

Analysis of chromosome lesions in women with gynecological disorders, residing in territories contaminated as a result of the Chernobyl accident.

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Abstract

Spontaneous levels of chromosome aberrations in peripheral lymphocytes of 95 women were analyzed in the course of medical examination of residents of Bryansk region territories (Klintsy district, and the town of Klintsy) contaminated after the Chernobyl NPP accident. All recognizable chromosome lesion types were scored in the first in vitro division metaphases stained with azure-eosin. The mean total aberration frequency in the sample studied was 5.1 ± 0.4 per 100 metaphases, the main contribution being made by chromatid deletions, which is typical for a normal spontaneous aberration pattern. Abnormal patterns, including significantly elevated aberration frequencies and/or presence of chromosome type exchange aberrations, were found in 13 women.

The aim of the study was to evaluate a possible correlation between cytogenetic anomalies and reproductive system disorders and/or the level of radiation contamination of the territory.

As a result of our estimation, it was shown that cytogenetic anomalies were in fact higher in subjects residing in territories with a higher radiation contamination; however, the difference was statistically insignificant. The frequency of cytogenetic anomalies was higher in women with gynecological disorders including hormone-dependent and inflammatory (17%) than in healthy women (7%). This difference was statistically significant for subjects with hormone-dependent diseases of the female reproductive system, but not in patients with inflammatory disorders.

Introduction

The explosion at the Chernobyl Nuclear Power Plant was one of the greatest known nuclear disasters of the 20th century. Four regions, namely Bryansk, Kaluga, Tula and Oriol, were among the most contaminated in Russia as a result of the accident at the Chernobyl Nuclear Power Plant in 1986. Exposure to caesium isotopes was thought to be more important for long-term health consequences, since these radionuclides have a much longer half life and exert their effects both locally, from deposition on plants, and by incorporation into vegetation through plant roots. Residents live in settlements surrounded by forests. The main food sources are locally produced (vegetables, milk) and/or include foods that can be foraged from the forests (mushrooms, berries and wild game) and swamps (cranberries). These wild foods are known to most readily concentrate radioactive elements (particularly ^{137}Cs). It is estimated that approximately 90 percent of the total lifetime radiation dose to individuals in the population is due to internal exposure to radiation from radiocesium ingested in contaminated foodstuffs. Particularly rich sources of ^{137}Cs include milk, meat, potatoes, mushrooms and wild berries [1,

2, 3, 4]. While the half-life of ^{137}Cs in agricultural products is 2.8–5.6 years, it is approximately 10 years for berries and 20 years for mushrooms [5].

Epidemiological studies performed since that time showed a statistically significant morbidity increase in residents of these territories compared to the whole country population. In particular, in contaminated territories in the Bryansk region (the most affected district in the Russian Federation), the total morbidity is currently 22% higher than in the whole country. In Klinty district 102,000 people (including 54,925 women) live in the area where the level of ^{137}Cs contamination ranges from 1 to 23.7 Ci/km² [2, 6].

Endocrine disorders (including a 3 to 5-fold thyroid cancer incidence growth) belong to diseases that make a major contribution to the morbidity increase [2, 7] and are more pronounced in subjects exposed as children and adolescents than in those who were adult in 1986. Women's reproductive system diseases are known to be closely related to endocrine disorders. Hence, it could be expected that the increase in endocrine morbidity rate in exposed children may be followed (with a 5-30-year lag) by an increase in reproductive system morbidity in women. Besides it was shown in the course of health monitoring of women residing in radiation contaminated regions that the Chernobyl NPP accident affected the immune system and chronic exposure to radiation increased the incidence of immune-related diseases [8].

Programs designed to explore these possibilities have recently been launched in the Russian Federation and Belarus.

One of the aspects of such studies is cytogenetic monitoring, which is of primary importance, since an increased spontaneous level of chromosome aberrations in peripheral lymphocytes is known as a biological indicator of adverse environmental health effects. Single and double chromosome fragments are typical spontaneous aberrations. They are about 90% of the total number of aberrations. The frequency of dicentric and rings is about 1 per 5000-10000 cells. They are known to be markers of radiation exposure [9, 10]. Human lymphocytes exposed to a dose of 0.1–0.15 Sv show 50-100 times higher dicentric frequency (1 per 100 cells) [11].

Furthermore, in a number of works it has been shown that chromosome instability revealed in intact peripheral lymphocytes can be associated with predisposition to cancer [12, 13, 14]. DNA strand breaks induced by ionizing radiation may give rise to chromosomal rearrangements such as translocations, inversions, insertions, or deletions. If these rearrangements do not cause cell death or are not repaired, they can result in malignant transformation via altered gene expression or loss of tumor-suppressor gene function. It is commonly accepted that exposure to moderate or high doses of radiation increases the risk of cancer in most organs. Risk estimates for thyroid, breast and lung cancers, for all solid cancers combined, and for leukemia are fairly precise, and associations have been found at relatively low doses (<0.2 Gy) [10, 15, 16]. According to preliminary estimates, the absolute risk of incurring tumors for every 0.01 Gy a year is approximately 2.1 for breast cancer [17].

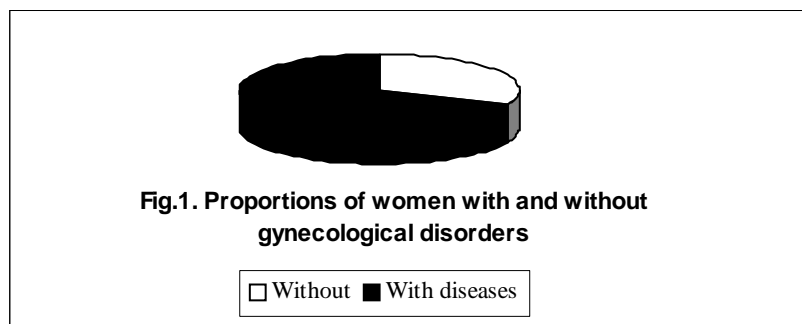
The aim of our study was to verify statistical hypotheses on possible correlations between cytogenetic anomalies, reproductive system disorders and/or the level of radiation contamination territory.

Methods and materials

The examination was conducted in November 2002. Data on cesium-137 contamination for each settlement were taken from an official reference-book [6]. Territory contamination with ^{137}Cs ranged 1.7 to 14.9 Ci/km² [1, 6].

The study population included only women who had been premenopausal at the time of the accident (mean age in 1986 was 20.5±9.9 years, ranging 4 to 48 years).

Medical examination showed that only 30 women out of 95 were free from disorders of one kind or another (Fig.1). Unfortunately, the similar results were obtained in course of breast diseases examination of the women from the Bryansk District. The number of women with benign breast diseases, including adenoma, fibroadenoma, mastopathy, and cysts was 67 %



[18].

For the purpose of further analyses, we divided all the disorders into hormone dependent (39 subjects), including myomas, cysts, endometrial hyperplasias, and menstrual cycle disorders, and inflammatory (26 subjects). The hormone dependent cases prevailed in the study population.

Our cytogenetic examination was undertaken with the aim of preliminary monitoring. Venous blood samples were taken into heparinized tubes. The cultures were prepared with 1-ml aliquots of the whole blood and incubated in Carrel's flasks at 37°C in RPMI-1640 medium with 20% bovine serum, phytohemagglutinin P (10 µg/ml), 0.09 mM BrdUrd and 100 IU/ml of penicillin (total volume, 9 ml, Sigma reagents). For the analysis of spontaneous aberration levels, cultures were harvested after 53-h incubation. Demecolcine (Sigma) was added to each culture for 3 h before harvest. Chromosome preparations were made following standard procedures and stained with azure-eosin. All aberrations recognizable without karyotype identification were scored in 100 first division metaphases with 45-46 chromosomes. Dicentrics, centric rings, detectable symmetrical translocations, double acentric rings and free double fragments (deletions) were registered as chromosome type aberrations, whereas all kinds of intra- and interchromatid exchanges, single fragments and isochromatid aberrations were ascribed to the chromatid type. Statistical data processing was performed using two-tailed t- and chi-square tests.

Results and discussion

The mean total aberration frequency in the whole study population was 5.4 ± 0.4 per 100 metaphases, the main contribution being made by chromatid deletions, which is typical for a normal spontaneous aberration pattern. Abnormal patterns, including aberration frequencies significantly (at $p < 0.05$) exceeding the mean group value and/or presence of chromosome type exchange aberrations, were found in 13 women (14%). The range of chromosome aberrations was 0 – 21.8 per 100 cells.

At first, we checked a hypothesis on a correlation between abnormal chromosome patterns and diseases. Results are presented in Table 1.

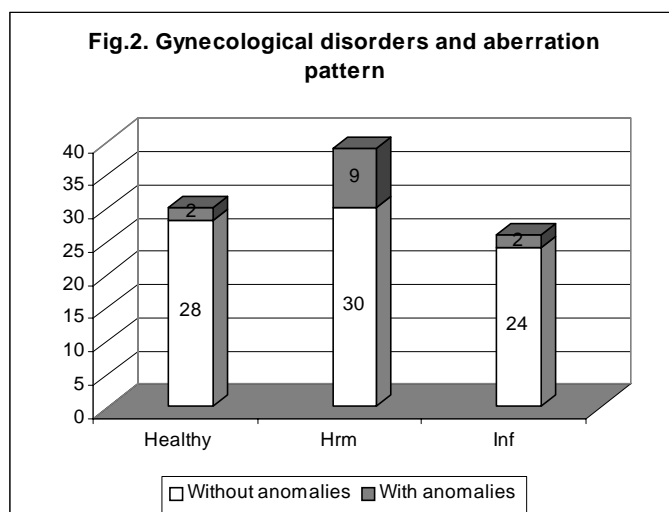
Table 1. Comparison of the abnormal chromosome patterns between healthy women and women with gynecological diseases.

Parameter	Study groups	
	Healthy	With diseases
Total number of women	30	65
With cytogenetic anomalies	2 (6.7%)	11 (16.9%)

The frequency of cytogenetic anomalies was higher in women with reproductive system disorders (11 cases out of 65 subjects) than in healthy women (2 cases out of 30 subjects). However, the difference between the respective frequencies (17 and 7%) did not reach the statistical significance level (the exact Fisher's test gave a p value of 0.13).

Subdivision of all gynecological disorders revealed in the population into two classes, hormone-dependent (Hrm, 39 cases) and inflammatory (Inf, 25 cases), allowed, further, to test hypotheses on elevated frequencies of chromosome anomalies in women with each of these disease classes as compared to healthy women. The difference between women with hormone-dependent disorders (23% of those showed chromosome anomalies) and healthy women (7%) proved to be statistically significant at $p=0.05$. In women with inflammatory diseases, chromosome anomalies were practically as frequent (8%) as in healthy women (Fig.2).

Thus, cytogenetic anomalies in peripheral lymphocytes of women with reproductive system disorders, residing in radiation contaminated regions, were more frequent than in healthy women residing in the same territory. This difference was statistically significant for subjects with hormone-dependent diseases of the female reproductive system, but not in patients with inflammatory disorders.



Then, we checked a hypothesis on a correlation between abnormal chromosome patterns and Cs-137 contamination levels. In a subgroup of women residing in regions with the higher contamination level atypical patterns (9 cases out of 59) were, in fact, more frequent as compared to those residing in less contaminated territories (4 out of 36), although the differences was statistically insignificant (Table 2). Similar results were obtained in the course of breast diseases examination in women residing in the same region [18]. These observations reveal the following tendency: abnormal chromosome patterns are encountered more frequently among residents of more contaminated territories. Other authors reported such tendencies as well [19, 20].

In addition, comparison of gynecological disorders frequencies between more and less contaminated territories did not give reliable significance levels (Table 2).

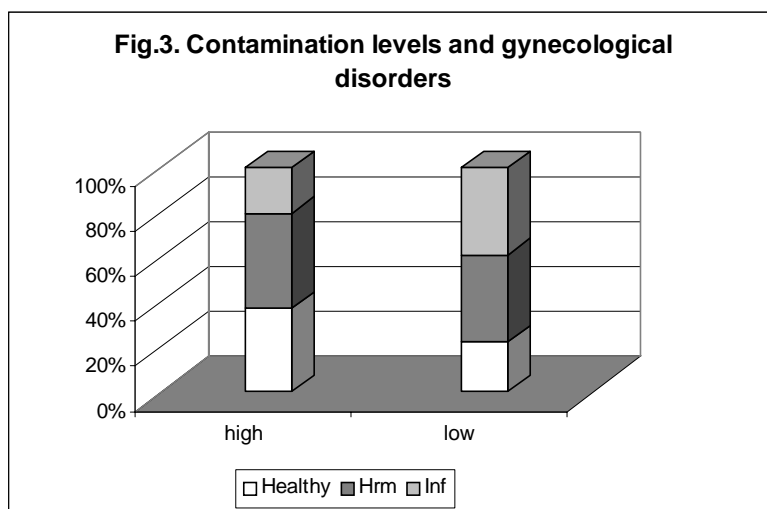
Table 2. Comparison of groups of women residing in territories with lower and higher ¹³⁷Cs-contamination levels

Parameter	Study groups	
	36	59
Number of women	36	59
Cs-137 level, [Ci/km ²]	1.7 - 4.9	5.0 - 14.9
Women with cytogenetic anomalies	4 (11,1%)	9 (15,3%)
Women with disorders	28 (77,8%)	37 (62,7%)

Unexpectedly, we found that incidence of inflammatory gynecological diseases was higher in less contaminated territories (14 cases out of 36; 40%), than in more contaminated regions (only 12 cases out of 59, 20%)(Fig.3).

It is possible that this observation relates to the different modulation of immune system due to exposure to various doses of radiation.

Accurate data on local contamination density would yield more reliable conclusion; however, unfortunately, such data were not available, and we used instead averaged data in one settlement or another. Probably, this is why we were not able to achieve a level of statistical significance.



Conclusions

1. The data are not indicative of a difference in spontaneous aberration patterns between residents of territories with two contamination levels (1 – 5 and 5 – 15 Ci/km²).
2. Frequency of atypical aberration patterns is increased in women with hormone dependent gynecological disorders.

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