CYTOGENETIC AND RADIOBIOLOGICAL EVALUATION OF ENVIRONMENTAL IMPACT OF ANTARCTIC CONDITIONS ON HUMAN

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Abstract
We have studied environmental impact of Antarctic congitions on residents of Ukraine by comparing parameters of genome instability and radionuclide content before and after wintering in Antarctica. Radionuclide content (^{137}Cs) was measured with "Human body radiation counter" ("SICh"). The level of chromosome aberrations in lymphocytes of peripheral blood and the rate of micronuclei in buccal cells were considered as parameters of genome instability. The average level of micronuclei before expedition was 3.52±0.63‰ and after expedition was 4.14±0.43‰. The average rate of metaphase cells with chromosome aberrations was 1.±0.38% before and 1.76±0.38% - after expedition. The average rate of chromosomal aberrations was 1.33±0.41% before and 1.85±0.39% after expedition. These figures (both of before and after expedition) are normal also some increasing take place.

Introduction
Human genome is exposed to ionizing radiation from the external and internal sources. Internal sources are different radionuclides, which get into human body with food or dust. They accumulate in organism and may cause somatic mutations and cancer [1]. The living on polluted Ukrainian territories accompanies with permanent getting of radionuclides into an organism and internal irradiation became constant and never-ending. The protection of the organism from radionuclides' penetration may promote to excretion them out of the organism. Such situation is indeed the case of participant of Antarctic expeditions (AE).

The members of Ukrainian AE long time (more then a year) live on clear Antarctic territory. According to data of the Ukrainian Antarctic Center, over-winterers after wintering have decreased radionuclides level in comparing with initial level. Thus the problem of influence of specific environmental conditions on their genome stability is very urgent. Actuality of the problem is how change genome instability as a consequence of wintering. The question may be answered after realization of the complex program of studies genome instability within frames of general program of bio-medical researches of Ukraine in Antarctica [2].

We used two methods for evaluation of genome instability of winterers: analysis of the general level of chromosome aberrations in metaphase of peripheral blood lymphocytes and the rate of micronuclei in smears of exfoliated buccal cells from the inner side of the cheek [3]. Analysis of metaphases is more informative and power, but needs more time, more qualified personnel, is more expensive and more laborious, than micronuclei-assays. In our work we have used both of the approaches. Before expedition we collected blood samples for chromosome analysis and buccal cells for micronucleus test. It will help us to find the initial levels of genome instability of winterers. During expedition the smears of buccal cells were periodically collected that will help to study dynamics of genome instability during wintering.

This article is devoted to studying of the set of parameters of genome instability of Ukrainian overwinterers before and after wintering in Antarctica.

Methods
Blood samples for chromosome assays and buccal cell smears were obtained from the group of candidates for VI UAE during medical examination in November 2000 (before expedition) and after expedition in February 2002. Additional slides with buccal cell smears were obtained during the wintering. Radionuclides content (^{137}Cs) was measured with "Human body radiation counter" ("SICh") before, immediately and two months later after returning from expedition.

Blood was collected by venepuncture. Samples of whole blood (1 ml) were cultivated in 5 ml of IMDM medium. PHA-P ("GENOME", Donetsk) - 5 μg per 1 ml of medium was used for blasttransformation of lymphocytes. Cultures were incubated at 37 °C for 52 h. [4]

Colchicine (1 μg/ml, final concentration) was added at 1.5 - 2 hours before the end of cultivation. After cultivation cells were treated with 0.075 M KCl and fixed in methanol : acetic acid (3:1) mixture. Metaphase plates were prepared by dropping of cell suspension onto cool moist slides. Slides were stained by routine Giemsa technique according to standard procedure.

Buccal cells were collected (scraped) from inner side of the both cheeks with a spatula. Immediately after scrapping cells were distributed on the slide surface, dried on air and fixed with 90% methanol. Fixed slides were kept in dry until cytogenetic analysis. Slides were stained with Giemsa solution. The chromatin particles were regarded as "micronuclei" if they were agreed with standards [3, 5, 6].

The level of structural and numeral chromosome anomalies in lymphocytes of peripheral blood and the rate of micronuclei in buccal cells were selected as parameters of genome instability. Frequency of metaphases with chromosome aberrations (AM), level of chromosome aberration (ChA), frequency of metaphases with premature centromere division (MPCD), levels of PCD and total PCD (TPCD), rate of polyploid mitosis (PM) and the micronuclei frequency (MNF) were selected as parameters of genome instability. Statistical calculations were performed with standard methods [7].

Results
Data on level of genome instability of winterers before and after expeditions are shown on Table 1. The level of genome instability was in general population limits before and after expedition.

Table 1. Parameters of genome instability before and after wintering

Parameters	AM %	ChA %	MPCD %	PCD %	TPCD %	PM %	MNF ‰
Before	1.26±0.38	1.33±0.41	0.83±0.29	1.65±0.70	0.02±0.02	0.07±0.04	3.52±0.63
After	1.76±0.38	1.85±0.39	0.53±0.25	0.53±0.25	0.46±0.09	0.1±0.04	4.14±0.43

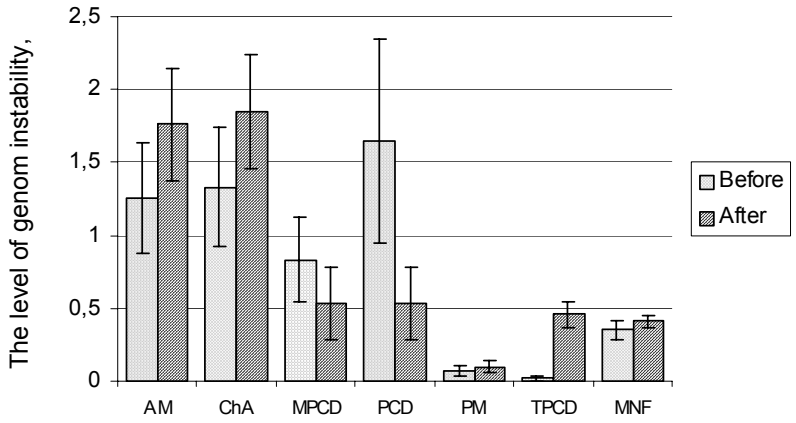

Fig 1 The genome instability of winterers before and after expedition.
The parameters of instability: AM - aberrant metaphases, ChA - chromosomal aberrations, MPCD - metaphase with premature centromere division, PCD - premature centromere division, PM - polyploid mitoses, TPCD- total premature centromere division, MNF - micronuclei frequency

The differences by level of chromosomal instability before and after expedition were statistically insignificant for AM, ChA, MPCD, PM, MNF. After wintering statistically significantly changed PCD and TPCD (Fig 1). The dynamics of micronucleus frequency of overwinterers during the wintering is shown on Fig. 2

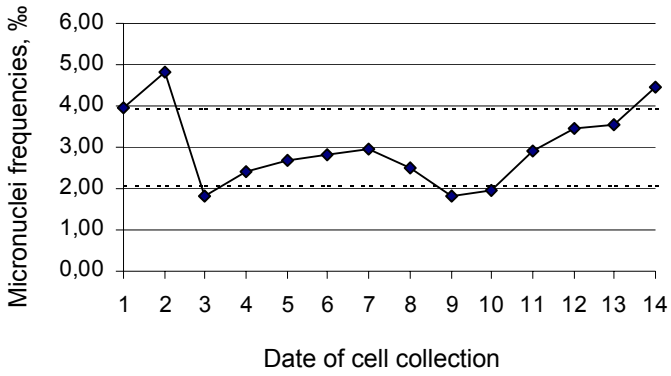

Fig. 2 The dynamics of the rate of micronuclei in buccal cells during the wintering. Dotted lines are limits of 95% confidence interval

We have studied the dynamics of the level of genome instability testing micronuclei frequency in exfoliated buccal cells. We selected this test due to of simplicity of slide preparation in field condition: buccal cells do not need previous cultivation and stimulation to mitosis. Buccal cells were collected once per month, because the cells that were collected were in dividing basal layer 1-3 weeks earlier.

The initial level of micronuclei before wintering was 3,96‰. This value is in general population limits (4 - 5‰). Over period of November 2000 –February 2001 the rate of micronuclei increased from 3,96‰ to 4,82‰. Over period of February 2001- Mart 2001 the rate of micronuclei decreased (Fig. 2). In April – July micronuclei frequency gradually increased from 2,43‰ to 2,96‰. From August to September it decrease to 1,81‰. Further time of staying in Antarctica was characterized by gradually increasing of MN frequency to initial level (at the start of expedition). After returning to Ukraine the micronucleus frequency again increased to 4.44‰ (Fig. 2).

Discussion

According to data of the Ukrainian Antarctic Center, over-winterers immediately after wintering had decreased radionuclides level in comparing with initial level (Fig.3). Changing of environmental conditions may influence on genome instability. One of the reason of changing of genome instability may be the changes of radionuclides concentration.

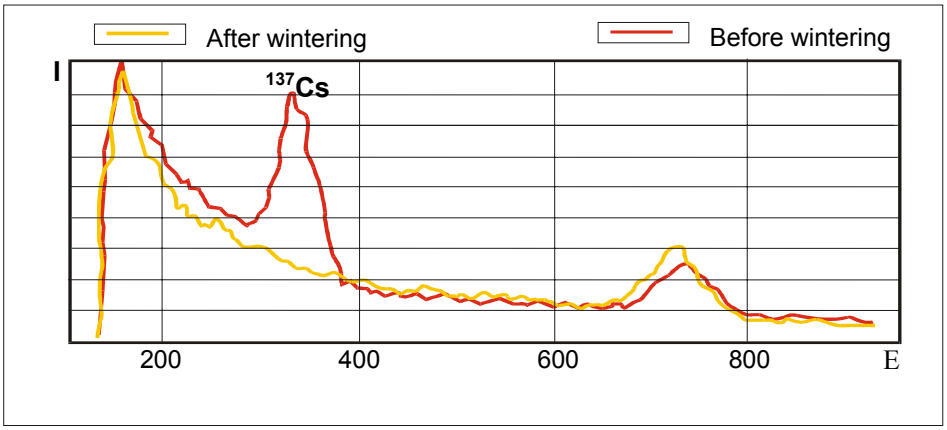

Fig 3. Contens of ^{137}Cs before and after wintering.
E – energy, kiloelectronvolt (keV); I – number of impulses per minutes.

To estimate the level of genome instability we used different, parameters, which give different information on the reasons of genome instability. If the metaphase test reflects events that took place 3 - 4 months before blood sampling, micronuclear testing reflects events that have occured about 1-3 weeks ago before cell sampling [3]. It can be explained by specificity of differentiation and life of lymphocytes and buccal epithelial cells. Thus, the two used approaches complement each other and let us to obtain different information on genome instability – the data of two test differ on causes of manifestations of genome instability and on the time when the corresponding events took place. Thus, we proposed to use the metaphase tests for basic evaluation of winterers' genome instability, and the micronuclear test for regular study of dynamics of the instability during expedition and wintering [2].

Obtained data suggest no correlation between changes of genome instability and ^{137}Cs concentration (in the studied limits) in human organism. Significant decreasing of the PCD (but not MPCD) and increasing TPCD may reflect another causes which could influence on processes of chromosome segregation. Studying of dynamics of micronuclei may provide information about gradually changes of genome instability and correlate these changes with radionuclides concentration.

During the wintering the certain decreasing of MNF was revealed. After returning to Ukraine MNF restored to initial level. The MNF decreasing during over-wintering may be explained by declining of ^{137}Cs penetration into the bodies due to specificity of nutrition, drinking water, air, etc. Though decreasing of MNF during Antarctic expedition may be explained by ^{137}Cs reduction, the increasing of MNF after returning to starting level may not be explained only by changes of radionuclides concentration. According to UAC data, the ^{137}Cs concentration reach to initial level two months after returning, while the growth of MNF was observed immediately after returning of over-winterers.

Conclusions

1. After expedition decreasing of PCD and increasing or TPCD were statistically significant. The other studied parameters of genome instability before and after wintering did not changed.

2. Investigation of the dynamic of micronucleus frequencies revealed decreasing of MN rate during the expedition and its restoring to initial level after returning to Ukraine.

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