 y. / controls	302 / 236	1.24 (1.18, 1.27) / 1.21 (1.16, 1.26)	427.36 / 363.28	0.004
1987–1991 yy./ controls	271 / 236	1.23 (1.19, 1.26) / 1.21 (1.16, 1.26)	270.04 / 235.58	0.008
1986 y. / 1987–1991 yy.	302 / 271	1.24 (1.18, 1.27) / 1.23 (1.19, 1.26)	291.15 / 282.37	0.526
Deactivation tasks / controls	231 / 236	1.24 (1.20, 1.27) / 1.21 (1.16, 1.26)	445.79 / 366.94	0.001
Other tasks / controls	353 / 236	1.23 (1.19, 1.27) / 1.21 (1.16, 1.26)	309.41 / 273.45	0.012
Deactivation tasks / other tasks	231 / 353	1.24 (1.20, 1.27) / 1.23 (1.19, 1.27)	305.31 / 284.12	0.138
High-risk group / controls	140 / 236	1.24 (1.19, 1.28) / 1.21 (1.16, 1.26)	445.54 / 366.94	0.002
Low-risk group / controls	444 / 236	1.23 (1.19, 1.27) / 1.21 (1.16, 1.26)	356.79 / 309.85	0.003
High-risk group / low-risk group	140 / 444	1.24 (1.19, 1.28) / 1.23 (1.19, 1.27)	305.78 / 288.31	0.285

^a For calculations Mann-Whitney test was used. ^b p – value characterizes differences between selected groups.

After grouping of workers into risk groups according to participation time and performed tasks, longer telomeres were found in high-risk group (1986-year participants performed “dirty” tasks) comparing with low-risk group (1987–1991 year participants performed other kinds of work), but the difference was not statistically significant ($p = 0.285$). So median RTL in the high-risk group was 1.24 U (IQR: 1.19; 1.28), but in the low-risk group – only

1.23 U (IQR: 1.19; 1.27). However, comparison with controls revealed significantly shorter telomeres in non-exposed persons (median RTL was 1.21 U (IQR: 1.16; 1.26)) than in both risk groups of CNPP accident workers ($p < 0.01$).

Despite the previously mentioned RTL differences between subgroups, the analysis of RTL concerning documented dose of radiation exposure revealed only very weak, insignificant correlation between telomere length and recorded dose in CNPP clean-up workers (Spearman's correlation coefficient $r_s = 0.051$, $p = 0.349$).

For assessment of aging effect on telomere length Spearman's correlation analysis was carried out. Weak negative correlation between RTL and documented age of CNPP clean-up workers was observed (Spearman's coefficient $r_s = -0.074$, $p = 0.073$). At the same time such correlation was not found in controls ($r_s = -0.007$, $p = 0.920$). The age effect on telomere length in 1986 and 1987–1991 year participants did not differ significantly and showed similar trends as found in total CNPP workers group. More notable difference between CNPP subgroups and controls was revealed after dividing CNPP accident workers according to performed job tasks. Surprising telomere lengthening with age was observed in “dirty” task workers ($r_s = 0.060$, $p = 0.362$), however in other job performers, significant negative correlation of RTL with age was found ($r_s = -0.157$, $p = 0.003$). Analogous coherence was observed in high and low risk CNPP workers – in the high-risk group statistically non-significant positive correlation of RTL with age was identified ($r_s = 0.104$, $p = 0.222$), while in the low-risk group statistically feasible negative correlation was noticed ($r_s = -0.142$, $p = 0.003$).

Detailed analysis of relative telomere length in CNPP clean-up workers according to health disturbances, which developed after returning from Chernobyl, revealed great differences. The results are summarised in Table 7,

where RTL of CNPP clean-up workers suffering from a definite disease was compared with RTL in persons without this disorder.

Table 7

Differences of relative telomere length (mean ranks of RTL) among CNPP accident clean-up workers suffering from particular disease comparing with liquidators without this disease

Comparable groups	Number of participants in corresponding groups	Mean ranks of RTL in corresponding groups	z- value	p-value
Malignant neoplasm present / absent	52 / 514	325.35 / 279.27	-1,936	0.053
Insulin independent diabetes present / absent	56 / 510	260.27 / 286.05	-1,120	0.263
Benign thyroid gland diseases present / absent	276 / 290	285.62 / 281.48	-0,301	0.763
Benign prostate hyperplasia present / absent	100 / 466	286.83 / 282.79	-0,224	0.823
Osteoporosis present / absent	41 / 525	263.37 / 285.07	-0,819	0.413
Senile cataract present / absent	221 / 345	269.83 / 292.26	-1,591	0.112
Atherosclerosis present / absent	90 / 476	268.62 / 286.31	-0,941	0.347
Chronic coronary heart disease and angina present / absent	145 / 421	260.95 / 291.27	-1,925	0.054

The longest telomeres were observed among CNPP clean-up workers with malignancies (median RTL was 1.25 U (IQR: 1.21, 1.28)). In persons with malignant neoplasms telomere length was longer than in persons without malignancies ($p = 0.053$) regardless of slightly older age of persons with neoplasms – mean age of oncologic patients was 58.33 ± 6.22 years, while in persons without neoplasms it was 54.60 ± 6.72 years. After adjusting of results for age significant difference was found ($p < 0.001$). On the other hand the

shortest telomeres were found in CNPP clean-up workers who suffered from chronic heart disease and angina pectoris (median RTL was 1.22 U (IQR: 1.18, 1.26)), as well as in persons with such aging-associated illnesses as atherosclerosis, osteoporosis and insulin independent diabetes, however comparing with persons without these disorders difference was not statistically significant. It was found that long telomeres owners had almost twice higher risk for developing of malignant neoplasm (OR 1.88; 95% CI: 1.02, 3.45) than people with short telomeres.

In addition, for evaluation of the effect of different kinds of protracted exposure to ionizing radiation on telomere condition, RTL was measured in 14 persons chronically exposed to ionizing radiation at work (so called group of radiologists). Median length of service under irradiation was 24 years (IQR: 8.5, 32.5) and median external exposure dose was 15.84 mSv (IQR: 5.74, 36.90). The age structure of these persons was similar to previous two groups. Significantly shorter telomeres were observed in persons exposed at work, comparing with CNPP clean-up workers ($p < 0.001$) and with control group ($p < 0.001$). However these results may be doubtful due to poor number of radiologists. At the same time weak positive correlation was observed between the age of radiologists and RTL (Spearman's coefficient $r_s = 0.351$, $p = 0.219$), as well as between the received dose and RTL ($r_s = 0.455$, $p = 0.187$).

The method of the real time q-PCR, used in the present study, has given us the possibility for effective and accurate measurement of a big number of samples, which revealed RTL differences between explored subgroups regardless of high inter-individual variability. Current investigation has revealed that CNPP clean-up workers have slightly longer telomeres than non-exposed persons. During the stay in Chernobyl certain amount of radioactive substances accumulated in the body of recovery workers through inhaling of the dust, ingestion of local food and drinking contaminated water [Yablokov *et al.*, 2009]. Workers, who performed decontamination and digging tasks, had

higher probability to incorporate larger amount of radionuclides due to closer contact with contaminated objects. The half-lives of some radionuclides like Cs-137 and Sr-90 released into the environment after Chernobyl disaster is approximately 30 years [Yablokov *et al.*, 2009], others – even more than 20 000 years [IARC, 2001]. The decay of such long-living radionuclides in human tissues may expose cells to a mixture of ionizing radiation of different types and the effect of such exposure may cumulate with time. A number of studies on biological effects of the ionizing radiation showed that radiation might damage DNA in the direct way by energy transfer from particles to a matter or in the indirect way by forming a pool of free radicals [Oikawa and Kawanishi, 1999; Petersen *et al.*, 1998; UNSCEAR, 2011; von Zglinicki, 2000]. Telomere region is rich in guanine that makes telomeres highly susceptible to oxidative damage [Hewitt *et al.*, 2012] and exposure to ionizing radiation may affect telomere structure. A number of extrinsic factors are known to cause telomere shortening, but telomere lengthening due to harmful exogenic exposure is less investigated. Currently available data on telomere length changes, induced by ionizing radiation, are ambiguous with little evidence of irradiation effect on telomere length regulation pathways.

Considering signs of premature aging, chronic psycho-emotional stress, exposure to toxic substances, and long-term low-dose exposure to various types of ionizing radiation from incorporated long-living radionuclides, telomere length was expected in CNPP clean-up workers to be shortened. However the results of our study surprisingly demonstrated a tendency to telomere elongation in CNPP recovery workers. Moreover our study revealed the tendency to elongation of telomeres in the persons who were more heavily exposed to ionising radiation. Telomere elongation was found even more than 20 years after termination of direct contact with radioactive objects. It is important to note that the most elongated telomeres appeared in persons with malignancies.

Ionizing radiation is known to be a proved carcinogen of the first group [IARC, 2009]. Our finding of long telomeres in CNPP clean-up workers after protracted exposure to ionizing radiation is consistent with Li *et al.* (2012) discovery of telomere elongation after chronic exposure through drinking water to such evidently carcinogenic substance as inorganic arsenic. Goytisolo *et al.* (2000) in study with mice cell lines demonstrated that short telomeres increase sensitivity to radiation. It was noticed in studies of radiotherapy effects on cancer and healthy human cells that after irradiation with sub-lethal doses cells enter stress-induced premature senescence, which is not triggered by dysfunctional telomeres (as it is usually seen in case of replicative senescence) and it is not associated with telomere shortening [Suzuki, 2001; Suzuki and Boothman, 2008]. It may be the possible explanation of our findings. Alternative pathways of entering senescence other than those associated with telomere shortening may be induced by a long-term exposure to small doses of ionizing radiation. In case of chronic exposure to radiation human tissues are likely to develop defensive reactions against permanently occurring DNA damage. One of such reactions may be the up-regulation of telomerase expression that leads to the elongation of telomeres. Moreover, the elongation of telomeres may be a significant factor for cancer development.

3.5. The Results of Transforming Growth Factor β (TGF β) Detection

The age structure of persons with TGF β analysed was similar in all groups: mean age of CNPP clean-up workers was 54.95 ± 6.76 years, radiologists – 54.67 ± 7.71 , and control group – 55.45 ± 7.80 years (independent samples *t*-test didn't reveal significant differences, $p > 0.05$). The level of TGF β was found to be significantly lower in CNPP clean-up workers comparing with control group ($p = 0.036$). This finding coincides with literature data, when low level of TGF β was observed in late period after exposure to ionizing radiation [Kajioka *et al.*, 2000; UNSCEAR, 2006d]. In the group of

radiologists the level of TGF β was similar to that in controls, showing tendency to be slightly higher than in previous two groups ($p > 0.05$). Moreover, TGF β was not detectable in 11.1% of CNPP clean-up workers (0 pg/ml); while in controls it was not detectable in 2.9% of cases, but in the group of radiologists – only in 2.8% of cases. Very high level of TGF β (≥ 8000 pg/ml) was in 10.7% of controls, 8.3% of CNPP clean-up workers, and 5.6% of radiologists ($p > 0.05$). Analysing TGF β by age, high inter-individual variability was observed even among persons of similar age, which influenced the validity of calculations; statistically significant correlation between level of TGF β and age was not found.

Evaluation of TGF β level by presence of certain disease revealed higher level of TGF β in CNPP clean-up workers with previous history of malignant neoplasm comparing with persons without cancers ($p = 0.063$). On the other hand, lower level of TGF β was observed in persons with atherosclerosis ($p = 0.122$), senile cataract ($p = 0.090$), insulin-independent diabetes ($p = 0.134$), coronary heart disease and angina pectoris ($p = 0.682$)¹. Slightly higher level of TGF β was found in CNPP clean-up workers suffering from benign thyroid disorders ($p = 0.184$), benign prostate hyperplasia ($p = 0.594$), and males with osteoporosis ($p = 0.314$)¹.

Simultaneous analysis of TGF β level and relative telomere length (RTL) was performed in 90 CNPP clean-up workers, 14 employees exposed to radiation at work, and 39 controls. The age and gender structure in each selected group was similar. The level of TGF β did not correlate with RTL in all study groups ($p > 0.1$). At the same time eight CNPP clean-up workers had malignancy in their previous history – among these persons weak insignificant positive correlation was observed between TGF β level and RTL ($r_s = 0,429$, $p = 0,289$); while in CNPP workers with benign prostate hyperplasia the level of

¹ Evaluated comparing with CNPP clean-up workers without corresponding disease

TGF β statistically significantly correlated with RTL ($r_s = 0,544$, $p = 0,029$). This finding might indicate similar features in development of cancer and benign proliferative diseases like prostate hyperplasia.

TGF β in normal cells acts as tumour suppressor, inhibiting cell proliferation and promoting cell differentiation or apoptosis. Low level of TGF β in serum of CNPP clean-up workers might reflect insufficient anti-cancer protection in their organism. TGF β together with other regulative factors participate in regulation of human telomerase reverse transcriptase (*hTERT*) gene [Andrews *et al.*, 2010]. In normal and malignant cells TGF β suppresses *hTERT* gene through Smad3 involvement [Li *et al.*, 2006], i.e., TGF β inhibits expression of telomerase with following decrease in telomerase activity and as the result telomeres remain non-elongated. Thereby longer telomeres in CNPP clean-up workers might be explained with insufficient inhibiting TGF β effect on expression of telomerase gene. On the other hand, high level of TGF β in persons with malignancies may indicate mutation in TGF β signalling pathway genes, which allows malignant cells to escape from anti-proliferative action of TGF β ; in addition, malignant cells actively produce TGF β by themselves, providing favourable environment for proliferation [Blobe *et al.*, 2000]. TGF β is an important factor for wound healing and tissue regeneration, because it participates in immune suppression and induces components of extracellular matrix. However overproduction of TGF β may cause formation of excessive fibrosis and scar tissue. Slightly higher level of TGF β in the group of radiologists might be explained with probable stimulation of immune system by intermittent exposure to low doses of ionizing radiation.

3.6. The Results of Nitrogen Monoxide and Iron Measurements in Hair

In the studied group of radiologists, cumulative dose was between 0.56 and 74.79 mSv (median 15.1 mSv), but the length of service under radiation exposure was from 5 to 41 year (median 18 years). Mean age of females ($n =$

21) in this group was 55.19 ± 9.78 years, but males ($n = 8$) – 50.63 ± 6.21 years. Females had higher irradiation doses (median 17.18 mSv, interquartile range (IQR): 5.85; 32.64) than males (5.42 mSv; IQR: 0.79; 12.80) as well as longer length of service under radiation exposure (females – 22 years, IQR: 13.5; 30.5; males – 10 years, IQR: 5.15; 25.25). In the group of radiologists doses of irradiation significantly correlated with length of service under exposure ($r_s = 0.507$, $p = 0.011$). Documented doses of exposure during work in Chernobyl among analysed CNPP clean-up workers were between 6 and 250 mSv (median 130 mSv). Mean age of male CNPP clean-up workers ($n = 55$) was 54.11 ± 6.38 years, females ($n = 3$) – 43, 56 and 60 years. In control group mean age was: 53.11 ± 9.39 years for females ($n = 64$), and 54.04 ± 9.42 years for males ($n = 23$). Assessment of NO-radical level in hair showed significant differences among genders in all groups ($p < 0.001$). In female hair NO level was significantly lower than in male hair. At the same time NO level feasibly correlated with level of iron ions in hair (EPR signal at $g = 4.2$; $r_s = 0.311$, $p < 0.001$). Moreover, the level of iron ions registered at $g = 4.2$ and $g = 2.3$ in females generally was lower than in males in all study groups.

The analysis of NO level revealed weak statistically significant correlation of NO with age (estimating both groups simultaneously: $r = 0.220$, $p = 0.007$; males: $r = 0.249$, $p = 0.024$, females: $r = 0.272$, $p = 0.024$). The weakest connection of NO with age was observed in CNPP clean-up workers – NO level in older persons was almost the same as in younger ones.

In total NO level in CNPP clean-up workers' hair was slightly higher than in males of other groups ($p > 0.05$). In radiologists-males the level of NO dramatically increased with increasing length of service under radiation exposure, but in radiologists-females such increase was not so evident. Strong statistically significant correlation of NO level with length of service under exposure was observed in radiologists-males ($r = 0.761$, $p = 0.028$); while in radiologists-females such correlation was not found. On the contrary,

documented doses received during stay in Chernobyl did not correlate with NO-radical level in group of CNPP clean-up workers.

Nitrogen monoxide is a gas, which immediately after its synthesis actively reacts with other substances. This is the reason why NO is difficult to detect and measure. Hair was chosen as a substrate for NO measurements in current work, because it is easy to obtain the material (non-invasive procedure) and bound form of NO is easier for detection. Moreover, hair cumulates information about long period of time due to slow growth. NO can bind and neutralize other free radicals, despite of the fact that NO itself has the structure of free radical; that is why it is accepted that NO may work as a radio-protective substance against damage caused by ionizing radiation [Soule, 2007]. In addition, NO acts as a powerful inhibitor of lipid peroxidation and may develop the anti-atherosclerotic effect [Halliwell and Gutteridge, 2007]. The results of our study demonstrated close relationship between NO level and iron metabolism in the human body. In addition, NO level was higher in those radiologists-males, who were older and worked longer time under exposure to ionizing radiation, which might indicate the protective function of NO; but NO level and age did not correlate in CNPP clean-up workers. Taking into account that long-living radionuclides accumulated in their organism during stay in Chernobyl and protractedly irradiated tissues with low doses of radiation for a long time, an assumption might be done that the process of NO synthesis could be disturbed in CNPP clean-up workers. Disturbance of NO synthesis and its regulation might cause insufficient radioprotective and antioxidative effect that could influence an ageing course.

4. MAIN CONCLUSIONS

1. The signs of premature aging were found in CNPP clean-up workers; moreover, aging process developed in heavier form and at younger age in humans, who underwent greater exposure to ionizing radiation:
 - young CNPP clean-up workers, who performed deactivation and digging tasks, had higher odds than older ones for developing of such age-dependent disorders like coronary heart disease, angina pectoris, myocardial infarction, degenerative disorders of nervous system, benign prostate hyperplasia, musculoskeletal disorders, osteoporosis, *etc.*;
 - total oncologic morbidity of CNPP clean-up workers in age group of 40–54 years significantly exceeded morbidity of age and gender matched general population during the period of time from 1998 till 2010; but mortality due to oncologic diseases was lower than in general population in all age groups of CNPP clean-up workers, that reflected effective medical care of this group;
 - urogenital malignancies (prostate, kidney, urine bladder, and testis) took first place, or 34.1% of all neoplasms, in the structure of CNPP clean-up workers' oncologic morbidity, that might be consistent with excretion of incorporated radionuclides through kidneys;
 - the incidence rate of prostate, thyroid and oral cavity malignancies in CNPP clean-up workers significantly exceeded the level of morbidity in general Latvian population with critical age for development of neoplasm from 45 to 59 years, but for stomach cancer – from 40 to 49 years;

- the mortality rate of CNPP clean-up workers went up significantly from 1986 to 2010, and proportion of age-related causes of death increased greatly with mortality maximum at age under 59 years during the time period from 1999 till 2009;
 - the critical age for death due to cardiovascular diseases in CNPP clean-up workers was between 35 and 44 years, when it significantly exceeded the mortality rate of general Latvian population.
2. Comparing with humans excessively non-exposed to ionizing radiation, the aging processes of CNPP clean-up workers overtakes the average pace of aging in general Latvian inhabitants.
 3. Relative telomere length in PBL of CNPP clean-up workers was slightly longer than in age and gender matched control group ($p < 0.01$); longer telomeres were found in persons more heavily exposed to ionizing radiation and in CNPP clean-up workers with malignancies.
 4. The level of TGF β was significantly lower in CNPP clean-up workers than in other groups ($p < 0.05$), that might indicate an insufficient anticancer protection in CNPP clean-up workers' organism.
 5. The low level of TGF β and telomere lengthening in persons protractedly exposed to ionizing radiation might be indicative of genome instability and activation of telomerase expression (also insufficient inhibition of telomerase expression by TGF β), as the result the predilection for malignant neoplasm development is likely to increase and stress induced premature aging could be activated.
 6. It was established, that protracted exposure to ionizing radiation is able to modify and accelerate the aging processes in humans.

7. The practical recommendations for health care improvement of the persons protractedly exposed to ionizing radiation were worked out on the base of the current study.

Confirmation of the Hypothesis of the Doctoral Thesis

1. The hypothesis “The humans, who underwent protracted ionizing radiation exposure, are likely to age faster than non-exposed persons, which may manifest with higher morbidity with age-dependent diseases and death at younger age” is confirmed, because mortality rate and structure as well as morbidity with age-dependent disorders of CNPP clean-up workers revealed faster rate of aging and signs of premature aging comparing with general Latvian population.
2. The hypothesis “Humans under various kinds of long-term irradiation might age with different intensity” is confirmed, because the analysis of epidemiological parameters and measurements of biological samples revealed significant differences between groups of differently exposed persons, indicating that aging process developed more heavily in humans, who underwent greater continuous irradiation.

5. PRACTICAL RECOMMENDATIONS

1. According to the findings of aging modulation and acceleration by protracted exposure to ionizing radiation, special attention of health care for adult irradiated persons should be paid to age-dependent disorders like coronary heart disease, degenerative diseases of nervous system, osteoporosis, *etc.*; the strategy should be aimed to the prevention and early detection of these disorders with following active treatment, taking into account that age-dependent disorders in

irradiated persons may develop at younger age than generally accepted in non-exposed population.

2. In health care of persons protractedly exposed to ionizing radiation, medical specialists should focus their attention on early revealing and timely initiated treatment of malignant neoplasms, because such approach allows them to reduce mortality.
3. Examinations with use of ionizing radiation should be avoided (computed tomography, X-ray examination, scintigraphy, *etc.*) in medical care of persons protractedly exposed to ionizing radiation. The methods of choice should be diagnostic procedures without X-rays – ultrasonography, magnetic resonance imaging, clinical analyses and other tests.
4. Radiosensitivity tests should be considered for administration in persons, who will be protractedly employed under exposure to ionizing radiation; this may help to recognise humans sensitive to unfavourable effects of radiation. Such test may be appropriate before decision making about future occupation and before starting training in occupation (*e.g.*, in radiologists).
5. Protractedly employing persons under exposure to ionizing radiation, possibility of work task and work condition shift should be considered to discontinue the exposure for some time, when the organism is allowed to regenerate. Such organization of work would be favourable for persons with long length of service under irradiation.
6. Regular ordination of the antioxidants should be considered periodically in risk groups (*e.g.*, protractedly employed under exposure to radiation) to reduce unfavourable effects of ionizing radiation on human organism.
7. Participation time in Chernobyl accident clean-up works and performed tasks during stay in Chernobyl should be taken into account

for evaluation of health condition and health care planning of CNPP clean-up workers, because 1986-year participants and those performed deactivation and digging tasks are more prone to develop broad spectrum of pathologies, besides at younger age than others.

8. Cardiovascular disorders should be prevented actively in CNPP clean-up workers, aiming early detection and minimizing risk factors; special attention should be paid to young CNPP clean-up workers, because critical age for early death from cardiovascular diseases among them is between 35 and 44 years, as well as their mortality remains high at older age.
9. Taking into account the high oncologic morbidity of CNPP clean-up workers, special attention in health care of this group should be paid to early disclosure of malignancies, therefore regular examinations should be done, paying attention to the distinct sites: prostate, kidneys and other organs of urogenital system (following examinations should be included: prostate specific antigen, ultrasonography of the abdomen, consultation of the urologist (on demand)); oral cavity and throat (visual examination of the oral cavity, consultation of the dentist); thyroid gland (detection of the thyroid hormones levels, ultrasonography of the thyroid gland, consultation of the endocrinologist (on demand)); stomach (fibrogastroscopy, especially in young accident workers). Colonoscopy should be obtained periodically in 1987-1991 year participants.

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