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FREQUENCY OF DICENTRICS IN UKRAINIAN CHILDREN AND ADOLESCENTS FROM PARENTS EXPOSED TO RADIATION FALL-OUT AFTER THE CHERNOBYL ACCIDENT

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Abstract

The accident of Chernobyl affected about 130 km north-west of Kyiv, 36,000 hectares of the territory covering 78 districts in 12 regions of Ukraine which have been contaminated with radionuclides with ^{137}Cs density exceeding 1 Ci/km^2. More than 1.8 million people inhabit the contaminated territories. Moreover, 502,377 children, residents of Ukraine, were born in the families where the parents have been exposed to the ionizing radiation. The aim of this study was to carry out a cytogenetical analysis of 55 Ukrainian children living in the areas around Chernobyl. Children were residents of Ukraine and they were born in the families where the parents have been exposed to the ionizing radiation due to nuclear accident.

Cytogenetical procedures were performed according to biological dosimetry assays. Analysis of 36 dicentrics from a total of 53,477 metaphases scored in these children reflected a very low frequency of dicentrics, maybe due to the relatively low doses of exposure in living areas. Finally, the estimated dose is below the detection limit; therefore, any overexposure has been detected by biological dosimetry.

Key words: Ukrainian children and adolescents, radiation, cytogenetic assessment

1. Introduction

After the 1986 Chernobyl nuclear power plant accident, populations of Ukraine, Belarus and Russia were exposed to Iodine (131I), Caesium (137Cs, 134Cs), Strontium (90Sr) and to a wide spectrum of short-lived isotopes which were not measured by physical dosimetry (Fucic *et al*. 2008). The explosion of the Chernobyl-4 reactor core led to the release of radioactivity that was deposited in the surrounding area as dust and debris. Ever since, the public in the affected areas has been exposed to radiation, both externally and internally via contaminated locally grown food, water and air, (Stepanova *et al*. 2008) being subjects to chronic exposure to low-level radiation which may still contribute to genome damage (Fucic *et al*. 2008). A special risk group was the children. It has been estimated that following the Chernobyl accident approximately 160,000 children aged 7 years or less were exposed to a variety of radioactive isotopes. Among the radionuclides involved in the accident 90Sr is specially incorporated in the skeleton of children at four- to six-fold higher rates than in adults (Fucic *et al*. 2008). After the accident various cytogenetic studies were performed in order to obtain some insight on the level of the human hazard due to such accidental exposure. The majority of these studies, carried out by investigators from the former USSR, concern workers involved in the explosion or in the clean-up operations/ liquidators or other individuals affected by acute radiation syndrome, that is, persons exposed to high dosages of ionizing radiations (Sevan'kaev *et al*. 2005). In addition, on Chernobyl clean-up workers and on people exposed to relatively low doses of ionizing radiation some studies also reported an increased frequency of chromosome aberrations, evaluated a short time after the accident (Snigiryova *et al*. 1997). Amog the studies concerning people exposed to

relatively low doses of ionizing radiation, some of them were directed towards of children from contaminated areas being reported an increased frequency of chromosomal aberrations in some of them (Padovani *et al*. 1997; Barale *et al*. 1998). Moreover, a dramatic increase in thyroid tumours in children was observed, as well as an increase in acute myelogenous leukemia (Barale *et al*. 1998). After these findings, genotoxicologists paid increasing interest in studies of children addressing the issue of whether they are more susceptible to environmental exposures to physical and chemical agents than adults (Fucic *et al*. 2008). In an attempt to assess health negative effects caused by radiation from Chernobyl accident, nearly 20 years after the disaster the World Health Organization found no evidence for an increased incidence of leukaemia in a report of the UN Chernobyl Forum. Nevertheless, the same report found a complete lack of analytical studies in which dose and risks were estimated on an individual level (Stepanova *et al*. 2008). One proved way to get information concerning absorbed radiation dose is to quantify cytogenetic effects. Biological dosimetry, based on the analysis of solid stained dicentric chromosomes, has been used since the mid-1960s. For many years the dicentric assay using blood lymphocytes was the only method of biological dosimetry available, and still today it is the technique most frequently used (IAEA 2011) to monitor individual acute doses down to about 0.1 Gy. The aim of this work is the assessment of the frequency of dicentrics in a group of Ukranian children and adolescents from some of the Chernobyl affected areas in order to elucidate a possible exposure to radiation coming from different contaminated sources.

2. Material and methods

2.1. Study location and subjects

Fifty-five Ukranian children and adolescents (29 boys and 26 girls), from parents exposed to radiation fall-out after the Chernobyl accident, with mean age ± standard deviation of 11.0±4.7 years completed the study which was carried out with the help of a local Non-Governmental Organization (NGO) (Fundación Juntos por la Vida) which has a program of hosting Ukranian children during summer with families from Valencia (Spain). Studied subjects were residents in areas very close to the region affected by the accident, and are in most borderline cases to the exclusion zone of 30 km. Figure 1 shows the location of the studied subjects. In this work, we have maintained the Ukrainian names of these places. The study protocol was approved by the Ethical Committee of Hospital La Fe (Valencia, Spain) and each head of household gave their verbal consent after the study had been fully explained to them. Access to the database was restricted to the researchers that participated in this study. Therefore, the information obtained in the study was considered as confidential, although the sanitary authorities have full access rights for inspection purposes.

2.2. Culture conditions and cytogenetic assessment

Peripheral blood samples were collected in sterile vacutainer tubes containing lithium heparin as anticoagulant. The analysis were carried out by mixing 0.75 ml of whole blood with 5 ml of PB-

Max Karyotiping medium and incubated 48h at 37 ºC. To analyze exclusively first-division metaphases, a final concentration of 12 μg ml^{-1} of bromodeoxyuridine was present since the setting up of the cultures. 150 μg of Colcemid was added 2 h before harvesting. Cells were treated with hypotonic solution (KCl, 0.075 M) and fixed with Carnoy's solution, methanol/acetic acid (3/1, v/v). C-banding assay from Fernandes *et al.* (2006) protocols was used to cytogenetic assessment. Briefly, three-day-old slides were placed in hydrochloric acid 0.2N at room temperature for 30 min and then washed three times in distilled water. Next, they were incubated in barium hydroxide 5% at 60 ºC for 1 min, washed for 2 min each in 0.2N HCl and finally in distilled water. Air-dried slides were stained with a solution of Giemsa 2% in phosphate buffer pH 6.8 for 5 min. The chromosome-type abnormalities considered were dicentric chromosomes (dic) and rings (r) and were taken into account only when an acentric fragment was present. Acentrics not related to a dicentric or a ring were recorded as extra acentrics (ace). The total number of analyzed cells ranged between 500 and 1000.

2.3. Statistical analysis

Data were analysed using the Statistical Package for the Social Sciences (SPSS) version 12.0 (SPSS Inc., Chicago, Ill., USA). For dose estimation, a previously established curve for gamma rays was used. The coefficients of the curve are $Y= 0.07 \pm 0.8 \times 10^{-2}$, $\alpha= 4.23 \pm 0.84 \times 10^{-2}$ and $\beta= 4.46 \pm 0.48 \times 10^{-2}$ (Montoro *et al*. 2005). The 95% confidence interval of dose estimations have been calculated according standard procedures (IAEA 2011), using the free software CABAS (Deperas *et al*. 2007).

3. Results and discussion

3.1. Cytogenetic assessment

After the analysis of 53561 metaphases, Table 1 shows the results of the cytogenetic study, including origin, sex, mean age, incidence of dicentrics, dicentrics frequency and absorbed dose, carried out on the 55 subjects. It has been also calculated the mean average and the standard error for the dicentric frequency and absorbed dose of the entire studied group. In our study, a mean dicentrics frequency of 0.0007 (0.7 dicentrics per 1000 cells) was obtained in the studied children and adolescents which is placed in the background level (around from 0.0005 to 0.0010) of dicentrics fixed by the IAEA (2011). For dose estimation, the coefficients of the curve are $Y= 0.07 \pm 0.8 \times 10^{-2}$, $\alpha= 4.23 \pm 0.84 \times 10^{-2}$ and $\beta= 4.46 \pm 0.48 \times 10^{-2}$ (Montoro *et al*. 2005) and the collective dose calculated was 0 (0-0.044) Gy, being this dose below the detection limit. The 95% confidence interval of dose estimations have been calculated according standard procedures (IAEA 2011), using the free software CABAS (Deperas *et al*. 2007).

Origin	Sex	Age (Mean±SD[a])	Incidence[b]	Y_{dic} ± SE[c]
Irpin	M	13.56 ± 4.82	4/9	0.0020 ± 0.0014[d] (2/4)
				0.0010 ± 0.0010[d] (2/4)
	F	10 ± 2.12	2/5	0.0010 ± 0.0010[d] (2/2)
Ivankiv	M	15.11 ± 5.51	5/9	0.0010 ± 0.0010[d] (3/5)
				0.0015 ± 0.0011[d] (1/5)
				0.0019 ± 0.0014[d] (1/5)
	F	15 ± 6.12	8/11	0.0010 ± 0.0010[d] (7/8)
				0.0016 ± 0.0010[d] (1/8)
Fenevichi	M	9.75 ± 3.10	1/4	0.0020 ± 0.0014[d] (1/1)
	F	-	-	-
Slavutych	M	15 ± 6.24	1/3	0.0010 ± 0.0010[d] (1/1)
	F	11.57 ± 2.64	3/7	0.0010 ± 0.0010[d] (2/3)
				0.0013 ± 0.0009[d] (1/3)
Hornostajpil	M	11 ± 0.00	2/4	0.0010 ± 0.0010[d] (1/2)
				0.0020 ± 0.0014[d] (1/2)
	F	10 ± 0.00	1/2	0.0020 ± 0.0014[d] (1/1)
Chernobyl	M	-	-	-
	F	9 ± 0.00	-	-
		12.18 ± 4.75	**27/55**	**0.0007** ± **0.0001**

[a] Standard deviation
[b] Incidence (subjects with dicentrics/total subjects in this row)
[c] Standard error
[d] Incidence (subjects with this value of $Y_{dicentrics}$/ subjects with dicentrics in this row)

Table 1. Results of the cytogenetic study in the 55 studied Ukrainian children and teenagers

The highest value of frequency of dicentrics is 0.0020 which is only obtained in the studied subjects from Irpin, Fenevychi and Hornostajpil. Theses cities (Figure 1), in comparison with others, are close to the Kiev Reservoir that receives water from the Pripyat river (which passes through the Zone of alienation around the Chernobyl reactor). Nowadays, this place has

radionuclide contamination which is referenced in the literature in the fish (Kaglyan *et al.*, 2009) and water (Yablokov *et al.*, 2009). Formerly, two laboratories, including Kharkiv (Ukraine) and Minsk (Belarus), published belatedly data collected in 1986 and subsequent years. The first laboratory (Maznik *et al.*, 1997) studied dicentric yields in evacuees from Prypiat, the town closest to Chernobyl, and followed their decline with time. The other laboratory (Mikhalevich *et al.*, 2000) evaluated children in villages outside the 30-km evacuation zone being the presence of dicentric chromosomes most frequently recorded in younger children (1.17% *vs* 0.67% for older group). Sevan'kaev *et al.* (1993) carried out cytogenetic examinations of children and teenagers from contaminated territories of the Kaluga region and observed that an increased level of unstable chromosomal aberrations was observed in 30-60% of the examined subjects but they did not discover an increase in the frequency of chromosomal aberrations with an increase in the dose load. On the other hand, Pilinskaya *et al.* (1994) established that the highest level of chromosomal aberrations was found in children living within a territory with a maximum level of exposure. Similar results were obtained by Kozenko *et al*. (2010) which observed that the presence of chromosomal aberrations in lymphocytes was increased by 53% in children living in contaminated areas and suggested that the presence of these long-standing changes in these children might suggest an impaired chromosome repair mechanism.

Figure 1. Location of the studied subjects.

Figure 2 shows data of frequency of dicentrics in children exposed to ionizing radiations after Chernobyl accident obtained from several authors. Fucic *et al.* (2008) reviewed that repeated measurements of chromosomal aberrations within a 4-year-period after accidental overexposure in children living in contaminated areas revealed a 53% increased average level of genome damage as measured by the chromosomal aberration assay. Therefore, up to 10 years after the accident children were still suffering from internal contamination. Cytogenetic studies showed that even the areas which were considered as unpolluted were actually contaminated with radionuclides at levels that are capable of increasing genome damage in children, speculating about an accumulation of stable genome damage in these children leading to long-term adverse health effects. At the end of the 20th century, an increase in the level of chromosomal aberrations in humans was found to be associated not only with direct exposure to ionizing radiation, but also with the phenomenon of radiation-induced genomic instability. Spontaneous and radiation-induced genomic instability in the cells of one or both irradiated parents can also be transgenerationally/gametically manifested in the somatic cells of their offspring cytogenetic examination of blood samples from the offspring of fathers-liquidators showed that their children had higher levels of chromosomal aberrations than the children of non-irradiated parents despite the fact that they were not directly exposed to ionizing radiation (Aghajanyan *et al.*, 2011).

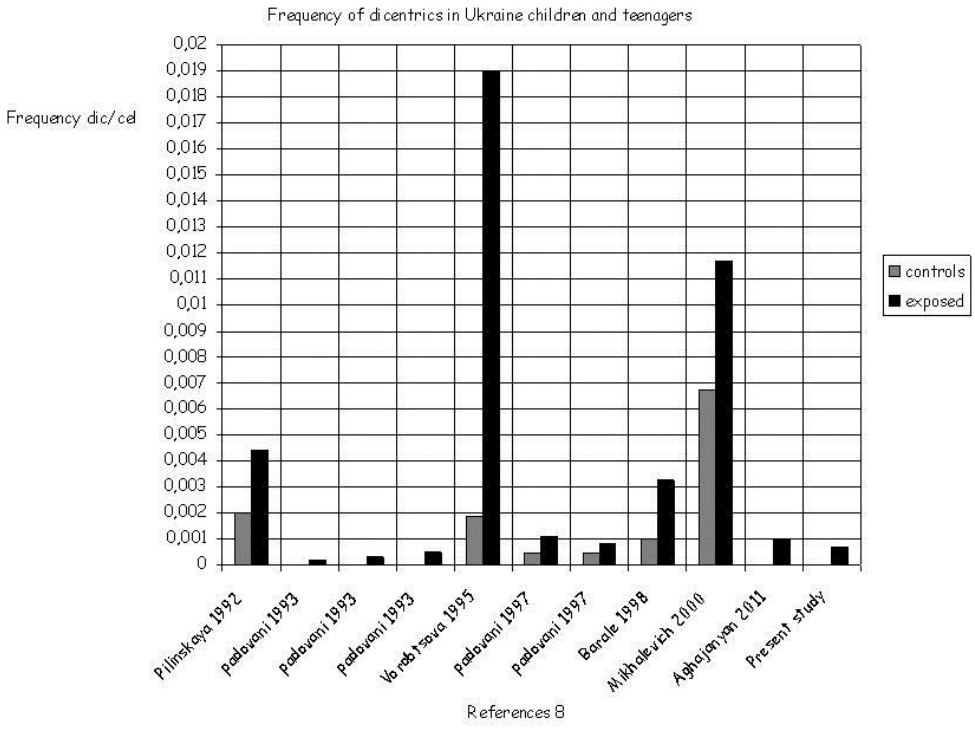

Figure 2. Frequency of dicentrics in Ukrainian children reflected in the literature.

4. Conclusion

Consensus has emerged that the background frequency of dicentrics is 0.0005-0.001 (IAEA 2011). In our study, we found a mean dicentrics frequency of 0.0007 ± 0.0001 which is between the background levels of dicentrics fixed by the IAEA in 2011. The colective absorbed dose was also estimated, although this dose is below the detection limit; therefore, any overexposure has been detected by biological dosimetry. It should be pointed that our study is the latest in literature from the best of our knowledge and therefore we expected that the effects of radiation in genomic damage were as low as possible and the frequency of dicentrics were close to background level.

Data from our cytogenetic study revealed that the study group from the area surrounding Chernobyl had not an increased frequency of dicentrics compared to the background internationally accepted. But further studies on other genomic biomarkers could show another type of chromosomal damage or radiation-induced genomic instability in this population.

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