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# Influence of the Chernobyl accident on the frequency of chromosomal damage and health status of Lithuanian clean-up workers

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Chromosomal damage and health status were analyzed in Chernobyl clean-up workers currently residing in Lithuania. Statistically significantly ($P < 0.05$) increased frequencies of chromosome-type aberrations (chromosome breaks, dicentric and ring chromosomes) as well as aberrant cells were found in the peripheral blood lymphocytes of clean-up workers when measured 6–8 years after the exposure. Significant health impairment was characteristic of these persons as well. On average, 5.6 diseases per patient were diagnosed in clean-up workers suffering from cardiovascular diseases. This high co-morbidity resulted in quite high rates of metabolic syndrome (16.7%). Among Chernobyl clean-up workers that had experienced post-traumatic stress disorder, 76% suffered from highly expressed sleep disturbances. Analysis of thyroid diseases among 500 clean-up workers has revealed that 27.6% individuals have different pathology of thyroid gland. Thus, even 20 years after the Chernobyl disaster, clean-up workers must be considered as a group of primary interest both for researchers and physicians.

**Key words**: Chernobyl clean-up workers, ionizing radiation, chromosomal damage, health status, metabolic syndrome, sleep disturbances

## INTRODUCTION

The nuclear reactor accident on 26 April 1986 at Chernobyl in the former Soviet Union released massive amounts of radioactivity over a period of 10 days. Due to this widely dispersed radioactivity, diverse and sometimes large populations were affected [1]:

– several hundred acutely exposed reactor operators, firefighters, and members of other emergency units who worked in or near the reactor during the accident. In 28 cases they received lethal whole body doses;

– hundreds of thousands of children with high radioiodine burdens in the thyroid due to a failure to implement countermeasures, such as blocking radioiodine incorporation by administering stable iodine and preventing cattle from grazing on contaminated pastures;

– tens of thousands of about 800,000 clean-up workers involved in decontamination and clearing of forests. Despite an official dose limit of 250 mSv, early exposures in the absence of full dosimetric controls might have been higher in some cohorts;

– about 240,000 inhabitants of highly contaminated areas and evacuated persons. Dose estimates range from 50 to 300 mSv.

The Lithuanian theritory was not highly contaminated (>37 kBq/m$^2$) with Chernobyl radioactivity [2]. Thus, Chernobyl clean-up workers from Lithuania could be considered as the most affected part of Lithuanian population. It is estimated that about 7,000 Lithuanian citizens took part in clean-up activities in the Chernobyl area in 1986–1988 [3].

Overexposure to ionizing radiation could cause various genetic and health effects. It is well documented that ionizing radiation increases the number of chromosmal damage in lymphocytes of exposed individuals [4]. There is also an evidence of a correlation between increased frequency of chromosome aberrations and cancer incidence in humans [5]. Thus, cytogenetic monitoring of human populations may be used for cancer risk assessment [6].

The main purpose of this paper is to summarize the results of cytogenetic studies of Chernobyl clean-up workers from Lithuania as well as to present some data on their health status. It is based on some papers presented during the conference “18 Years

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after the Accident of the Chernobyl Nuclear Power Plant" (April 26th, 2004, Vilnius, Lithuania) [7].

## MATERIALS AND METHODS

### Cytogenetic studies

*Study group.* Five hundred and ten male Chernobyl clean-up workers from Lithuania, 127 never-smokers, 67 ex-smokers and 275 smokers, were analyzed in 1991–1994 for the frequency of chromosome aberrations (CA) in their peripheral blood lymphocytes. Thus, analysis of chromosomal damage in lymphocytes of Chernobyl clean-up workers was performed 3–8 years after the irradiation. The individual workers spent, on average, 1 to 6 months at Chernobyl and were allowed to receive up to 0.25 Sv of external γ-radiation. For 283 clean-up workers, individual doses estimated by local dosimetric services at Chernobyl were available. These dose estimations ranged from 0.004 to 0.476 Sv, mean 0.13 ± 0.08 Sv. However, the uncertainty of these dose estimations may be quite high, because a significant proportion of the clean-up workers had no personal dosimeters and their dose estimations were made on the basis of the time spent in zones with different contamination levels [2]. For 17 clean-up workers repeated blood samples were taken with the interval of approximately 1 year. Forty-three male subjects, 16 never-smokers, 2 ex-smokers and 25 smokers of approximately the same age, health and occupational history were chosen as controls.

*Cytogenetic procedures.* The study was conducted with peripheral blood lymphocytes obtained by venipuncture. Heparinized whole blood was diluted in the ratio 1:15 with RPMI 1640 Auto-Mod culture medium supplemented with 12% heat-inactivated newborn calf serum, 50 μg/ml gentamycin, 10 μg/ml 5-bromo-2'-deoxyuridine and 8 μg/ml phytohaemagglutinin P. All reagents were purchased from Sigma St. Louis, MO, USA. Cells were cultured in sterile amber-colored glass bottles for 72 h at 37 °C in complete darkness. Colchicine was added to the culture during the last 3 h at a final concentration of 0.6 μg/ml. Culture harvest and fixation, preparation of flame-dried slides and differential staining of sister chromatids by the fluorescence-plus-Giemsa (FPG) technique were carried out exactly as previously described [8].

### Analysis of health status

*Analysis of thyroid diseases.* Thyroid diseases were analyzed in 500 of clean-up workers during their annual visits to the Sapiega Hospital. Their age ranged from 38 to 65 years. In 138 cases ultrasound analysis was performed. Forty-two thyroid nodules were analyzed cytologically.

*Evaluation of co-morbidity rates.* One hundred and sixty five patients were randomly selected from the group of clean-up workers suffering from cardiovascular diseases and treated in Sapiega Hospital in 1996–2003. The average age of the patients was 48.8 years. Their morbidity rates were assessed by the analysis of their health records made at Sapiega Hospital.

*Analysis of sleep quality.* Fifty-seven patients with post-traumatic stress disorder (PTSD) participated in the study. All of them had been treated in Sapiega Hospital in Vilnius. All data were collected in 2003. The Pittsburgh Sleep Quality Index (PSQI) [9] was used to evaluate the sleep quality of patients. The parameters of anxiety and/or depression were assessed by the Hospital Anxiety and Depression (HAD) scale [10]. The Beck Depression Inventory (BDI) scale [11] was applied to estimate the severity of depression.

## RESULTS AND DISCUSSION

The frequency of CA of various types in Chernobyl clean-up workers and control individuals is shown in

Table 1. **Number of individuals, analyzed cells and chromosomal aberrations of different types in control persons and Chernobyl clean-up workers**

| Indices | Controls | | Clean-up workers | |
|---|---|---|---|---|
| | Number | Per 100 cells | Number | Per 100 cells |
| Number of individuals | 43 | – | 510 | – |
| Number of cells | 5594 | – | 60486 | – |
| Aberrant cells | 135 | 2.41 | 2299 | 3.80* |
| Total chromosomal aberrations | 137 | 2.44 | 2441 | 4.04* |
| Chromatid breaks | 83 | 1.48 | 883 | 1.46 |
| Chromatid exchanges | 17 | 0.30 | 224 | 0.37 |
| Chromosome breaks | 26 | 0.46 | 1087 | 1.80* |
| Dicentric and ring chromosomes | 7 | 0.13 | 223 | 0.37* |
| Chromosome translocations and inversions | 4 | 0.07 | 24 | 0.04 |

*$P < 0.01$ as compared to controls. Probabilities were calculated using normal approximation of binomial distribution.

Table 2. **Frequency of aberrant cells in lymphocytes of the same donors at different samplings (interval between samplings ~1 year)**

| Donor ID | Aberrant cells, % ± S.E.M. | | |
|---|---|---|---|
| | 1st sampling | 2nd sampling | 3rd sampling |
| 101 | 2.75 ± 0.82 | 2.00 ± 0.99 | 0.67 ± 0.47* |
| 158 | 4.50 ± 1.46 | 1.00 ± 0.99 | 3.00 ± 1.21 |
| 174 | 4.00 ± 1.39 | 9.00 ± 2.02* | 1.00 ± 0.50*,** |
| 184 | 2.50 ± 1.10 | 2.67 ± 0.93 | 2.83 ± 0.68 |
| 208 | 0.00 | 3.00 ± 1.21 | 2.00 ± 0.99 |
| 221 | 3.00 ± 1.21 | 5.00 ± 1.54 | 7.00 ± 1.47* |
| 315 | 1.00 ± 0.99 | 5.00 ± 1.54* | 1.00 ± 0.45** |
| 316 | 3.00 ± 1.71 | 5.50 ± 1.14 | 3.00 ± 0.70 |
| 401 | 5.00 ± 1.54 | 3.00 ± 1.21 | 2.00 ± 0.57 |
| 431 | 4.00 ± 1.96 | 7.00 ± 1.80 | 1.67 ± 0.52** |
| 457 | 6.00 ± 1.68 | 2.30 ± 0.86* | 3.20 ± 0.79 |
| 462 | 6.00 ± 1.68 | 3.50 ± 1.30 | 2.20 ± 0.66* |
| 481 | 2.00 ± 0.99 | 4.00 ± 1.39 | 6.50 ± 1.74* |
| 486 | 6.00 ± 2.37 | 4.00 ± 1.39 | 2.00 ± 0.99 |
| 491 | 4.00 ± 1.39 | 6.50 ± 1.74 | 3.00 ± 1.21 |
| 510 | 5.00 ± 1.54 | 7.00 ± 1.80 | 5.00 ± 2.18 |
| 513 | 5.50 ± 1.61 | 4.00 ± 1.39 | 2.33 ± 0.87 |
| Mean ± S.E.M. | 3.78 ± 0.43 | 4.38 ± 0.51 | 2.85 ± 0.43** |
| Repeated measures ANOVA: | | F = 2.836 | P = 0.0735 |

* P < 0.05 as compared to the 1st sampling.
** P < 0.05 as compared to the 2nd sampling.

Table 1. Statistically significant differences were found in the frequency of aberrant cells, chromosome breaks, dicentric chromosomes and total frequency of CA per 100 cells. Thus, even 3–8 years after the Chernobyl accident, radiation-induced chromosomal damage is still present in lymphocytes of Chernobyl clean-up workers. As expected [4], the tendency of the increased frequency of dicentric and ring chromosomes was statistically significant. An increased frequency of acentric fragments has been reported in individuals occupationally exposed to low levels of ionizing radiation [12, 13]. The same was noted in individuals exposed to radiation from Chernobyl fallout [14] and in another study of Chernobyl clean-up workers [15]. In the present study, the frequency of chromosome breaks was the main type of radiation-induced CA.

Repeated blood sampling (Table 2) indicated that the number of CA could change from sampling to sampling. A weak tendency of declining (F = 2.836, P = 0.0735, repeated measures ANOVA) of the mean number of aberrant cells was detected. This observation is in agreement with our previous findings about a drop in the mean frequency of chromosome breaks in lymphocytes of Chernobyl clean-up workers in relation to time elapsed since exposure [16].

Our previous findings [16–19] indicated an increased frequency of chromatid-type aberrations in lymphocytes of Chernobyl clean-up workers as well. It was interpreted as quite unexpected finding, since radiation exposure in non-dividing lymphocytes induces exclusively chromosome-type aberrations [4]. In our previous paper [16] we speculated that this increase might be most probably explained by the influence of some unrecognized factors related to life-style, health status or occupational exposure.

Table 3. **Co-morbidity among 165 randomly selected Lithuanian Chernobyl clean-up workers suffering from cardiovascular diseases treated in Sapiega Hospital in 1996–2003**

| Diseases | Number | Frequency ± SEM (%) |
|---|---|---|
| Cardiovascular diseases | 362 | 38.9 ± 1.6 |
| Obesity | 115 | 12.4 ± 1.1 |
| Musculoskeletal and connective tissue diseases | 105 | 11.3 ± 1.0 |
| Behavioral and mental diseases | 77 | 8.3 ± 0.9 |
| Endocrine diseases | 71 | 7.6 ± 0.9 |
| Neurological diseases | 57 | 6.1 ± 0.8 |
| Diseases of digestive system | 49 | 5.3 ± 0.7 |
| Chronic respiratory diseases | 44 | 4.7 ± 0.7 |
| Urological diseases | 27 | 2.9 ± 0.6 |
| Other diseases | 23 | 2.5 ± 0.5 |
| Total | 930 | 100 |

Indeed, in this study we used a control group different from previous studies, its significant proportion consisting of individuals matched with Chernobyl clean-up workers by their health status (17 out of 43 control individuals were Sapiega Hospital patients suffering from the same disease as clean-up workers but not exposed to Chernobyl radiation). As shown in Table 1, no increase of chromatid-type aberrations in lymphocytes of Chernobyl clean-up workers was found.

This may indicate that the health status of Chernobyl clean-up workers may be important for the frequency of chromosomal damage in their lymphocytes. Indeed, a more detailed analysis indicated a serious health impairment of Chernobyl clean-up workers.

Analysis of thyroid diseases among 500 clean-up workers has revealed that 27.6% individuals have different pathology of thyroid gland: 4.8% suffered from Hashimoto thyroiditis with hypothyroidism, 11.2% from thyroid nodules, 11.6% from diffuse thyroid enlargement, 0.2% from thyroid tumour. A slightly lower frequency of thyroid disorders (15.3%) was reported in clean-up workers from Latvia [20]. Interestingly, in our previous (carried out in 1991–1995) study [21] a lower prevalence (3.7%) of thyroid nodularity in Lithuanian Chernobyl clean-up workers was reported. Thyroid tumours are rather rare, so, it is quite difficult to evaluate whether their incidence is increased in the cohort of Lithuanian clean-up workers. Literature data show no increase of thyroid tumours in Estonian clean-up workers [22]. However, a singnificant increase was characteristic of clean-up workers from Russia [23].

The analysis of co-morbidity rates (Table 3) indicated that for Chernobyl clean-up workers it was characteristic to have several diseases at once. One hundred and sixty-five clean-up workers suffering from diseases of the cardiovascular system also exhibited other various diseases, a total of 930 diagnoses. The average morbidity was 5.6 diseases per patient. A similar tendency of multiple morbidity has recently been noted for Chernobyl clean-up workers from Latvia [24]. Parallel to cardiovascular diseases, quite frequently clean-up workers have obesity (12.4 ± 1.1%), musculoskeletal and connective tissues diseases (11.3 ± 1.0%), behavioral and mental diseases (8.3 ± 0.9%), and endocrine diseases (7.6 ± 0.9%).

Such high co-morbidity rates may indicate that a certain proportion of clean-up workers could suffer from the so-called metabolic syndrome which is commonly defined as a group of risk factors or abnormalities closely associated with insulin resistance, which markedly increase the risk of both coronary heart disease and diabetes [25]. Indeed, after the analysis of 165 patients, we can estimate that according to WHO definitions [26] the frequency of metabolic syndrome cases among Chernobyl clean-up workers suffering from cardiovascular diseases should be about 16.7%.

It has recently been shown that the increased prevalence of metabolic syndrome could be associated with sleep apnoea [27]. Therefore we assessed sleep quality and its relationship to the psychoemotional condition of 57 Chernobyl clean-up workers that had experienced post-traumatic stress disorder (PTSD). According to PSQI assessment, highest sleep disturbances with scores more than 10 points were determined for 76% of patients. Moderately severe sleep disturbances (scores 6–10 points) were confirmed in 18% of patients. Only 6% of patients did not have any sleep disturbances. Patients with PTSD had significant symptoms of anxiety and depression as well: 85% of the patients suffered from anxiety and 75% from depression. There was some correlation among anxiety, depression and sleep disturbances: high-level sleep disturbances were characteristic of 69.6% of patients with clearly expressed anxiety, 46.4% of patients with deep depression, and of as many as 71.4% of patients having both anxiety and depression. Sleep disturbances were much less common in patients without anxiety (10.7%) and depression (26.8%).

As has previously been reported [3], the prevalent (>40%) causes of death among Chernobyl clean-up workers in Lithuania are external reasons such as accidents, suicides, murders, etc. In that context, it is quite interesting to note that sleep disturbances have recently been associated with increased occupational accidents [28, 29]. Thus, sleeping disturbances observed in Chernobyl clean-up workers may also contribute to high rates of death due to external reasons among this population.

To sum it up, Chernobyl clean-up workers from Lithuania still exhibit relatively high rates of chromosomal damage. Various health problems are very common in this group of people as well. Thus, even 20 years after the Chernobyl disaster, clean-up workers must be considered as a group of primary interest for both researchers and physicians.

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## ČERNOBYLIO AVARIJOS ĮTAKA CHROMOSOMŲ PAŽAIDŲ DAŽNIUI IR AVARIJOS LIKVIDUOTOJŲ IŠ LIETUVOS SVEIKATOS BŪKLEI


S a n t r a u k a

Tyrėme Černobylio avarijos padarinių likviduotojų iš Lietuvos sveikatos būklę ir chromosomų pažaidas jų limfocituose. Rasta statistiškai patikimai ($p < 0,05$) daugiau chromosominio tipo (chromosomų trūkių, dicentrikų ir žiedinių chromosomų) aberacijų, taip pat ir aberantinių ląstelių černobyliečių periferinio kraujo limfocituose praėjus 6–8 metams po apšvitinimo. Šie žmonės taip pat turėjo gana ryškių sveikatos sutrikimų. Nustatyta, kad vidutiniškai vienam pacientui, sergančiam kardiovaskulinėmis ligomis, diagnozuojama 5,6 įvairių ligų. Toks didelis sergamumo lygis yra gana aukšto dažnio (16,7%) metabolinio sindromo priežastis. 76% černobyliečių, turinčių potrauminio streso sindromą, taip pat būdingi miego sutrikimai. Černobyliečių skydliaukės tyrimai rodo, kad įvairių jos patologijų dažnis siekia 27,6%. Taigi net praėjus ir 20-iai metų po Černobylio avarijos, jos metu nukentėję asmenys turi sulaukti tiek mokslininkų, tiek ir gydytojų išskirtinio dėmesio.